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Role of microenvironment in resistance to therapy in AML

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Abstract

As part of the dynamic interactions between leukemic cells and cells of the bone marrow microenvironment, specific niches provide a sanctuary where subpopulations of leukemic cells evade chemotherapy-induced death and acquire a drug-resistant phenotype. This review focuses on the cellular and molecular biology of the leukemia stem cell (LSC) niche and of microenvironment/leukemia interactions. Key emerging therapeutic targets include chemokine receptors, adhesion molecules, the sympathetic nervous system, and hypoxia-related proteins, as well as the genetic and epigenetic abnormalities of the leukemia-associated stroma. The complex interplay between LSCs and microenvironment components provides a rationale for appropriately tailored molecular therapies designed to improve outcomes in leukemia. Further understanding of the contribution of the bone marrow niche to the process of leukemogenesis may provide new targets that allow destruction of LSCs without adversely affecting normal stem cell self-renewal.

Keywords

Bone marrow microenvironment; acute myeloid leukemia (AML); leukemia stem cell (LSC); hematopoietic stem cell (HSC); endosteal niche; vascular niche

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Introduction

The chemotherapy-based and targeted therapies have driven significant progress in human acute myeloid leukemia (AML) over the past decade. However, relapse after an initial response remains common, which indicates the persistence of chemoresistant leukemia stem cells (LSCs) in specific niches in the bone marrow (BM) microenvironment. More than 30 years ago, a specialized regulatory BM microenvironmental “niche” was proposed, where stem cells reside, receive appropriate support for maintaining self-renewal and multi-lineage differentiation capacity, and are protected from environmental stress (1). These niches are part of a complex of BM cells, including bone-lining cells (osteoblasts and osteoclasts), mesenchymal stem cells (MSCs), sinusoidal endothelium and perivascular stromal cells, nonmyelinating Schwann cells, and immune cells, that play different roles in hematopoietic regulation (2, 3). The BM niche maintains normal hematopoietic stem cells (HSCs) primarily in a quiescent state by providing signals that inhibit cell proliferation, and only upon receipt of a stimulating signal does the stem cell become activated to divide and proliferate. Expansion of the leukemic clone is known to associate with impairment of normal hematopoiesis, resulting in severe anemia, thrombocytopenia, and immunodeficiency (4). Understanding the mechanisms underlying the regulation of leukemia-initiating LSCs by the BM niche and the alteration of the BM microenvironment by LSCs is crucial to eradicating AML (5).

BM niches fuel the growth of leukemia cells and contribute to the therapy resistance and metastatic potential of leukemia cells by shielding LSCs (6). It has become increasingly evident that myeloproliferative neoplasia and myelodysplastic syndrome (MDS) cells as well as AML cells remodel the BM niche into a malignant niche that contributes to their development through cellular, structural, and functional changes. Not only a “microenvironment-induced oncogenesis” but also a “malignancy-induced microenvironment” has been proposed (7). This review describes the key components and regulation, via cytokines, chemokines, adhesion molecules, the sympathetic nervous system, and hypoxic conditions, of the BM endosteal and vascular niches in healthy and in leukemic BM. The genetic abnormalities of leukemia-associated stroma, which are currently a subject of intense investigation to understand their contribution to LSC survival and expansion, will be also discussed.

Components of microenvironmental niches for normal hematopoietic stem cells

Historically, two distinct anatomical niches for HSCs have been described: the endosteal and vascular niches (8-11). The endosteal and vascular niches are closely related to distinct vascular structures, arterioles and sinusoids (12, 13), respectively, and work in concert (14, 15). Recent advances in microscopy, flow cytometry, and transgenic animal models have revolutionized the understanding of these niches (16) and the intrinsic and extrinsic cues that regulate the behavior of HSCs.

Arterioles run in proximity to the endosteal surface, accompanied by sympathetic nerve fibers ensheathed by nonmyelinating Schwann cells, and quiescent HSCs associate with

periarteriolar niches found within the endosteal BM (12). In turn, sinusoids, fenestrated and lined by reticular-shaped sinusoidal cells, are associated with less-quiescent HSCs re-localizing to perisinusoidal niches (12, 13).

Hypoxia promotes maintenance of HSCs in a quiescent and pluripotent state(17), while HSCs that reside in close proximity to the vascular niche are actively cycling and are responsible for replenishing circulating cells (18). Hypoxia-Inducible Factor α (HIF-1 α), the master transcriptional regulator of hypoxia response, maintains HSCs in a quiescent state by promoting expression of various target genes, including vascular endothelial growth factor (VEGF) and C-X-C chemokine receptor type 4 (CXCR4) (3, 19, 20). On the other hand, the high abundance of capillaries in cancellous bone is incompatible with the hypoxia in BM niches, and histologic images of HSCs indicate that HSCs reside in proximity to the proliferative progenitor cells throughout the BM (9). A recent quantitative imaging study of HSCs further demonstrated that, despite their preferential endosteal localization, HSCs closely interact with BM microvessels and yet exhibit a hypoxic profile (21). These novel observations suggest that regulation of the hypoxic HSC state is mediated through a cell-intrinsic system rather than lack of blood supply.

The endosteal niche

Osteoblasts—The surface of the endosteum is lined by osteoblasts and osteoclasts. Osteoblasts are progenitor bone-forming cells derived from pluripotent MSCs; they work in tandem with osteoclasts in the process of osteogenesis (22). Osteopontin (OPN) and N-cadherin, extracellular matrix (ECM) proteins of endosteal osteolineage cells, mediate HSC localization (23). Signaling through Jagged-1 (Jag-1) on osteoblasts and its receptor Notch on HSCs is involved in the expansion of the HSC pool (8). On the other hand, interaction of angiopoietin-1 (Angpt1) in osteoblasts with its receptor Tie-2 on HSCs results in activation of β 1-integrin and N-cadherin and enhanced adhesion between the niche cells and the HSCs, which contributes to the maintenance of stem cell quiescence (24). The C-X-C motif chemokine 12 (CXCL12) produced by osteoblasts is the major chemoattractant for HSCs (25).

Osteoclasts—Bone-resorbing osteoclasts participate in the initial formation as well as the maintenance of the cavities that constitute the endosteal niche (22, 26). Osteoclastic bone resorption produces abundant active transforming growth factor beta (TGF- β) from bone, the largest latent reservoir of TGF- β (27). Osteoclast inhibition by bisphosphonates causes severe depression of HSC formation and delay of hematopoietic recovery (28). The relevance of osteoclasts in retaining high calcium concentration, which is critical for localization of calcium-sensing receptor-expressing HSCs on the endosteal surface, has been demonstrated in a mouse model (10).

Sympathetic nervous system—The sympathetic nervous system is responsible, via norepinephrine signaling, for regulation of HSCs residing in the periarteriolar position (29). HSCs residing in contact with nonmyelinating Schwann cells, ensheathed autonomic nerves, are reported to be highly quiescent. Mechanistic analysis showed that nonmyelinating Schwann cells are responsible for activation of latent TGF- β produced by a variety of BM

cells, including osteoblasts and MSCs. TGF- β /Smad signaling plays a critical role in HSC maintenance, and autonomic denervation reduced the number of active TGF- β -producing cells, resulting in rapid loss of HSCs from the BM (30). Consistent with these findings, chemotherapy-induced nerve injury impaired hematopoietic regeneration in a murine model (31).

Mature hematopoietic cells and niche regulation—T-regulatory (Treg) cells provide a relative immune sanctuary for HSC on the endosteal surface (13, 32), in which co-localized accumulation of HSCs with Treg cells has been shown to be lost after depletion of Treg cells (32). Other multiple adjunct cell types, including macrophages and megakaryocytes, participate in HSC localization and regulation of HSC quiescence (13).

The vascular niche

The perivascular niches are the important resident area of HSCs (1). The candidate niche cells include CXCL12-abundant reticular (CAR) cells (33), Nestin-positive (Nestin+) MSCs (34), and leptin receptor-positive (LepR+) MSCs (11), which exhibit significant overlap and express multiple soluble and membrane-bound factors that regulate HSC self-renewal and retention (35).

CXCL12-abundant reticular cells—Components of the BM niches, including osteoblasts, CAR cells, and Nestin+ MSCs, are known to be CXCL12-secreting cells. In the sinusoidal areas of the BM where HSCs predominantly reside, the HSCs are in direct contact with CAR cells, which secrete higher levels of CXCL12 than osteoblasts. Most CAR cell populations express PPAR γ , Runx2, and Osterix in the BM, and short-term ablation of CAR cells *in vivo* severely impaired the adipogenic and osteogenic differentiation potential of BM cells, indicating that CAR cells are adipo-osteogenic bipotential progenitors (36).

CXCL12 attracts HSCs expressing its cognate receptor CXCR4. CXCL12-CXCR4 signaling is involved in homing of HSCs into BM and in adhesion through activation of several integrins, and supports survival of HSCs (33). Downregulation of CXCL12 or depletion of CXCR4 leads to mobilization of HSCs into the peripheral blood and reduction of the HSC population (33), suggesting that CXCL12-CXCR4 chemokine signaling plays an essential role in maintaining the HSC pool.

Nestin-positive MSCs and leptin receptor-positive MSCs—MSCs are identifiable by expression of indicator genes from promoters for Nestin, LepR, Prx-1, or Mx-1, with overlaps between the populations not well defined (13). Specific Nestin+ MSCs, which co-localize with HSCs and adrenergic nerve fibers (34), have been reported to participate in the regulation of BM niches (7). Depletion of Nestin+ MSCs significantly reduced BM homing of hematopoietic progenitors and HSC content in the BM in an *in vivo* model (34). The conditional deletion of stem cell factor (SCF) from LepR+ perivascular stromal cells, including Nestin+ MSCs and CAR cells, significantly reduced HSC number (11), whereas SCF deletion from osteoblasts did not affect HSC frequency and function (11).

Bone marrow niche for leukemic stem cells

LSC behaviour, like that of HSCs, is modulated by interactions and signals received within their BM niches (8, 24, 37). Although LSCs share the molecules with HSCs to mediate the interaction with the BM niches, recent studies indicate that LSCs create their “foster home,” inducing reversible changes in niche cell function or composition that contribute to LSC engraftment into the niches and subsequent leukemia development, survival, and drug resistance (7, 38). Suppression of normal hematopoiesis in leukemia patients with relatively low tumor burden may reflect disruption of normal HSC BM niches and creation of leukemia niches by leukemic cells (39).

Homing to the BM niche: CXCL12-CXCR4 signaling

Interaction of LSCs and BM niches is recognized as the major cause of AML relapse. Many *in vitro* and *in vivo* studies demonstrated that inhibition of CXCL12-CXCR4 interactions results in abolishment of CXCL12-induced Chemotaxis, inactivation of prosurvival signaling pathways, and decreases in stromal protective effects on chemotherapy-induced apoptosis in AML cells (40-42). CXCR4 expression has been reported to be higher in Flt3/internal tandem duplication AML than in Flt3/wild-type AML (43), and CXCR4 inhibition increased sensitivity of FLT3-mutated leukemic cells to the FLT3 inhibitor sorafenib under stromal co-culture conditions (44). Similarly, treatment with CXCR4 inhibitor plerixafor combined with TGF- β -neutralizing antibody and cytarabine decreased leukemia burden and prolonged survival in a leukemia mouse model, proving that TGF- β and CXCL12, produced abundantly in the BM niche, play a role in AML chemoresistance (45). In AML cells, chemotherapy-induced upregulation of surface CXCR4 has been demonstrated, which caused stromal protection from additional chemotherapy-induced apoptosis (46). These results suggest that CXCL12-CXCR4 interactions in the BM microenvironment contribute to the chemoresistance of leukemic cells and that disruption of these interactions by CXCR4 inhibitors represents a rational strategy for blocking LSC homing to a BM niche and/or sensitizing AML cells to chemotherapy or kinase inhibitors.

Adhesion to the BM niche

Adhesion to the BM niche directly protects LSCs from damage by chemotherapy or kinase inhibitors, and cell adhesion receptor integrins are required for lodging of LSCs in the BM niche (47). Integrins are known to link to CD44 through their interactions with selectins. The transmembrane glycoprotein CD44, existing as a standard isoform (CD44s) and a range of variant isoforms (CD44v), modulates interactions of LSCs with ECM components hyaluronan and a range of growth factor ligands (41, 48). Jin *et al.* demonstrated that administration of CD44 antibody markedly reduced leukemic repopulation in human AML-transplanted mice (48). As an elemental mechanism in promoting leukemic progression and chemoresistance, CD44 signaling modulates microRNA expression, which regulates promoter methylation status and gene expression (49), and induces leukemia cell reprogramming to exhibit a more stem cell-like LSC phenotype (49).

Hypoxia/HIF-1 α signaling

The overexpression of oxygen-regulated component HIF-1 α has been shown in clusters of leukemic cells in BM specimens (50). One of the accepted functions of hypoxia and HIF-1 α is upregulation of growth factor VEGF and stimulation of angiogenesis. The microvasculature is an active component of the BM microenvironment, supplying appropriate oxygen and nutrients. VEGF secreted by leukemic cells activates receptors on both leukemic and endothelial cells and plays a vital role in the growth of leukemia cells (51). The direct HIF-1 α inhibitor PX-478 decreased hypoxia-mediated VEGF expression in tumor xenografts, resulting in antitumor activity (52). Furthermore, HIF-1 α has been demonstrated to regulate *CXCL12* gene expression in endothelial cells, which increased migration and homing of circulating CXCR4-positive progenitor cells into the ischemic tissue (53), and hypoxia upregulates CXCR4 expression in AML cells (54).

Sympathetic nervous system

Leukemic cells have been shown to alter the niche in a manner that affects normal HSC migration, pool size, and differentiation (39). Schepers *et al.* demonstrated that the malignant myeloid cells reprogrammed the endosteal BM osteoblasts into the pro-inflammatory BM niche that supports LSCs while creating an inhospitable environment for normal HSCs (55).

Induction of neoplastic niche alteration by AML cells through sympathetic neuropathy has been demonstrated recently (56). Hanoun *et al.* showed that MLL-AF9 AML cells rapidly transform the HSC niche, reducing the numbers of arteriole-associated niche cells and the density of their sympathetic nerve network, which is critical for MSC quiescence. HSCs isolated from AML mice had decreased BM repopulation capacity, with a greater extent in the peripheral blood and spleen, indicating that AML colonization of the BM significantly alters the HSC niche (23).

A similar role for sympathetic neuropathy has been demonstrated in malignant cells during disease progression in myeloproliferative neoplasia (MPN) (57). The destruction of sympathetic nerve fibers in the perivascular niche by MPN cells induces Nestin⁺ MSC apoptosis, HSC niche alteration, and MPN pathogenesis. Interleukin-1 β produced by MPN cells destroy Schwann cells, which support the sympathetic nerve fibers, followed by the apoptotic loss of Nestin⁺ cells and reduction of HSC maintenance factors in the microenvironment, such as CXCL12, resulting in peripheral mobilization of HSCs and acceleration of MPN cell expansion in the BM.

Both studies provide new insight into the critical role of the sympathetic nervous system in regulating the HSC niche and in malignant niche transformation during disease progression in AML and MPN.

Modulation of LSC niche

LSCs that receive the support of a BM niche for their survival may in turn contribute to deregulation of the BM niche by their dominant proliferation-promoting signals. Recently, MSCs have been recognized as participating in the malignant process. MSCs in the BM

constitute a heterogeneous population (58), and heterotypic signaling from diseased “reprogrammed” MSCs may affect other cells in the BM. MDS is characterized by BM failure and predisposition for evolution into AML (58). Medyouf *et al.* demonstrated that MSCs are implicated in MDS initiation and progression (59). CD34⁺ hematopoietic cells derived from MDS patients, co-injected with their corresponding MSCs into the BM cavity of mice, demonstrated greater reconstitution ability than CD34⁺ cells alone or co-injected with normal MSCs. Transcriptional profiling revealed the aberrant gene expression implicated in intercellular crosstalk, osteo/adipogenesis, inflammation, and fibrosis in MDS MSCs, indicating that MSCs are required to drive disease initiation and progression (58). Similarly, deficiency of phosphatase and tensin homolog (PTEN), a tumor suppressor and antagonist of the PI3K pathway, in both hematopoietic cells and the microenvironment resulted in myeloproliferation that progressed to overt leukemia/lymphoma (60). Notably, PTEN deletion in hematopoietic cells in the presence of a wild-type BM microenvironment promoted HSC depletion without evidence of myeloproliferation or leukemic development (60).

These findings indicate the importance of interactions between hematopoietic cells and the BM niche/microenvironment and suggest that additional genetic mutations within the BM microenvironment may be necessary for leukemic transformation. Raaijmakers *et al.* demonstrated that conditional knockout of *DICER1*, a gene that regulates microRNA processing, in osteoblastic precursors results in BM failure and leukemia predisposition.

DICER1 deletion caused reduced expression of *SBDS*, the gene mutated in Schwachman-Bodian-Diamond syndrome. Deletion of *SBDS* in mouse osteoprogenitors induced myelodysplasia and the development of AML (7). Other genetic changes in the BM microenvironment also have been demonstrated to contribute to or be required for leukemogenesis. Walkley *et al.* reported that dysfunction of the retinoblastoma protein, a central regulator of the cell cycle and a tumor suppressor, or of retinoic acid receptor γ in the BM microenvironment contributes to development of preleukemic myeloproliferative disease (61, 62). These findings highlight the suggestion that primary stromal dysfunction can result in secondary neoplastic disease, supporting the concept of niche-induced oncogenesis.

It has been shown that β -catenin signaling, NF- κ B activation, and Jagged/Notch activation play critical roles in the development of AML LSCs. Wang *et al.* showed that the Wnt/ β -catenin signaling pathway was required for self-renewal of murine LSCs derived from MLL-AF9-induced AML (63). Very recently, Kode *et al.* demonstrated that activation of β -catenin in mature osteoblastic cells induces AML via Notch activation (64). The activation of NF- κ B or the absence of its inhibitor I κ B α in myelopoietic cells changed the nonhematopoietic compartment, resulting in increased numbers of dysplastic hematopoietic cells with progression into secondary AML via upregulated perinatal expression of Jag-1 in I κ B α hepatocytes and activation of Notch 1 in neutrophils (65). Very recently, the interaction between vascular cell adhesion molecule 1 (VCAM-1) and very late antigen-4 (VLA-4) has been shown to play an integral role in the activation of NF- κ B in the stromal and leukemia cell compartments (66).

Intriguingly, recent studies have indicated the link between microenvironment and AML metabolism. In an *in vitro* screening of 14,718 compounds in LSC-enriched AML cells, the cholesterol-lowering drug lovastatin induced cell-autonomous inhibition of LSCs in a co-culture system with MSCs but did not inhibit AML cells cultured alone. Lovastatin pretreatment of LSC-stromal co-cultures also prolonged the survival of mice injected with these cells (67, 68). In turn, AML cells alter the immune microenvironment via release of high concentrations of arginase II, which suppresses T cell proliferation, polarizes surrounding monocytes into a suppressive M2-like phenotype, and finally inhibits proliferation and differentiation of murine granulocyte-monocyte progenitors and human CD34⁺ progenitors (69). These findings imply that metabolic features supporting the AML BM niche may yield novel therapeutic targets.

Conclusion

Recent and current studies are elucidating the role of the BM microenvironment in the pathogenesis of hematologic tumors. Identification of the precise mechanisms involved in leukemia-host interactions that contribute to drug resistance will provide important insights for development of novel therapies targeting both leukemic cells and the cells in their surrounding microenvironment. Although the impacts of the leukemic microenvironment on therapeutic outcome or the potential targets of the leukemic microenvironment have not been well characterized, the increasing insights into LSC development in their specific BM microenvironment will ultimately result in novel therapeutic strategies in a framework for targeting niche cells to attenuate leukemic progression or targeting LSCs without adversely affecting normal stem cell self-renewal.

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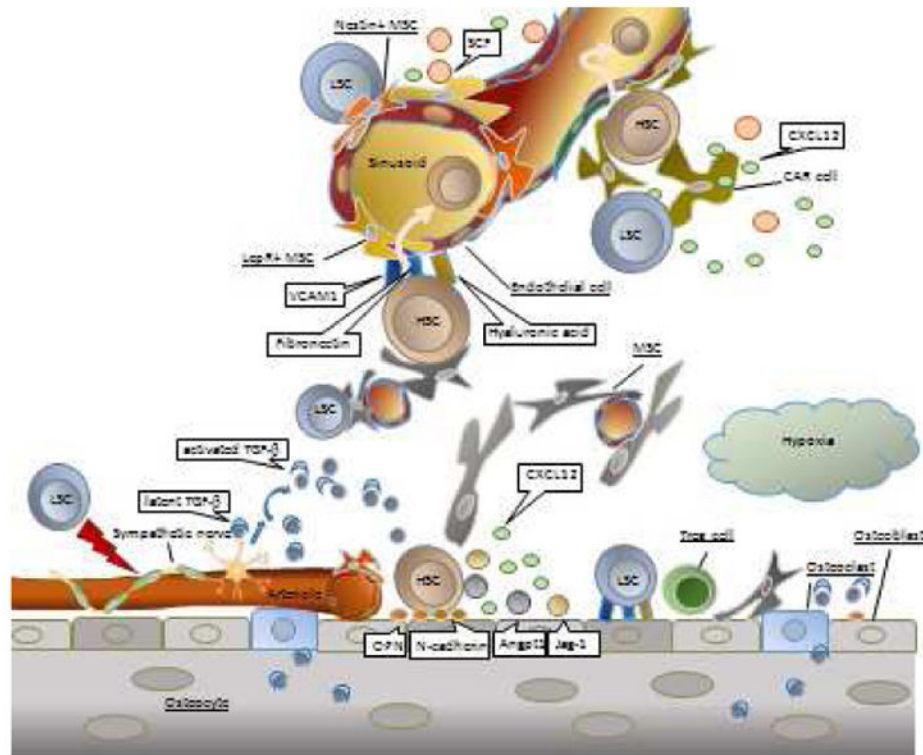


Figure 1. Key components of the leukemic bone marrow microenvironment

Normal hematopoietic stem cell (HSC) niches consist of multiple cell types, including osteoblasts, CXCL12-abundant reticular (CAR) cells, Nestin-positive mesenchymal stem cells (Nestin+ MSCs), leptin receptor-expressing MSCs (LepR+ MSCs), endothelial cells, and Schwann cells wrapping sympathetic nerve fibers. Leukemia stem cells (LSCs) hijack HSC bone marrow (BM) spaces, including perivascular and endosteal niches, partially by sympathetic nerve system denervation. The BM stromal cells and osteoblasts produce complex extracellular matrix, comprising molecules such as vascular cell-adhesion molecule-1 (VCAM1), fibronectin, and hyaluronic acid, which facilitates engraftment and adhesion of LSCs. Osteoblasts within endosteal niches generate osteopontin (OPN), N-cadherin, angiopoietin-1 (Angpt1), and Jagged-1 (Jag-1), which in turn promote leukemia cell dormancy and decrease their chemosensitivity. Osteoclastic bone resorption preserves high calcium concentration as well as transforming growth factor beta (TGF- β) from bone, which are critical for localization of HSC. T-regulatory (Treg) cells provide an immune sanctuary for HSCs on the endosteal surface. Latent TGF- β is activated by the sympathetic nervous system, and autonomic denervation by LSCs induces rapid loss of HSCs from the BM. CAR cells, Nestin+ MSCs, LepR+ MSCs, and endothelial cells may play roles in leukemia cells migration to the perivascular microenvironment via interaction with cytokines, chemokines, and adhesion molecules.