Therapeutic Targeting of Microenvironmental Interactions in Leukemia: Mechanisms and Approaches

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Bone marrow (BM) microenvironment plays a critical role in the process of leukemogenesis with the dynamic interactions between leukemic cells and cells of the bone marrow microenvironment. In the BM microenvironment, two kind of BM specific niches, "osteoblastic (endosteal)" and "vascular" niches, provide a sanctuary for subpopulations of leukemic cells to evade chemotherapy-induced death and allow acquisition of a drug-resistant phenotype. The key components and regulatory mechanisms (via cytokines, chemokines, adhesion molecules, and hypoxic conditions) of these niches contribute to the process of leukemogenesis. The understanding of the molecular pathways critical for microenvironment/leukemia interactions and the contribution of the BM niches to the leukemogenesis may provide a rationale for appropriately tailored molecular therapies targeting not only leukemic cells but also their microenvironment to ensure improved outcomes in leukemia. At the same time, the better understanding of the nature of hematopoietic stem cells, leukemia stem cells and their niches is expected to provide an alternative approach to the treatment of various serious diseases, including leukemia, in clinical practice.

Key words: bone marrow microenvironment, stem cell niche, leukemia, molecular targeted therapy, drug resistance

Introduction

Hematopoietic cell development is tightly regulated by bone marrow (BM) stromal cells (BMSCs) through production of cytokines, chemokines, and intracellular signals initiated by cellular adhesion. BMSC encompass a variety of cell types, including osteoblasts, osteoclasts, endothelial cells, perivascular reticular cells, and mesenchymal stem/stromal cells (MSCs), all of which are critical for the regulation of hematopoietic stem cells (HSCs) maintenance and localization. Cytokines and chemokines produced by BMSC concentrate in particular niches. Of these, CXCL12/stromal cell-derived factor-1alpha (SDF-1α) positively regulates HSC homing. BM engraftment involves subsequent cell-to-cell interactions through the BMSC-produced complex extracellular matrix (ECM). HSCs reside and self-renew in the specialized microenvironmental "niche" had been proposed in 1978 by Schofield.

"stem cells stay in the sanctuary niche, where they receive good support for maintaining self-renewal and multilineage differentiation capacity and be protected from environmental stress such as hypoxia.”

Key components of the BM microenvironment

Interaction with BM stroma cells

Hypoxia

Figure-1 Hematopoietic stem cell niche
area of the BM microenvironment called the niche. The BM niche modulates HSC quiescence, proliferation, differentiation, and migration. HSCs interact with the niche by exchanging various molecular signals, including adhesion mechanisms. The two distinct microenvironmental niches within the BM, the endosteal and vascular niches, have been demonstrated to work in concert.\(^6\)

**Components of the osteoblastic (endosteal) niche**

The surface of the endosteum is lined by osteoblasts and osteoclasts. In the osteoblastic niche, signaling through Jagged-1 on osteoblasts and its receptor NOTCH on HSCs is involved in the expansion of the HSC pool.\(^3\). Angiopoietin-1 in osteoblasts interacts with its receptor Tie-2, a type of receptor tyrosine kinase expressed in HSCs, results in activation of \(\beta_1\)-integrin and N-cadherin. This enhanced adhesion between the niche cell and the stem cell contributes to the maintenance of stem cell quiescence.\(^2\). CXCL12 produced by osteoblasts is the major chemoattractant for hematopoietic stem and progenitor cells (HSPCs).\(^7\). Bone-resorbing osteoclasts form the cavities that constitute the endosteal niche,\(^8\), and participate in the initial formation as well as the maintenance of the HSC niche. The critical role of TGF-\(\beta\)/Smad signaling in HSC maintenance was also demonstrated. Glial nonmyelinating Schwann cells, a component of the BM niche, were shown to be responsible for activation of latent TGF-\(\beta\) produced by a variety of BM cells.\(^9\). In immune-suppressive BM niches called immune-privileged sites, regulatory T cells participate in creating a relative sanctuary from immune attack and supports stem-cell function.\(^10\) (Figure-2).

**Components of the vascular niche**

Cells pass in and out of the circulation through sinusoids, a reticular network of fenestrated vessels that provides the nutrient-rich microenvironment with high concentrations of oxygen and growth factors. CXCL12-abundant reticular (CAR) cells which reside predominantly in sinusoids secrete...
high levels of CXCL12. CXCL12 is a chemokine that functions through its receptor CXCR4. CXCL12–CXCR4 signaling is involved in homing of HSC into BM during ontogeny as well as survival and proliferation of colony-forming progenitor cells. Nestin-positive mesenchymal stem cells (MSCs) also constitute an essential HSC niche component, co-localizing with HSCs and adrenergic nerve fibers. Thus, heterogeneous stromal cells contribute to HSC maintenance through various mechanisms in vascular niche (Figure 2).

**Microenvironmental niches as a “foster home” for leukemia stem cells (LSC)**

The proof for the concept that a subpopulation of leukemic stem cells (LSCs) is solely responsible for maintenance of the leukemia was derived from the study of human acute myeloid leukemia (AML). A small fraction of myeloid leukemic cells, LSCs, exhibits the capacity for long-term self-renewal within the BM microenvironment, which is required for maintenance of the malignant clone. LSCs are able to generate leukemic blasts, and the leukemic clone is organized as a hierarchy. BMSC are capable of promoting the growth, survival and drug resistance of leukemic cells by providing the necessary cytokines and cell contact-mediated signals to LSCs. The molecular mechanisms for maintaining quiescence of normal stem cells may also facilitate LSC survival. Whereas LSCs share certain features of self-renewal and differentiation with HSCs, LSCs differ in their dysregulated proliferation and ability to invade and spread. Recent studies indicate that BM niche components contribute to LSC engraftment into the niches, to leukemia development, survival, and drug resistance; and to determination of leukemia phenotype by providing the necessary cytokines and cell contact–mediated signals to LSCs. On the other hand, LSCs themselves create their “foster home,” inducing reversible changes in BM stromal cell function or composition that result in survival of the leukemic cells.

**CXCR4/CXCL12 interactions and migration of leukemic cells**

CXCL12–CXCR4 signaling is involved in homing of HSCs into BM during ontogeny as well as survival and proliferation of colony-forming progenitor cells. CXCR4 levels are significantly elevated in leukemic cells from patients with B-cell chronic lymphocytic leukemia (B-CLL), and to a lesser degree in AML. Inhibition of CXCL12–CXCR4 interactions resulted in abolishment of CXCL12–induced chemotaxis; inactivation of prosurvival signaling pathways, including phosphorylation of p44/42 mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription 3 (STAT3); and decreases in stromal protective effects on chemotherapy-induced apoptosis in CLL and AML cells (Figure 3). A small-molecule reversible inhibitor of CXCL12/CXCR4, AMD3100, completely blocked CXCL12-induced chemotaxis, attenuated the migration of pre-B-ALL cells into BMSC layers and enhanced the cytotoxic and antiproliferative effects of vincristine and dexamethasone. In primary CLL and AML cells, CXCR4 specific inhibitor AMD3465 abolished CXCL12-induced chemotaxis, inactivated prosurvival signaling pathways, and partially abrogated the protective effects of BMSC on chemotherapy-induced apoptosis. In a murine model of acute promyelocytic leukemia (APL), administration of AMD3100 in combination with chemotherapy triggered an increase of circulating APL cells with decreased tumor burden and improved overall survival compared to chemotherapy alone. These results suggest that disruption of CXCL12–CXCR4 interactions by CXCR4 inhibitors represents a rational strategy for blocking LSC homing to a BM niche and/or sensitizing leukemic cells to chemotherapy or kinase inhibitors.

**Modulation of the LSC stem cell niche via hypoxia/HIF-1α signaling**

Leukemic cells proliferate even under hypoxic conditions, indicating that the cells are able to adapt to hypoxic conditions. Further, overexpression of the oxygen-regulated component Hypoxia-Inducible Factor-1α (HIF-1α), has been observed in clusters of leukemic cells in BM. Notably, HIF-1α was recently found to regulate CXCL12 gene expression in endothelial cells, resulting in selective in vivo expression of CXCL12 in ischemic tissue. In AML, total and surface CXCR4 expression were
upregulated under hypoxic conditions in leukemic cell lines and patient samples. In this context, HIF-1α may represent an important molecular target within the tumor microenvironment.

Conclusion and future directions

Despite significant progress achieved over the past decade in the chemotherapy-based and targeted treatments of several leukemia subsets, the relapse remains common after an initial response, indicating resistance of leukemia stem/progenitor cells to current therapies. By elucidating the role of the BM microenvironment in the pathogenesis of hematologic tumors, recent studies have provided the framework for identifying and validating novel therapies that target both leukemic cells and cells in their surrounding microenvironment. The underlying molecular mechanisms involved in stem cell activation and homing to the niche will provide important insight into the precise mechanisms involved in tumor–host interactions that contribute to drug resistance. This understanding will provide a framework for the rational combination of agents in clinical trials to overcome drug resistance and improve patient outcomes. In particular, further understanding of the contribution of the microenvironmental niche to the process of tumorigenesis may provide new targets aimed at destroying cancer stem cells without adversely affecting normal stem cell self-renewal.

References


