Abnormal bone marrow microenvironment: the “harbor” of acute lymphoblastic leukemia cells

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Abstract

Bone marrow (BM) microenvironment regulates and supports the production of blood cells which are necessary to maintain homeostasis. In analogy to normal hematopoiesis, leukemogenesis is originated from leukemic stem cells (LSCs) which gives rise to more differentiated malignant cells. Leukemia cells occupy BM niches and reconstruct them to support leukemogenesis. The abnormal BM niches are the main sanctuary of LSCs where they can evade chemotherapy-induced death and acquire drug resistance. In this review, we focus on the protective effects of BM niche cells on acute lymphoblastic leukemia cells.

Keywords: acute lymphoblastic leukemia, bone marrow microenvironment, osteoblastic niche, vascular niche

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a hematologic neoplasm characterized by clonal proliferation of B or T malignant cells (B-ALL or T-ALL). The normal bone marrow (BM) microenvironment is disrupted by leukemia cells\textsuperscript{1} and then switches to an abnormal niche to protect leukemia cells from therapy-induced cell death, resulting in disease progression.\textsuperscript{2}

There are two distinct BM niches. One is the osteoblastic niche which includes osteoblasts, stromal cells, adipocytes, macrophages, and regulatory T (Tregs). The other is the vascular niche which consists of vascular endothelial cells and perivascular stromal cells\textsuperscript{3,4} (Table 1). The dynamic interactions between niche cells and ALL cells are through cells and non-cellular components (eg, extracellular matrix). A better understanding of the abnormal niche to the progression of ALL may offer new therapeutic potentials.

1.1. Osteoblastic niche

1.1.1. Osteoblasts. Osteoblasts, attached to endostium, are critical components of the osteoblastic niche.\textsuperscript{5} Osteoblasts play a key role in the proliferation, differentiation, and maintenance of hematopoietic stem cells (HSCs) by releasing various growth factors, such as granulocyte colony-stimulating factor (G-CSF), mSCF (stem cell factor),\textsuperscript{6,7} so the absence of osteoblasts leads to the loss of HSCs. In counterpart to the normal microenvironment, osteoblasts secrete cytokines, chemokines to protect leukemic stem cells (LSCs) from death induced by multi-drugs,\textsuperscript{8} showing an indispensable impact on leukemogenesis. For example, osteopontin is an extracellular matrix molecule secreted by osteoblasts, which could maintain LSCs in a dormant state, resulting in the evasion of death from cytotoxic agent cytarabine (Ara-C).\textsuperscript{9}

To investigate the functional impact of osteoblasts on ALL cells, Moses et al\textsuperscript{10} described a 2D co-culture model and found that only ALL cells buried beneath osteoblasts went into quiescence and showed significant resistance to chemotherapy. This was related to reduced expression level of BCL6 protein, a proto-oncoprotein in ALL cells.

Altered miRNA expression has provided valuable insight into the molecular mechanisms of leukemia.\textsuperscript{11} Manipulating miR-221/222 could induce quiescent cells to cell cycle, and increase the sensibility of S phase cells to chemotherapeutic drugs.\textsuperscript{12} When co-cultured with osteoblasts, expression of miR-221/222 in ALL cells decreased, which created a quiescent chemo-resistant ALL phenotype.\textsuperscript{13} However, due to the lack of unique markers, the characterization
of MSCs is still a mystery. What counts is that BM stromal cells (BMSCs) are key players in the transformation of the niche to favor the survival of leukemia cells. Primary LSCs are unable to proliferate and survive without BMSCs.

It is known that many adhesive interactions between leukemia cells and BMSCs contribute to the self-renewal and survival of leukemic cells, such as lymphocyte function-associated antigen (LFA-1)/intercellular adhesion molecule-1 (ICAM-1)-mediated adhesion, very late antigen-4 (VLA-4)/vascular cell adhesion molecule-1-mediated adhesion, and N-cadherin/β-catenin mediated adhesion. Besides, BMSCs show pro-function to ALL through cytokines and molecules. Activin A, a member of the transforming growth factor-β (TGF-β) family secreted by BMSCs, was highly expressed when co-cultured with ALL cells, resulting in modulating the proliferation, migration, and progression of ALL cells. Naderi et al found that BMSC-derived prostaglandin E2 (PGE2) could inhibit DNA damage-induced p53 accumulation by activating cAMP-PKA signaling, thereby accelerating leukemogenesis and protecting against therapy-induced cell death. Yu et al confirmed that overexpression of HO-1, a cytoprotective enzyme, in BMSCs could enhance the resistance of B-ALL cells to vincristine, which was induced by PI3K/AKT signaling pathway. In addition, asparagine secreted by BMSCs was absorbed by ALL cells to protect themselves from asparaginase cytotoxicity.

Chemotherapeutic drugs, including paclitaxel, anthracyclines, ara-C, and methotrexate induced apoptosis of leukemia cells through upregulating the level of reactive oxygen species (ROS). Upregulating the ROS level, particularly in mitochondria which was the prime source of ROS, was a feasible strategy of killing ALL cells. Cai et al found that reduced mitochondrial ROS level was related to BMSC coculture, which induced chemotherapy resistant. T-ALL cells cultured with BMSCs led mitochondrial metabolism to switch toward the glycolytic phenotype, which was initiated by phosphorylation of Drp1. In addition, intracellular oxidative stress may be elicited by chemotherapeutic drugs. T-ALL cells transferred more mitochondria through tunneling nanotubes that were protrusions extending from cell membrane to BMSCs, but received fewer mitochondria from BMSCs, resulting in chemoresistance. ICAM-

### Table 1

**Types of BM niche cells.**

| Niche cells types       | Function in normal BM                                                                 |
|-------------------------|---------------------------------------------------------------------------------------|
| Osteoblastic niche cells| Regulate normal hematopoiesis                                                         |
| BMSCs                   | Origin of BM niche cells and regulate hematopoiesiyan                                |
| Adipocytes              | Negatively regulate normal hematopoiesis                                              |
| Macrophages             | Support HSCs survival and retention                                                  |
| Tregs                   | Mediate immune responses and prevent immune attack                                     |
| Vogueal niche cells      | Regulate angiogenesis                                                                  |
| CAR cells               | Secret abundant CXCL 12, maintain HSCs pool, and HSCs proliferation                   |
| NES/MSC cells           | Express and regulate HSCs maintenance genes, and promote HSCs homing                 |
| Leptin+ cells           | Express SF, and maintain HSCs numbers                                                 |

BM = bone marrow; CAR = CXCR12-abundant reticular, HSCs = hematopoietic stem cells, MSCs = mesenchymal stem cells, SCF = stem cell factor.
controls whereas the leptin levels of BM-derived plasma from levels were markedly higher in ALL patients than healthy patients. Leptin, an adipocyte-secreted hormone that was involved in hematopoiesis. 31 Increased adiposity is frequently coincident with reduced hematopoiesis. BMAs produce numerous factors, such as stromal cell-derived factor 1α (SDF-1α), leptin, adiponectin, and FFA 32 to progress leukemia. Adipocytes were energy storage to provide a suitable safe area to protect them from chemotherapy-induced apoptosis. Pramanik et al 38 demonstrated that ALL cells migrated into adipose tissue via upregulation of survival genes Bcl-2 and Pim-2.

On the other hand, adipocytes attract ALL cells to migrate to a safe area to protect them from chemotherapy-induced apoptosis. Pramanik et al 38 demonstrated that ALL cells migrated into adipose tissue via adipocyte-derived SDF-1α, and thus gained a survival advantage within BM niche. Additionally, ALL cells were protected from chemotherapy in vitro by adipose tissue 38 via upregulation of survival genes Bcl-2 and Pim-2.

Leptin, an adipocyte-secreted hormone that is involved in hematopoiesis, 39,40 can activate many signaling pathways, including Jak/Stat, MAPK/ERK1/2, and PI3K signaling pathways to stimulate cell proliferation and protect leukemia cells from apoptosis. 41,42 Besides, Le et al 43 reported that upregulation of Lepr could inhibit ALL progress. Serum leptin levels were markedly higher in ALL patients than healthy controls 44 whereas the leptin levels of BM-derived plasma from children ALL were significantly lower than healthy controls at diagnosis. Leptin levels were normalized after complete hematologic remission of ALL. 44

In summary, although various studies have been carried out to investigate the effect of BMAs in tumorigenesis, BMAs as a cell-based therapy for ALL are less reported. Therefore, much more studies are necessary to develop targeted therapy based on the interaction between BMAs and ALL.

1.1.4. Macrophages. Macrophages, which are considered as pivotal components of immune responses, 4,5 play significant and distinctive roles in hematopoiesis. Macrophages can be divided into two subsets, M1 and M2. M1 macrophages produce proinflammatory cytokines (IL-12, IL-1β, TNFa, IL-6, and IL-23) and immune activation factors to encourage inflammation and tumor suppression. 46,47 M2 macrophages secrete anti-inflammatory cytokines (IL-10, IL-13, and TGF-β) and PGE2 to suppress inflammation and promote the invasion, growth, and metastasis of tumors. 47,48

The role of tumor-associated macrophages (TAMs) had been extensively investigated in solid tumors whereas few reports in hematopoietic malignancies. In leukemic microenvironment, TAMs are called leukemia-associated macrophages (LAMs), which mostly display an M2-like phenotype 49 and promote leukemia. In Notch1-induced mouse model of T-ALL, the gene expression patterns and phenotypes of LAMs from BM, spleen, and peritoneum showed significant differences. LAMs from spleen accelerated T-ALL progression. Compared with LAMs from BM and spleen, peritoneum-derived LAMs expressed more M1 associated genes. 50 Valencia et al 51 highlighted that the ALL cells released bone morphogenetic protein 4 (BMP4) to induce macrophages to polarize toward an M2-like macrophage with pro-tumoral features, which in turn stimulate ALL cells. Taken together, increasing evidence suggest that ALL cells actively engage in crosstalk with LAMs to regulate their progression. So, repolarizing macrophages into M1 phenotype may be a promising therapeutic strategy in ALL.

1.1.5. Regulatory T cells. Among immunocytes in BM microenvironment, Tregs are the mostly well-characterized type, which are pivotal regulators in various inflammatory conditions and secure peripheral T-cell tolerance. 52 Tregs which comprise around 2% to 10% of human CD4+ T-cells, 53 are characterized by positive expression of CD4, CD25, the transcription factor forkhead box P3 (Foxp3), and negative expression of CD127. Tregs suppressed T-cell activities through the production of immunosuppressive cytokines, including IL-10, TGF-β, and IL-35. 54,55

Tregs could be activated by CD19+ B-ALL cell lines and primary B-ALL blasts. 56 It is known that the percentage of Tregs cells was higher in patients with B-ALL and the expression of cytotoxic T lymphocyte-associated antigen-4, glucocorticoid-induced tumor necrosis factor receptor, and lymphocyte activation gene 3 increased, suggesting that the activation of Tregs might be an initiator to immune escape. Additionally, there was a positive correlation between patients’ age and Tregs numbers. The more Tregs cells ALL patients had, the worse prognosis they got, indicating that a higher percentage of Tregs in ALL predicted a worse immunological reaction. Helios in combination with FoxP3, are suitable markers for discriminating functional Tregs. 56 Li et al 57 demonstrated patients with pre-B ALL had a higher percentage of Helios’ FoxP3+CD4+ Tregs, and the expression level of Helios was correlated positively with the inhibition of Tregs. Thus, Helios may be a novel target to manipulate Treg activity in ALL immunotherapy.

1.2. Vascular niche

The vascular niche, also known as the endothelial niche, is mainly served as a location where the proliferation, differentiation, and mobilization of short-HSCs take place. 58 The vascular niche is comprised of many different cell types that ensure normal homeostasis with their mutual cooperation, including vascular endothelial cells, CXCR12-abundant reticular (CAR) cells, Nestin+ MScs, and Lepr+ MScs. 59

Angiogenesis is necessary for the progress of tumor growth and metastatic dissemination. Leukemia-derived VEGF connects with
VEGFR expressed on endothelial cells to activate proliferation, migration, survival, and vascular permeability. Lyu et al revealed that the expression level of VEGF was higher in relapsed ALL patients compared to standard or high-risk ALL, suggesting that VEGF is associated with an adverse prognosis. Ang/Tie signaling pathway exerts a vital and rate-limiting role in tumor vascularization. Tie-1 and Tie-2 tyrosine kinase receptors are expressed specifically on vascular endothelial cells. Angiopoietin1-4 (Ang1-4), the ligand of Tie-2 receptor, are expressed on perivascular cells. The level of Ang1 in BM plasma was lower and Ang2 was higher than control, suggesting that the remission induction is associated with increasing Ang1/Ang2 ratio. Hence, Ang1 and Ang2 can serve as biomarkers to monitor the effectiveness of chemotherapy.

Most vascular niche cells are originated from MSCs. CAR cells, which secret CXCL12, surround sinusoidal endothelial cells or locate near the endosteum. CAR cells have a strong overlap with leptin receptor-expressing (LepR+) cells. Leukemia cells are in direct contact with CAR cells to promote leukemogenesis. Uy et al showed that treatment with G-CSF led to a marked decrease in pro-B and pre-B cells through decreasing production of CAR cells related factors, including CXCL12, IL-7, and insulin-like growth factor-1. Additionally, CXCL12 deletion in vascular endothelial cells impeded tumor proliferation, suggesting that CXCL12 was necessary for T-ALL progression (Figure 2).

2. CONCLUSIONS

Coordination between ALL cells and osteoblastic/vascular niches creates a suitable leukemic microenvironment, promoting ALL cell proliferation and resistance to chemotherapeutic drugs. Various BM niche cells are involved in the progression of ALL, and these cells protect ALL cells from chemotherapy-induced death and help them to acquire drug resistance. Fortunately, increasing numbers of potential targets of niche-directed treatments are now starting to emerge, gaining great efficiency for the treatment of ALL. In conclusion, each of the alterations in the abnormal niche may be effectively targeted by various therapeutic procedures, to be the basis for the development of innovative strategies.

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