REVIEW

Bone marrow niche-mediated survival of leukemia stem cells in acute myeloid leukemia: Yin and Yang

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ABSTRACT

Acute myeloid leukemia (AML) is characterized by the accumulation of circulating immature blasts that exhibit uncontrolled growth, lack the ability to undergo normal differentiation, and have decreased sensitivity to apoptosis. Accumulating evidence shows the bone marrow (BM) niche is critical to the maintenance and retention of hematopoietic stem cells (HSC), including leukemia stem cells (LSC), and an increasing number of studies have demonstrated that crosstalk between LSC and the stromal cells associated with this niche greatly influences leukemia initiation, progression, and response to therapy. Undeniably, stromal cells in the BM niche provide a sanctuary in which LSC can acquire a drug-resistant phenotype and thereby evade chemotherapy-induced death. Yin and Yang, the ancient Chinese philosophical concept, vividly portrays the intricate and dynamic interactions between LSC and the BM niche. In fact, LSC-induced microenvironmental reprogramming contributes significantly to leukemogenesis. Thus, identifying the critical signaling pathways involved in these interactions will contribute to target optimization and combinatorial drug treatment strategies to overcome acquired drug resistance and prevent relapse following therapy. In this review, we describe some of the critical signaling pathways mediating BM niche-LSC interaction, including SDF1/CXCL12, Wnt/β-catenin, VCAM/VLA-4/NF-κB, CD44, and hypoxia as a newly-recognized physical determinant of resistance, and outline therapeutic strategies for overcoming these resistance factors.

KEYWORDS

Bone marrow niche; leukemia stem cell; acute myeloid leukemia; Yin and Yang

Introduction

Acute myeloid leukemia (AML) is characterized by the accumulation of clonal or oligoclonal undifferentiated leukemic blasts in the bone marrow (BM) and/or blood that exhibit uncontrolled growth, differentiation block, and a reduced ability to undergo apoptosis. The main focus of leukemia research has been the elucidation of genetic and epigenetic features of these blasts, and how these changes affect cell proliferation, differentiation, and survival. The current paradigm of leukemogenesis implies a multistep process that involves at least two different types of genetic alterations. One typically involves the deregulation of transcription factors that modulate hematopoietic development, such as GATA-1 and C/EBPα, the second affects the intracellular signaling pathways that mediate cell survival and proliferation, and includes factors like Fms-like tyrosine kinase 3 (FLT3) and the KIT1-4. In support of this, a pre-leukemic hematopoietic stage has recently been identified that is characterized by DNMT3A mutations in hematopoietic stem cells (HSC)5. However, leukemogenesis occurs in a bone marrow environment that is functionally altered and can by itself induce AML.

Concomitant with the characterization of the different components comprising the bone marrow (BM) niche, an increasing number of studies emphasize the biologic significance, and potential clinical relevance, of these constituents in leukemogenesis, especially since the BM niche is critical to the maintenance and retention of HSC, in particular leukemia stem cells (LSC). Furthermore, crosstalk between LSC and the associated BM stroma represents a powerful relationship that influences leukemia initiation, progression, and response to therapy2-7. In fact, our group was the first to discover that mesenchymal stromal cells (MSC), which originate in the BM and migrate to solid tumors, can be used as delivery vehicles for anti-tumor agents8,9. They also contribute critically in leukemic cell drug
resistance. In this review, we will focus on the signaling pathways involved in BM microenvironment-mediated survival of LSC in AML.

The BM niche and HSC

Biological niches are local tissue microenvironments that maintain stem cells. HSC niches are present in the aorta-gonad-mesonephros (AGM) region, placenta, fetal liver, and spleen throughout development, and the BM is the primary post-natal reservoir for HSC maintenance and hematopoiesis. Ray Schofield proposed the hypothesis of the BM niche as a specialized location for reconstituting HSC, and during the past four decades this hypothesis has been validated with additional characterization of the cellular and molecular components of this niche.

Molecular crosstalk between HSC and the cellular constituents of BM niche controls the balance between HSC self-renewal and differentiation. Stromal cell-derived factor-1 (SDF1/CXCL12) is a chemokine essential to maintaining the quiescent HSC pool via CXCL12-CXCR4 signaling in adult BM. CXCL12-abundant reticular (CAR) cells adjacent to sinusoids were first shown to co-localize with HSC throughout the BM. By conditional deletion of CXCL12 and stem cell factor (SCF), it was established that HSC maintenance and self-renewal are provided by perivascular, nestin-positive, immature MSC and endothelial cells. Other cell types, including sympathetic nerves, non-myelinated Schwann cells, macrophages, and osteoclasts also regulate the HSC niche. As the first cell population shown to influence HSC self-renewal, osteoblasts do so directly via the secretion of CXCL12 and other factors. However, recent data are suggestive of a different MSC critical for HSC maintenance. This cell is nestin-negative, termed MSC-Prx1, and secretes SDF1/CXCL12. Its knockout results in loss of normal HSC. Additional research has identified HIF-1α and HIF-2α as determinants of HSC maintenance suggesting hypoxia (i.e., <2% O2) in the BM niche. Together, these data revealed a BM niche created by MSC and endothelial cells that is perivascular and located in trabecular bone adjacent to sinusoids (Figure 1). Constituents of this niche secrete SCF, CXCL12, and other factors that promote HSC

**Figure 1** HSCs and LSCs in bone marrow niche. Normal HSCs and LSCs reside in a BM niche created by MSC and endothelial cells that is perivascular and located in trabecular bone adjacent to sinusoids. The endothelial sinusoid is surrounded by nestin and/or leptin receptors positive MSC with high expression of the chemokine CXCL12, which is a chemoattractant for CXCR4-expressing HSCs. CD146 mesenchymal progenitors facilitate transendothelial migration, homing, proliferation, and differentiation of normal HSCs and LSCs. Bone marrow niche is hypoxic regulated by HIF-1 and HIF-2. LSC proliferation results in expansion of hypoxic microenvironmental niches.
maintenance and preserve the balance between HSC self-renewal and differentiation.

Yin and Yang: crosstalk between LSC and the BM niche

In Chinese philosophy, Yin and Yang describe how opposite or contrary forces are actually complementary, interconnected, and interdependent in the natural world, and how they give rise to each other as they interrelate to one another. Many tangible dualities (such as light and dark, fire and water) are thought of as physical manifestations of the duality symbolized by Yin and Yang. This duality lies at the origins of many branches of classical Chinese science and philosophy, as well as being a primary guideline of Traditional Chinese Medicine (TCM). Inspired by the interdependence or mutual rooting between Yin and Yang, herein, we are employing the term Yin and Yang to describe the dynamic interactions between leukemic cells and their BM microenvironment, in contrast to the "seed and soil" hypothesis proposed by the English surgeon Stephen Paget to describe the complex, interdependent relationship between tumor cells and their microenvironment\textsuperscript{16,17}.

In the context of Yin and Yang theory and TCM, Yang generally represents for function and activity, as Yin stands for physical elements. As for the relationship of LSCs and BM niche, LSCs are believed to be able to initiate and perpetuate leukemia and BM niche support the maintenance and survival of LSCs. LSCs or leukemia cells, which we will call Yang, are critically dependent on the BM niche, which we will call Yin. Yin is in turn functionally altered by the presence of the transformed cells and responds by further supporting leukemogenesis. Indeed, like normal HSC, LSC/leukemia cells remain dependent on signals from the BM niche for survival and proliferation, and the niche serves as a sanctuary for resistance to chemotherapeutic agents\textsuperscript{2,4,9,17,18}. Moreover, recent research has illustrated that CD34\textsuperscript{+} LSC engraftment and proliferation in BM alters the stromal microenvironment sufficiently to essentially create a "malignant niche" that is inhospitable to normal HSC\textsuperscript{19}. To shed light on the BM niche reprogramming induced by leukemic cell interaction, we performed proteomic, gene expression, and microRNA analysis on global change in MSC in AML compared with normal MSC in vitro and in vivo. We found that survival pathway-related genes (i.e., GSKA, STAT1, STAT5, PP2A, CDKN1A, and CDK4) were significantly over-expressed in AML MSC. Notably, gene expression analysis showed CXCL12, complement-related genes (i.e., C4A, C4B and Serpin G1), and inhibitor of osteoblast differentiation-IGFBP5 were significantly upregulated. Furthermore, AML MSC expressed reduced levels of let-7g, let-7c, miR 21, and miR93, and an elevated level of miR410. Collectively, our evaluations demonstrated leukemic cell-induced MSC changes consistent with pro-survival, growth-stimulatory signals that mimic an inflammatory response, and potentially contribute to LSC therapy resistance\textsuperscript{20} (Figure 2).

Interdependence between each other is core theory of Yin and Yang. In the context of LSCs and BM niche, interchange from supporting role of BM niche to leading actor for leukemia initiation is an amazing presentation of interdependence between Yin and Yang. Accumulating reports have also demonstrated that alterations in the BM niche can lead to the development of myeloproliferative disease in mice. For example, Walkley et al.\textsuperscript{21,22} demonstrated that mice deficient in retinoic acid receptor γ in BM stromal cells developed myeloproliferative syndrome, and similarly the deletion of Dicer1 in mouse osteoprogenitors induced myelodysplasia and secondary leukemia. Recently, Kousten's group has reported that β-catenin and FOXO1 in osteoblast can induce AML\textsuperscript{23,24}. These data supported the concept of niche-induced oncogenesis. Furthermore, presumably microenvironment-initiated leukemogenesis following allogenic stem cell transplantation was more pronounced in BM MSC from leukemia expressing the MLL-AF4 oncogenic fusion gene\textsuperscript{25}, and patients with monosomy of chromosome 7, MSC were found to express the MLL-AF4 fusion gene\textsuperscript{25} and in other studies were shown to harbor cytogenetic aberration\textsuperscript{26-29}. Moreover, the leukemia microenvironment provides critical extrinsic cues that dictate transformation potential during chronic myeloid leukemia progression. Sontakke et al.\textsuperscript{30} showed predominant transplantable lymphoid leukemia was induced by BCR-ABL/BMI1 co-expressed CD34\textsuperscript{+} cells in the NOD/Lt-scid/IL2Rnull (NSG) xenograph mouse model. Interestingly, myeloid leukemia development was not observed. Similar lymphoid-biased leukemia occurred in the SCL-tTA/BCR-ABL transgenic chronic myeloid leukemia mouse model\textsuperscript{31,32}. However, in NSG mice transplanted with a humanized BM niche, both lymphoid and myeloid leukemia could be induced\textsuperscript{30}. These data indicate that different components of the BM niche activate corresponding signaling pathways that contribute to lymphoid or myeloid tumor development.

Consequently, leukemia-induced microenvironment reprogramming and microenvironment-induced leukemogenesis provided the basis for our Yin and Yang comparison in the development of these malignancies. Furthermore, interdependence between Yin and Yang and
Crosstalk of LSCs and BM niche not only shed light into the understanding of leukemogenesis, but also provide new insight into novel strategy of targeting both LSCs and BM niche.

Signaling pathways involved in BM niche-mediated survival of AML LSC

The stromal cells in BM niche can provide a sanctuary in which leukemic cells acquire a drug-resistant phenotype and evade chemotherapy-induced death. In order to dissect the underlying mechanism of this resistance, the first challenge is to simulate the BM microenvironment both in vitro and in vivo. Considering the critical role of MSC in BM niche for both HSC and LSC, Konopleva et al. developed an in vitro MSC co-culture system that mimics the BM microenvironment in vivo by culturing leukemic cells derived from human leukemia cells lines or primary human leukemia samples with mouse stromal cells (Figure 3). This was further refined and updated to an entirely human co-culture system by replacing the mouse MSC with BM-derived MSC from healthy human donors.

In vivo, the NSG or NSG-S xenograph mouse models are currently the gold standard for the determination of human hematopoietic cell engraftment and studies of the development of human leukemia. However, is the mismatch between human leukemia cells and the mouse BM niche appropriate? In view of this potential shortcoming, several humanized xenograft mouse models have been reported. For example, Sontakke et al. reported a NSG mice model in which scaffolds coated with culture-expanded human MSC were implanted subcutaneously to allow the development of a humanized niche containing mineralized bone-matrix, osteoblasts, stromal cells, as well as the appropriate tissue vascularization. Furthermore, our group successfully developed a novel in vivo model of a genetically-controlled hematopoietic microenvironment using human MSC and endothelial colony-forming cells implanted subcutaneously into NSG mice, which also leads to the development of extramedullary bone and BM in mouse. Together, these MSC co-culture systems and the humanized microenvironment NSG models are useful tools for the study...
of leukemia biology, as well as the development of novel anti-leukemic therapeutic modalities aimed at modifying this microenvironment. In regard to the latter, the following sections detail some of the signaling pathways we believe are intimately associated with BM niche-mediated survival of LSC and chemoresistance in AML (Figure 4).

**SDF-1α/CXCR4 signaling pathway**

SDF-1α, also known as C-X-C motif chemokine 12 (CXCL12), is a chemokine protein that is encoded by the CXCL12 gene in humans. SDF-1α is a growth factor for B-cell progenitors and a chemotactic factor for T-cells and...
monocytes. Interaction of SDF-1α and its receptor CXCR4 play a key role in not only crosstalk between HSC and the BM niche, but also in leukemic cells in this microenvironment. Konopleva et al.9 and others39 have reported that stromal cells protect AML and chronic lymphocytic leukemia cells from apoptosis induced by chemotherapeutic agents. Although the mechanisms of stroma-mediated leukemic cell protection involve a complex interplay between stromal cytokines/chemokines and adhesion molecules, the SDF-1α and CXCR4 axis has emerged as critical mediator of this process.40,41

Interrupting the leukemic cell and microenvironment interaction by targeting the SDF-1α/CXCR4 axis has become an attractive approach to AML therapy. Different novel CXCR4 inhibitors are available, including the RCP168, LY2510924, and BL8040 (Bioline) peptides and the small molecules AMD3465 and AMD3100, to test the hypothesis that CXCR4 inhibition enhances AML cell sensitivity to chemotherapy.40,43 Zeng et al.40,41 identified the synthetically and modularly modified chemokine RCP168, which acts by blocking SDF-1α-induced chemotaxis and suppressing survival signaling, as a potent anti-leukemic agent. Most importantly, our results indicate that CXCR4 inhibition can overcome leukemic cell protection from chemotherapy conferred by stromal cells. Additionally, Zeng et al.40,41 study showed that the CXCR4 inhibitor antagonized stroma-induced leukemic cell chemotaxis and inhibited pro-survival signaling in these cells.

Activating mutations of FLT3 occur in 30% of AML patients and are associated with poor prognosis. Accordingly, FLT3 inhibitors have been used as a tool to possibly improve AML therapeutic response and disease prognosis. Clinical trials with various FLT3 inhibitors have shown that BM anti-leukemic responses are less common than those observed in the peripheral blood. One potential explanation may be resistance to the FLT3 inhibitors afforded to the leukemic cells by their interaction with the BM microenvironment. CXCR4 inhibition increased the sensitivity of FLT3-mutated leukemic cells to sorafenib, the prototypical FLT3 inhibitor, and resulted in markedly prolonged survival in mice.44,45 In a recent clinical trial combining the CXCR4 inhibitor plerixafor with G-CSF (which cleaves CXCL12, VLA-4, and CD44), and sorafenib resulted in 36% CRs in a highly refractory group of patients with FLT3 mutations.46 Of all AML, those with FLT3 mutations express the highest levels of CXCR4.

The protective effect of stromal cells was significantly reduced by pre-exposure to the HDM2 inhibitor nutlin-3a. p53 activation by nutlin-3a reduced CXCL12 mRNA levels and secretion of CXCL12 by 70% partially through p53-mediated HIF-1α down-regulation in MSC. p53 activation in stromal cells blunts stromal cell-mediated resistance to FLT3 inhibition, in part through the down-regulation of CXCL12.45 These findings indicated that SDF-1α/CXCR4 interactions contribute to the resistance of AML to sorafenib and other chemotherapeutic agents. Hence, combinations of CXCR4 inhibitors, or HDM2 antagonists, and FLT3 inhibitors may be effective in the treatment of FLT3-mutant AML.

Although CXCR4 inhibitors significantly increase the sensitivity AML cells to chemotherapy, the mechanisms involved in this progress are not fully understood. Chen et al.47 examined the role of miRNAs in targeting the SDF-1α/CXCR4 axis in AML and demonstrated that the human miRNA let-7a, which negatively regulates BCL-XL expression, is regulated by SDF-1α/CXCR4 signaling in human AML cells. He identified the transcription factor Yin Yang 1 (YY1) as a link between SDF-1α/CXCR4 signaling and let-7a, since YY1 was upregulated by SDF-1α and downregulated by treatment with a CXCR4 antagonist. Inhibiting CXCR4 or overexpressing let-7a in AML cells led to reduced expression of BCL-XL and enhanced cytarabine-induced AML apoptosis both in vitro and in vivo. Based on these data, the authors proposed that CXCR4 induces AML chemoresistance by downregulating let-7a via YY1, resulting in transcriptional activation of MYC and BCL-XL.47 The novel CXCR4 inhibitors LY2510924 and BL8040, mentioned previously, have vastly superior receptor occupancy and anti-CXCR4 activity and are presently in clinical trials.42,43

**Wnt/β-catenin signaling pathway**

WNT (Wingless and INT-1) signaling is involved in virtually every aspect of embryonic development and it also controls homeostatic self-renewal in a number of adult tissues. Recent research showed that the Wnt/β-catenin signaling pathway is required for self-renewal of LSC that are derived from either HSCs or more differentiated granulocyte-macrophage progenitors.48,49 Because the Wnt/β-catenin pathway is normally active in HSC but not in the latter cells, these results suggested that reactivation of β-catenin signaling is required for the transformation of progenitor cells by certain oncogenic triggers. The Wnt/β-catenin pathway is active in AML, and essential for LSC function, while adult HSC do not require constitutively active β-catenin. Thus, targeting the Wnt/β-catenin pathway may represent a novel therapeutic mechanism to eradicate AML blasts as well as their progenitor LSC.
Interestingly, Wnt signaling plays a vital role in the regulation of proliferation and differentiation of MSC. In fact, exogenous in vitro application of Wnt3a to MSC, as well as human adipose-derived stem cells, results in both increased self-renewal and decreased sensitivity to apoptosis induction in these cells. Furthermore, the Wnt/β-catenin signaling pathway plays a critical role in microenvironmental leukemic cell chemotherapy resistance through its activity in vicinal stromal cells like MSC. In this context, β-catenin inhibitors represent an effective therapeutic choice that can target both the leukemic and stromal cells. Indeed, C82 is a novel β-catenin/CBP (cAMP-response element binding protein) modulator that, via its binding to CBP, inhibits the interaction of β-catenin and CBP. This action disrupts Wnt/β-catenin/CBP-mediated cell proliferation and self-renewal signaling. Moreover, an open-label, dose-escalation phase 1/2 study of PRI-724 (the active metabolite of C82) for advanced myeloid malignancies is currently enrolling patients at MD Anderson Cancer Center and other cancer centers. Preliminary results from this trial have shown PRI-724 is well tolerated and it has an acceptable toxicity profile. A mechanistic evaluation of patient samples showed that treatment down-regulated CD44 and survivin expression, which could serve as biomarkers for drug activity. Perhaps most importantly, an activating mutation of β-catenin in osteoblast, not in HSC, induced the development of AML. What better proof for relevance of the BM microenvironment in leukemogenesis and AML drug resistance can be envisioned than these findings? So far, no mutations have been reported in leukemia stroma in MSC, but cytogenetic abnormalities have indeed been reported.

Moreover, recent studies indicated that galectin might serve as a link to connect BM niche and LSC via activation of β-catenin. Galectin-3 is a member of the β-gal-binding galectin family of proteins, which can be specifically induced by bone marrow-derived MSCs. MSCs induced galectin-3 up-regulation, promoting β-catenin stabilization and thus activating the Wnt/β-catenin signaling pathway in ALCs, which is critical in cytotoxic drug resistance of leukemia. Additionally, Kikushige et al. revealed that galectin-9 and its receptor Tim-3, constitute an autocrine loop critical for BM MSC markedly reduced the stromal-mediated drug resistance in leukemic cells both in vitro and in vivo. In particular, this unique in vivo model of human leukemia BM microenvironment illustrated a direct link between NF-κB activation and stroma-associated chemoprotection. Mechanistic in vitro studies revealed that the interaction between VCAM-1 and VLA-4 played an integral role in the activation of NF-κB in the stromal and tumor cell compartments. In support of the role of VLA-4 in the sensitivity of AML cells to chemotherapy, a neutralizing VLA-4 antibody, in conjunction with cytarabine, prevented the development of AML in a xenograft model. Although the small-molecule inhibitors of VLA-4/VCAM-1 interactions caused impressive mobilization of normal HPCs, alone or in combination with granulocyte colony-stimulating factor or CXCR4 inhibitors, this approach has yet to be explored in leukemias. Together, these results suggested that reciprocal NF-κB activation in BM MSC and leukemia cells was essential for promoting chemoresistance in the transformed cells. Consequently, targeting NF-κB or VLA-4/VCAM-1 pathway could be a clinically relevant mechanism to overcome stroma-mediated chemoresistance in BM-resident leukemia cells.

**PI3K/AKT/mTOR signaling**

Direct contact between leukemic cells and BM-derived MSC triggers a pleiotropic spectrum of proliferative and/or anti-apoptotic signaling pathways, including the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway (PI3K/AKT/mTOR), which attenuates the response of AML cells to conventional chemotherapy. Jacamo et al. confirmed that multiple survival signaling pathways, including PI3K/AKT/mTOR, were upregulated in primary AML cells co-cultured with stromal MSC. An mTOR kinase inhibitor, PP242, effectively induced apoptosis in primary samples cultured with or without stroma. Mechanistically,
PP242 attenuated the activities of mTORC1 and mTORC2, sequentially inhibited phosphorylated AKT, S6K, and 4EBP1, and concurrently suppressed chemokine receptor CXCR4 expression in primary leukemic cells and in stromal cells cultured alone or co-cultured with leukemic cells. In mouse model, PP242 inhibited mTOR signaling in leukemic cells and demonstrated a greater anti-leukemia effect than rapamycin. These findings and other studies indicate that disrupting mTOR/AKT signaling with selective mTOR kinase inhibitors could effectively target leukemic cells within the BM microenvironment\(^{56-58}\). Furthermore, the PI3K/AKT/mTOR signaling pathway also plays a critical role in stromal cell-mediated resistance to FLT3 inhibition. Indeed, combined treatment with selective PI3K inhibitor GDC-0941 and sorafenib reversed the protective effects of BM stromal cells on FLT3-mutant AML cells in hypoxia. This activity was also associated with the downregulation of Pim-1 and Mcl-1 expression\(^{59}\).

**Signaling via CD44/HA**

CD44 is a ubiquitously expressed transmembrane glycoprotein that is extensively alternatively spliced resulting in the production of many isoform variants\(^{60-62}\). CD44 mediates cell-cell and cell-extracellular matrix interactions through binding to its main ligand, hyaluronan. Hyaluronan is a glycosaminoglycan that is highly concentrated in the endosteal region of bone. Beyond its adhesion function, the CD44-hyaluronan interactions can also promote tyrosine kinase (TK) activity of HER2 and the non-receptor kinase, Src, and also activate RhoA and Rac1. CD44 has also been shown to bind Tiam1 and Vav2\(^{60-62}\). Furthermore, CD44 is a key regulator of LSC homing to microenvironmental niches and is also suppressing differentiation of these cells\(^2\). Notably, elevated CD44 expression on leukemic cells has been documented, and the expression of certain of its variants is associated with poor disease prognosis. Ligation of the CD44 adhesion molecule inhibits drug-induced apoptosis in human myeloid leukemia cells. Furthermore, ligation of CD44 with the H90 monoclonal antibody resulted in a marked reduction of the leukemic burden in NOD-SCID mice transplanted with primary AML cells. This was believed to occur through alteration of LSC fate and abrogation of homing. In vivo administration of this antibody to NOD/SCID mice transplanted with human AML markedly reduced leukemic cell growth compared to the control group, and the absence of leukemia in serially transplanted mice demonstrated that the LSC was directly targeted in this experiment. Proposed mechanisms underlying this suppression included interference with homing to stem cell-supportive microenvironmental niches, and the alteration of LSC fate, suggesting CD44 as a key regulator of LSC\(^{60-62}\). Additionally, the finding that LSC requires interactions with the niche to maintain stem cell properties provides a therapeutic strategy to eliminate quiescent LSC that may be applicable to other types of cancer stem cells as well\(^{60}\).

**Warburg effect and hypoxia**

The link between metabolic alteration and cancer was first made by Otto Warburg when he provided the provocative observation that cancer cells preferentially consume glucose and metabolize it to lactate even in the presence of oxygen\(^{63}\). Aerobic glycolysis was viewed as a corruption of the more highly efficient ATP-generating oxidative phosphorylation associated with normal cells. More modern interpretations argue that the cancer cell uses a modification of metabolic pathways adaptively, shifting in favor of the production of macromolecules needed to meet the biomass demands of rapidly dividing cells. Several studies provide evidence that at least some leukemias use the Warburg effect\(^64\). HIF1-α, an inducer of the Warburg effect, has been implicated in AML pathogenesis. Isocitrate dehydrogenase 1 (IDH1) and IDH2 mutations in AML are associated with promotion of aerobic glycolysis through induction of HIF1-α stability and with production of an oncometabolite that inhibits a chromatin modifier, thereby also connecting metabolism with epigenetic control of gene expression\(^64,65\).

HSC are exposed to low levels of oxygen in the bone marrow niche resulting in the induction of hypoxia-inducible factors, which are the main regulators of cellular responses to this environment\(^2\). Hypoxia and other factors, can induce hypoxia-inducible factor α (HIFα). HIF-1α regulates CXCL12 gene expression in endothelial cells resulting in selective in vivo expression of CXCL12 in ischemic tissue. This can increase migration and homing of circulating CXCR4-positive progenitor cells into this tissue\(^66\). Furthermore, CXCR4 expression was upregulated under hypoxic conditions in AML cell lines and patient samples. These data suggested that a hypoxic BM microenvironment represents a conditional stem and progenitor cell niche in which HIF-1α-induced stabilization and activation of CXCL12-CXCR4 signaling facilitates recruitment and retention of LSC.

Remarkably, HIF-1α also downregulated miR-17/20a by directly targeting p21 and STAT3 that ultimately interfered with AML cell differentiation\(^67\). HIF-2α also has a key role in the maintenance and survival of human HSC and primary AML cells\(^68\). Indeed, knockdown of HIF-2α, and to a much
lesser extent HIF-1α, impeded the long-term repopulating ability of HSC in the BM. HIF-2α deregulation also significantly decreased engraftment of human AML cells, and HIF-2α overexpression protected both HSC and AML cells from apoptosis induced by ER stress. In this context, HIF-1α appears to represent an important molecular target within the tumor microenvironment, and several strategies specifically targeting HIF-1α are now being explored in solid tumors. These include a novel antisense oligonucleotide against HIF-1α and small-molecule HIF-1α inhibitors. The applicability of this strategy to the LSC niche remains to be determined.

Additional prominent signaling pathways in AML

The apoptosis repressor with caspase recruitment domain (ARC) protein is a member of caspase recruitment domain (CARD) containing proteins that have diverse functions such as anti-apoptosis, regulation of NF-κB activity, and promotion of leukemia-stromal interactions by increasing the expression of CXCR4 in AML cells and of CXCL12/SDF-1 in MSC\(^69,70\). Carter et al.\(^69\) profiled ARC expression in newly diagnosed AML patients using a validated, robust reverse-phase protein array, and correlated ARC levels with clinical outcomes. Multivariate analysis indicated that ARC was a statistically significant independent predictor of disease prognosis in AML. These results suggested that ARC is a potential therapeutic target in AML. Furthermore, Carter et al.\(^70\) found CXCL12, CCL2, and CCL4 are highly expressed and upregulated by ARC induction in MSC. CCL2 and CCL4 triggered the migration of AML cells, which was antagonized by blocking antibodies or small molecule inhibitors. These co-culture conditions greatly increased the levels of CXCL12, CCL2, and CCL4 in the MSC. The effect was diminished when AML cells were co-cultured with ARC KD MSC. These findings suggested that multiple ARC-regulated receptor/ligand pairs play important roles in leukemia-stromal interactions, and that the elucidation of novel mechanisms to disrupt this interaction could be of great importance in the therapy of AML.

Additionally, transforming growth factor-β (TGF-β) is a potent pleiotropic cytokine that functions as a formidable barrier to the development of cancer hallmarks in normal cells and tissues\(^71\). Paradoxically, tumorigenesis counteracts the tumor suppressing activities of TGF-β, thus enabling TGF-β to stimulate cancer invasion and metastasis\(^72\). In hematologic malignancies, including leukemias, myeloproliferative disorders, lymphomas, and multiple myeloma, resistance to these homeostatic effects of TGF-β develops\(^73\). Mechanisms for this resistance include mutation or deletion of members of the TGF-β signaling pathway and disruption of the pathway by oncoproteins. These alterations define a tumor suppressor role for the TGF-β pathway in human hematologic malignancies. Advances in the TGF-β signaling field should enable targeting of the TGF-β signaling pathway for the treatment of hematologic malignancies.

Future directions

Tumor metabolism in the leukemia microenvironment is emerging as an attractive drug target in AML. For example, IDH mutations in AML are associated with promotion of aerobic glycolysis through induction of HIF1-a stability, and with the production of an onco-metabolite, 2-hydroxyglutarate, that inhibits a chromatin modification, thereby connecting glutamine metabolism with the epigenetic control of gene expression\(^64,65\). Additionally, a recent study revealed that AML cells create an arginase-dependent immunosuppressive microenvironment, which can be modulated through small-molecule inhibitors of arginase and inducible nitric oxide synthase, suggesting a novel therapeutic target in AML\(^74,75\). Oxidative phosphorylation and metabolic changes in leukemic cells and their microenvironment are also promising new areas of study for the therapy of AML\(^76\).

In summary, dissection of the crosstalk between leukemic cells and the BM microenvironment not only contributes to a better understanding the leukemogenesis, but also helps to ascertain novel approaches to attack microenvironment-mediated LSC survival and drug resistance in leukemic cells. With respect to this, we believe it logical to develop combinational therapies that simultaneously target survival pathways in the leukemic cells and the cytoprotective mechanisms afforded by their interaction with their stromal neighbors. It is anticipated that a combinational approach would be superior by preventing relapse due to the development of microenvironment-mediated survival of leukemia cells.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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