Bone Marrow Microenvironment-mediated Resistance to Chemotherapy in Leukemia

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Many studies have demonstrated the ability of bone marrow stromal cells to protect leukemia cells from spontaneous and chemotherapy-induced apoptosis. Accumulated evidence suggests that a “crosstalk” between leukemia cells and BM stromal cells is responsible for the reciprocal modulation of each other’s functions to create a leukemia-promoting “soil” that is able to nurture the malignant “seed” cells. This crosstalk is achieved by an intricate communication system that involves integrin-mediated signals, soluble factors (cytokines, chemokines, growth factors, etc.) exosomes and transcriptional reprogramming of cells. We present here a short overview of the role of integrins, chemokines/cytokine-receptor signals and transcriptional changes that regulate the interactions of leukemia cells with their stroma and describe how they contribute to the development of chemoresistance. Journal of Nature and Science, 1(8):e145, 2015.

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Introduction

Within the architecture of the bone marrow (BM), leukemia cells receive protection from apoptosis-inducing chemotherapy agents by a complex concert of cells that represent the BM microenvironment. Extensive experimental evidence accumulated over the last 20 years has demonstrated that BM mesenchymal stromal cells (BM-MSC), a key component of the BM microenvironment (BME), can interfere with chemotherapy-induced apoptosis of leukemic cells by providing them with an array of cytokines, chemokines and tumor promoting factors that favors leukemia progression. Undoubtedly, this chemoresistance-enhancing effect has profound clinical significance in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and other types of leukemia (1-4), since it promotes post-therapy residual disease that retains potential for relapse.

Although co-culture models of leukemia cells and BM-MSC have been used to study the intricate crosstalk between the leukemic blasts and stromal cells and to elucidate the mutual exchange of growth factors and cytokines involved in such encounter (5, 6), the process by which leukemia-stroma interactions confer chemoresistance to leukemia cells is not fully understood. This communication promotes changes in the BM stroma and shapes the microenvironment into a niche prone to enhance leukemia progression (7). In this short article we provide an overview of how leukemia cells and BM stroma cells communicate via cell-adhesion and soluble factors, the repercussion of such communication and possible therapeutic avenues.

The role of integrins

Cell–cell adhesion between BM-MSC and leukemia blasts initiate cell adhesion-mediated signals that can alter normal hematopoiesis and promote malignant hematopoietic cell survival (8-12). These cell-to-cell interactions involve adhesion molecules, such as integrins, on the surface of leukemia cells. Integrins are glycoproteins composed of two subunits, alpha and beta (13), and are responsible for cell adhesion to the extracellular matrix (ECM) and to integrin receptors on the surface of other cells. One of these integrins, integrin beta3 (ITGB3), has been recently found to be selectively required for the growth and survival of LSC but not normal hematopoietic cells. In this study, Miller, et al. showed that the knockdown or genetic deletion of ITGB3 caused a dramatic growth-inhibitory effect of primary leukemia cells in vivo and prolonged the survival of tumor-bearing mice, suggesting that ITGB3 mediates the interaction of leukemia stem cells with the bone marrow microenvironment and it is critical for tumorigenesis (14). Another integrin, and probably the best studied example, is the very late antigen-4 (VLA-4), composed of an integrin alpha4-chain (CD49d) associated non-covalently with a beta1 integrin chain, CD29. VLA-4 binds to integrin receptors such as vascular cell adhesion molecule-1 (VCAM-1), osteopontin (OPN) and fibronectin (15). These types of adhesive interactions trigger the activation of pro-survival and proliferative pathways in both the blasts and stromal cells that are critical for blast survival (Figure 1).

An example of such changes is the activation of NF-xB in tumor-surrounding stroma upon interaction with tumor cells (17-19). Many different factors can promote classical activation of NF-xB through the stimulation of the IkB kinase complex to phosphorylate and degrade IkB, leading to NF-xB nuclear translocation and subsequent target gene expression (20). We have recently reported that co-culture of human BM-MSC with human leukemia cells induce transcriptional changes in both, the stromal and the leukemia cells, through the reciprocal activation of NF-xB (16).

Furthermore, NF-xB activation resulted in the ubiquitous up-regulation of VCAM-1 in the BM-MSC unveiling a possible mechanism that involves integrin engagement and soluble factor-mediated signaling as responsible for the stroma-mediated chemoresistance of leukemia cells (21). Consistent with these observations, we showed that interference of VLA-4/VCAM-1 interaction with a VLA-4 blocking antibody efficiently impaired the co-culture mediated activation of NF-xB in BM-MSC. Along these lines, it is important to highlight that VLA-4 mediates adhesion of hematopoietic stem cells (HSC) to vascular cell adhesion molecule-1 VCAM-1 within the BME (22) and that in preclinical studies, administration of anti-VLA-4 antibodies effectively mobilized HSC progenitors into the peripheral blood of treated subjects (23, 24). Moreover, the combination of Plexixa and VLA-4 blocking antibodies showed greater mobilization potential than either agent alone (25). Other VLA-4 blocking agents like Natalizumab, Firastrateg and BIO5192 have been tested with some degree of success in Multiple Sclerosis (MS), Crohn’s disease and/or HSC mobilization (26-29), but have yet to prove efficacy in AML or other types of leukemia. The relevance of interfering with VLA-4/VCAM-1 not only relies in the physical mobilization of leukemia cells from the chemoprotective BM sanctuary, but also in the chance of interrupting the downstream signaling events triggered by such interaction which are apparently critical for the survival and resistance to chemotherapy of leukemia cells.

The SDF-1α/CXCR4 axis

It is evident that cross-talk between leukemia and stromal cells in the BME plays a critical role in chemo-resistance in AML cells. BM stromal cells constitutively secrete the chemokine stromal cell-derived factor-1 alpha (SDF-1α), also named CXCL12.

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The role of NF-κB

NF-κB plays a central role in inflammation (41), cell proliferation and differentiation (42), as well as apoptosis and cell death (43). Its activation can be triggered by a myriad of stimuli and many signaling pathways. As a result of its activation, NF-κB regulates an intricate network of target genes encompassing growth factors, cytokines, chemokines, adhesion molecules, intracellular signaling effectors, transcription factors and miRNAs. As we mentioned above, we demonstrated that cell-cell interactions between leukemia cells and BM-MSCs induce the activation of NF-κB leading to a series of transcriptional changes that conditions stroma cells to favor leukemia progression. As a result of the NF-κB activation in BM-MSC, we found up-regulation of IL-8, IL-6 and Chemokine (C-C motif) ligand 2 (CCL2) among other NF-κB-target genes (21). Interestingly, CCL2 is one of the key chemokines that regulate migration and infiltration of myeloid derived suppressor cells (MDSC) and tumor-associated macrophages (TAMs). Recent studies have shown that in AML patients, baseline CCL2 levels are significantly higher than in normal controls (44). Similar findings were reported in Acute Lymphoblastic Leukemia (ALL) (45) and Chronic Lymphocytic Leukemia (CLL) (46). Burgess et al. demonstrated that CCL2 and CXCL2 levels are high in CLL, particularly in the presence of the BME and their expression appears to correlate with sustained leukemia cell survival. Although there’s extensive literature in solid tumors indicating that MDSCs and TAMs play a significant role in tumor progression (47), there’s very little evidence for such phenomenon in leukemia. We are currently conducting experiments to test this hypothesis.

A large number of miRNAs have been shown to be transcriptionally regulated by NF-κB in AML. It has been recently found that high expression of miR-155 independently predicts poor outcome in cytogenetically normal (CN) AML patients (49). Up-regulation of miR-155 causes the down-regulation of its target SHP1 leading to the activation of the PI3K/Akt pathway and a reduction in myeloid differentiation. Interestingly, treatment of AML cells with MLN-4926, a neddylation inhibitor presently used in clinical trials, decreases binding of NF-κB to the miR-155 promoter and down-regulates miR-155 with significant anti-leukemic effects (50). In addition, the activity of NF-κB can be regulated by miRNAs targeting its upstream regulators or even some members of the NF-κB family creating feedback mechanisms that fine-tune the activity of NF-κB. Furthermore, NF-κB induces the synthesis of proteins that regulate miRNAs. Two well-known examples are the NF-κB-dependent induction of Lin28 and YY1. Lin28 is an RNA binding protein that has been shown to play a master regulator of stemness and differentiation in HSCs (51). A critical function of Lin28 is to inhibit the processing and maturation of let-7 miRNAs. It has been shown that NF-κB can directly activate Lin28 transcription and rapidly reduce let-7 microRNA levels. Let-7 miRNAs targets include OCT4, SOX2, IL-6 and, as mentioned above, BCL-XL and Myc. Therefore, a reduction of let-7 leads to higher levels proteins that regulate differentiation, apoptosis and further activation of NF-κB (through IL-6) generating a positive feedback loop (52).

It was already mentioned that let-7a expression can be negatively regulated by the transcriptional factor/repressor YY1 through the activation of the SDF-1/CXCR4 axis in AML. But YY1expression can also be up-regulated by activated NF-κB leading to decreased expression of mir-29b, a miRNA that acts as a tumor suppressor and is significantly down-regulated in AML (53). In addition, it has been found that NF-κB also regulates CXCR4 expression at both, the transcriptional and cell-surface protein levels. Furthermore, SDF-1α stimulation leads to a rapid nuclear translocation of NF-κB protein constituting a positive feedback loop (54).

Another key chemokine regulated by NF-κB is interleukin 8 (IL-8) or CXCL8. The importance of IL-8 and its receptor, CXCR2, in the pathogenesis of cancer comes from studies in solid tumors and in particular preclinical studies in breast cancer in which IL-8 seems to have a relevant microenvironmental role (55-57). In leukemia, the expression of IL-8 in B-cell CLL it has been found to be elevated and associated with prolonging survival in an autocrine fashion (58, 59). In ALL, it has been reported that the transcriptional levels of IL-8 and other cytokines and chemokines are elevated in BM-MSC derived from patients compared to healthy subjects and demonstrated that elevated IL-8 enhanced the capacity of BM-MSC to support adhesion of ALL cells (45). In normal hematopoiesis, IL-8 seems to play an inhibitory role in proliferation and differentiation (60, 61) and this could possibly explain why leukemia cells thrive in an IL-8 rich microenvironment while normal hematopoietic cells are suppressed. Jacamo et al. reported elevated IL-8 mRNA levels in BM-MSC derived from AML and ALL patients compared to healthy donor-BM-MSC. In addition, co-culture of normal BM-MSC with AML and ALL cell lines and primary samples induced the expression of IL-8, in an NF-κB-dependent manner, as determined.
Figure 1. Schematic representation of leukemia cells–BM-MSC crosstalk. Communication between leukemia cells and BM-MSC involves a complex network of transcription factors/transcriptional repressors, miRNAs, adhesion and signaling molecules, cytokines and chemokines. Binding of SDF-1α to CXCR4 and/or engagement of VCAM-1/VLA-4 can activate NF-κB and other survival signaling pathways. Many survival components including kinases (e.g. ERK, AKT), transcription regulators (e.g. YY1, NF-κB), and anti-apoptotic molecules (e.g. BCL-XL, MYC) are activated. In addition NF-κB up-regulates pro-survival miR-155 and contributes to the blockade of let-7a through the induction of YY1. Altogether, this exquisite array of molecules contributes to resistance to chemotherapy in leukemia cells.

by qRT-PCR (21). Schinke et al. found elevated transcriptional levels of IL-8 and CXCR2 in purified stem cells from AML and myelodysplastic syndromes (MDS) and indicated that the expression of CXCR2 is associated with worse prognosis in both AML and MDS (62). In addition, they showed that the genetic or pharmacologic inhibition of CXCR2 resulted in decreased viability of AML/MDS stem cells in vitro and in vivo suggesting that CXCR2 could be a potential therapeutic target against AML and MDS stem cells.

The role of exosomes
Exosomes are naturally occurring extracellular vesicles (EV) which size can range from 70 to 140 nm and are released by exocytosis upon fusion of multivesicular bodies with the plasma membrane. Exosomes are secreted by multiple cell types, including cancer cells, and can contain proteins (including cytokines, chemokines and other extracellular messengers), mRNA, microRNAs and even DNA of high molecular weight. They can also pack macromolecular complexes like the RNA-induced silencing complex (RISC) and other microRNA-processing enzymes like Dicer, AGO2, and TRBP. Hence, exosomes can be considered master messengers in intercellular communication. Recent studies show that cancer-derived exosomes play a role in the migration and metastasis of cancer cells (63, 64) and can further induce an oncogenic effect by subjugating the surrounding microenvironment to participate in cancer development and progression (65, 66). In leukemia, it has recently been shown that exosomes modulate the crosstalk between leukemia cells and the bone marrow microenvironment (67, 68) and can reprogram the BM niche (69, 70) to favor leukemia progression. Our preliminary data suggest that BM-MSC can also secrete exosomes and transfer their contents to leukemia cells possibly contributing to resistance to chemotherapy (71).

Discussion
Leukemia cells are protected from chemotherapy-induced apoptosis by their interactions with bone marrow (BM) mesenchymal stromal cells (BM-MSC). Our recently published data (21) demonstrate that leukemia-stroma interaction induces NF-κB activation in both leukemic and stromal cells and that such activation plays a key role in the development of stroma-mediated chemo-resistance. A complex network of transcription factors/transcriptional repressors, miRNAs, adhesion and signaling molecules, cytokines and chemokines and intricate signaling pathways are triggered as a result of these interactions (Figure 1). Cooperatively, these events contribute to the survival and proliferation of leukemia cells. Several clinical trials have suggested beneficial effects of CXCR4 inhibition in AML. However, their impact was limited by the poor pharmacokinetic properties of the pioneer CXCR4 inhibitor Plerixafor which has receptor occupancy of only 1.5 hrs. Plerixafor has been used in combination with granulocyte colony-stimulating factor (G-CSF) in two clinical trials to improve mobilization of leukemia stem cells from the bone marrow (72, 73). In spite of the subtle mobilization effects achieved by the early generation of CXCR4 inhibitors, the potential of blocking the SDF-1α/CXCR4 axis in AML should remain an attractive therapeutic tool as the inhibition of CXCR4 downstream signaling (PI3K/AKT, MAPK, PKC and JAK/STAT) and transcriptional events (YY1, NF-κB, miRNAs, etc.) can certainly be of benefit. Combination therapy with VLA-4 inhibitors (Natalizumab, Firategrast and BIO5192) and/or NF-κB inhibitors (MLN-4926, Bortezomib, etc) could also be considered. With regards to NF-κB inhibition, it is important to highlight that most of the compounds that have been shown to successfully eradicate LSCs are known to be inhibitors of NF-κB (74).

In this short review we focused on the leukemia-stroma communication factors that can contribute to the development of
chemoresistance in leukemia cells. It is important to highlight that there are other microenvironmental factors that can affect the response to chemotherapy agents, such as hypoxia. In solid tumors, hypoxia is often associated with poor prognosis, metastatic behavior and resistance to chemotherapy with hypoxia-inducible factor 1 (HIF-1) being the master transcriptional regulator of hypoxia response (75, 76). Although there is a large body of evidence suggesting that the BM niche where HSC and the hematopoietic progenitor cells reside is hypoxic with respect to the oxygen concentration usually found in circulating blood (77, 78), the role of hypoxia in hematological diseases is still largely unexplored. Early indications that hypoxia plays a role in leukemia progression come from a rat leukemia model demonstrating that leukemia cells reside in markedly hypoxic areas of the BM (79). A later study indicated that HIF-1α was up-regulated in the CD34+ CD38– leukemia stem cells (LSC) fraction of AML and that genetic or chemical inhibition of HIF-1α significantly increased apoptosis of LSCs and impaired their ability to reconstitute the disease upon xenotransplantation into immunocompromised recipient mice (80). More recently, Benito et al. showed a marked expansion of hypoxic BM areas in immunodeficient mice engrafted with acute lymphoblastic leukemia (ALL) cells and found that hypoxia promotes chemoresistance in various ALL derived cell lines. In this study, through administration of PR-104, the hypoxia-activated dinitrobenzamide mustard currently in phase I/II clinical to treat refractory/relapsed AML and ALL (81), prolonged the survival and decreased leukemia burden of ALL-bearing immune-deficient mice (82). Collectively, these findings indicate that hypoxia is yet another key feature of the leukemic microenvironment and suggests that targeting hypoxia should be considered in the treatment of acute leukemias.

In summary, a deeper understanding of interactions between leukemia cells and microenvironmental components of the BM may provide insights that can lead novel emerging therapies for the treatment of acute leukemias.


4. Wu S, Korte A, Kebelmann-Betzing C, Gessner R, Henze G, Seeger K. Genetic or chemical inhibition of HIF-1α was up-regulated in the CD34+ CD38– leukemia stem cells (LSC) fraction of AML and that genetic or chemical inhibition of HIF-1α significantly increased apoptosis of LSCs and impaired their ability to reconstitute the disease upon xenotransplantation into immunocompromised recipient mice (80). More recently, Benito et al. showed a marked expansion of hypoxic BM areas in immunodeficient mice engrafted with acute lymphoblastic leukemia (ALL) cells and found that hypoxia promotes chemoresistance in various ALL derived cell lines. In this study, through administration of PR-104, the hypoxia-activated dinitrobenzamide mustard currently in phase I/II clinical to treat refractory/relapsed AML and ALL (81), prolonged the survival and decreased leukemia burden of ALL-bearing immune-deficient mice (82). Collectively, these findings indicate that hypoxia is yet another key feature of the leukemic microenvironment and suggests that targeting hypoxia should be considered in the treatment of acute leukemias.

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