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Role of CXCL12 and CXCR4 in the pathogenesis of hematological malignancies

Amnon Peled[a,*], Shiri Klein[a], Katia Beider[b], Jan A. Burger[c], Michal Abraham[a]

[a] Goldyne Savad Institute of Gene Therapy, Hebrew University Hospital, P.O.B 12000, Jerusalem 91120, Israel
[b] Hematology Division, Chaim Sheba Medical Center and Tel Aviv University, Tel-Hashomer, Israel
[c] Department of Leukemia, The University of Texas Houston, TX, USA

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ABSTRACT

The chemokine receptor CXCR4 and its ligand stromal cell-derived factor-1 (SDF-1/CXCL12) are important players in the cross-talk among lymphoma, myeloma and leukemia cells and their microenvironments. In hematological malignancies and solid tumors, the overexpression of CXCR4 on the cell surface has been shown to be responsible for disease progression, increasing tumor cell survival and chemoresistance and metastasis to organs with high CXCL12 levels (e.g., lymph nodes and bone marrow (BM)). Furthermore, the overexpression of CXCR4 has been found to have prognostic significance for disease progression in many type of tumors including lymphoma, leukemia, glioma, and prostate, breast, colorectal, renal, and hepatocellular carcinomas. In leukemia, CXCR4 expression granted leukemic blasts a higher capacity to seed into BM niches, thereby protecting leukemic cells from chemotherapy-induced apoptosis, and was correlated with shorter disease-free survival. In contrast, neutralizing the interaction of CXCL12/CXCR4 with a variety of antagonists induced apoptosis and differentiation and increased the chemosensitivity of lymphoma, myeloma, and leukemia cells. The role of CXCL12 and CXCR4 in the pathogenesis of hematological malignancies and the clinical therapeutic potential of CXCR4 antagonists in these diseases is discussed.

1. Introduction

Stromal cell-derived factor-1 (SDF-1/CXCL12) was initially identified as a pre-B cell growth-stimulating factor [1]. CXCR4 (CD184) is the chemokine receptor specific for CXCL12. Genetic disruption of either CXCL12 or CXCR4 in mice results in similar lethal phenotypes [2–5]. Both animals die in the perinatal period and have defects in their ventricular septum, gastric vasculogenesis, cerebellar development, bone marrow myelopoiesis, and B cells but have normal T cells and lymphopoiesis. CXCR4 is widely expressed on most hematopoietic cell types, including neutrophils, monocytes, T lymphocytes, B cells, B cell precursors, $CD34^+$ progenitor cells from the blood and bone marrow, blood-derived dendritic cells, Langerhans cells, T cells, macrophages, and both immature and mature T cells in the thymus [6–8]. Using different CXCR4 antagonists, it has been demonstrated in animal models as well as in humans that under physiological conditions the CXCL12/CXCR4 axis is critical to the retention of hematopoietic cells including CD34+ stem cells, progenitors, B cell precursors, neutrophils, monocytes, natural killer (NK) cells, NKT cells, and immature dendritic cells in the bone marrow. Furthermore, it has also been shown that CXCR4 plays a role in the trafficking, tissue dissemination and localization of T cells and other mature hematopoietic cells.

Further support for the physiological role of CXCR4 is found in patients with WHIM syndrome (warts, hypogammaglobulinemia, infections, myelokathexis). This rare autosomal-dominant inheritance disease is characterized by diverse symptoms indicative of an aberrantly functioning immune system and neutrophil retention in BM. WHIM is caused by mutations in the chemokine receptor CXCR4 that lead to the increased responsiveness to CXCL12 [9].

The overexpression, compare to normal cells, of CXCR4 on the surface of hematological and solid tumors has been shown to be responsible for disease progression, including an increase in cell survival and chemoresistance and metastasis to organs with high CXCL12 levels (e.g., lymph nodes, bones, and BM) [10–12]. Furthermore, the overexpression of CXCR4 was found to have prognostic significance for disease progression in many type of tumors including lymphoma, myeloma, leukemia, prostate, breast, glioma, colorectal, renal, and hepatocellular carcinomas [11,13,14]. In leukemia, CXCR4 expression granted leukemic blasts a higher capacity to seed into BM niches, thereby protecting leukemic cells from chemotherapy-induced

* Corresponding author at: Hadassah Hebrew University Hospital, Gene Therapy Institute, P.O. Box 12000, Jerusalem 91120, Israel.
E-mail address: peled@hadassah.org.il (A. Peled).

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apoptosis, and was correlated with shorter disease-free survival [15–19]. Conversely, neutralizing the interaction of CXCL12 with CXCR4 disrupted metastasis, induced apoptosis, and increased chemosensitivity in solid cancers and leukemia [20–22].

2. Role of CXCL12 and CXCR4 in the pathogenesis of lymphomas

Waldenstrom's macroglobulinemia (WM) is an incurable lymphoma in which tumor cells accumulate in the BM, lymph nodes and spleen [23]. The most intriguing finding from recent studies has been the discovery of two activating somatic mutations effecting toll-like receptor (TLR) and CXCR4 receptor signaling [24,25]. Most of the WM patients (90–95%) carry the MYD88 L265P activating mutation that triggers Bruton's tyrosine kinase (BTK) and cell growth [26]. As many as 30% of WM patients carry an activating somatic mutation in CXCR4 that involves the C-terminus [25]. These activating somatic mutations are similar to those observed in patients with WHIM syndrome. These mutations have been shown to enhance sustained AKT, ERK and BTK signaling following CXCL12 stimulation of WM cells and to increase the migration, adhesion, growth and survival of WM cells [27]. A study testing the efficacy of using ulocuplumab (a fully human anti-CXCR4) together with ibrutinib (a Bruton's tyrosine (BTK) kinase inhibitor) in symptomatic patients with mutated CXCR4 Waldenstrom's macroglobulinemia is open (NCT03225716).

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma among adults. DLBCL typically presents as a nodal or extranodal mass with rapid growth. CXCR4 expression has been correlated to disease progression in 11 (26%) cases of primary testicular DLBCL [28] and to poor survival in 48 out of 94 (50%, $p < .05$) DLBCL cases [29]. In 20 patients with non-Hodgkin lymphoma, a significant decrease in CXCR4 mRNA expression in the BM after treatment correlated with a significantly lower risk of death [30]. In a large cohort of 743 patients with de novo DLBCL who received the standard rituximab-CHOP therapy, the expression of CXCR4 was associated with the male gender, bulky tumor volume, a high Ki-67 index, an activated B-cell-like (ABC) subtype, and the overexpression of Myc, Bcl-2 or p53. Furthermore, CXCR4 was an independent risk factor for predicting a worse progression-free survival in germinal center B-cell-like (GCB)-DLBCL but not in ABC-DLBCL; this association was also observed in patients with an IPI ≤ 2 but not in those with an IPI > 2. Furthermore, concurrent CXCR4 and BCL2 translocation showed dismal outcomes resembling but independent of MYC/BCL2 double-hit DLBCL [31].

We have previously demonstrated that the high affinity short synthetic peptide CXCR4 antagonist BL-8040/BKT140 (4F-benzoyl-TN14003) antagonist BL-8040/BKT140 alone can directly induce the apoptosis of NHL cell lines and primary NHL cells. Furthermore, combining BL-8040 with rituximab synergistically increased the anti-lymphoma effect. Moreover, using an in vivo model for lymphoma, we demonstrated that disrupting the CXCR4/CXCL12 axis using the CXCR4 antagonist BL-8040 was an effective way to abrogate the BMSC-mediated resistance of NHL cells to rituximab and to target NHL in the BM microenvironment [32]. In support of our observations, it was also reported that the CXCR4 antagonist plerixafor can enhance the effect of rituximab in DLBCL cell lines in vitro [33].

We have recently reported that treating leukemic or neuroblastoma (NB) cells with the CXCR4 antagonist BL-8040 induces the apoptosis of AML blasts and NB cells by downregulating BCL-2 expression and by upregulating miR-15a/16-1 expression. Furthermore, we have found that BL-8040 treatment downregulates Myc expression, an oncogene that was previously shown to positively control the expression of both BCL2 and miR-15a/16-1. These findings provide the scientific basis for future clinical studies aiming to test the efficacy of CXCR4 antagonists in a selected subset of DLBCL patients in which CXCR4 is expressed, and these results also support a crucial role for CXCR4 in the development and progression of disease.

3. CXCR4/CXCL12 axis in multiple myeloma

Multiple myeloma (MM) is a neoplasm characterized by the clonal proliferation of plasma cells (PC) in the bone marrow (BM) [34]. The development of novel agents, including proteasome inhibitors, immunomodulatory drugs and antibodies [35,36], has led to notable changes in therapeutic strategies for this disease and has improved survival, yet MM remains incurable. Interactions between tumor MM cells and BM niche components as well as alterations in the BM microenvironment are tightly associated with MM pathogenesis [37]. The CXCR4/CXCL12 axis plays a crucial role in normal PC development. CXCR4-expressing, mature B cells home to the BM niche, completing their maturation and residing there long-term in contact with CXCL12-expressing BM stromal cells [38,39]. Similarly, to normal PCs, myeloma cells utilize CXCR4 for homing to the BM and for maintenance [40]. CXCL12-mediated signals induce the proliferation of MM cells and protect them from drug-induced apoptosis [41,42]. CXCR4 expression on MM cells is correlated with disease progression [43] and poor prognosis [44], while an increase in the CXCL12 serum expression level is associated with increased osteolytic disease [45] and increased BM angiogenesis [46]. Furthermore, the secretion of CXCL12 by MM cells and BM stromal cells was shown to recruit tumor-supportive monocytes in a CXCR4-dependent manner, therefore potentially affecting the immune MM milieu composition [47]. Moreover, the recruitment of angiogenic monocytes to MM tumor sites can be driven by CXCR7, a second receptor for CXCL12 [48]. Finally, it was shown that persistent chemo-resistant minimal residual disease (MRD) PC clones were enriched in CXCR4 [49], further strengthening the role of CXCR4 in MM therapy refractoriness.

An increasing number of studies have targeted CXCR4 in vitro and in vivo using various specific inhibitors. The CXCR4 antagonist plerixafor (AMD3100) was shown to interfere with the protection provided by the BM, inducing chemo-sensitivity to the proteasome inhibitor bortezomib in MM cells [41,43], and is under clinical trial (NCT00903968). Furthermore, CXCR4 inhibition with BL-8040 directly inhibited MM cell viability induced apoptosis in vitro and suppressed MM xenograft growth in mice [50]. In addition, the CXCR4-neutralizing antibody ulocuplumab was shown to reduce MM dissemination in mice [51] and had activity in relapsed/refractory MM patients. Similarly, a fully human anti-CXCR4 antibody (BMS-936564/MDX-1338) demonstrated anti-MM activity in vitro and in vivo [52].

Similar to CXCR4, CXCL12 inhibition with anti-CXCL12 L-ribonucleotide NOX-A12 significantly reduced the tumor burden and acted synergistically with bortezomib, probably by making the BM niche less permissive to MM [51]. Furthermore, in a clinical phase IIa study, treatment with NOX-A12 induced myeloma cell mobilization and enhanced the clinical activity of bortezomib/dexamethasone in relapsed MM patients [53].

4. Role of CXCL12 and CXCR4 in the pathogenesis of CLL and ALL

Chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia and is characterized by the accumulation of B-leukemic cells in BM [54]. CXCR4 is overexpressed in CLL cells and is thought to play an important role in the interaction of these cells with the BM microenvironment [55].

Indeed, CXCR4 was consistently expressed on circulating B-CLL cells from CLL patients ($n = 51$) with a fluorescence intensity that was five-fold greater than in cells from healthy volunteers ($n = 42$). However, CXCR4 was not found to be higher in those individuals with bad prognosis (Binet's stage C) or individuals with a diffuse infiltration pattern [56] . CXCR4 was recently identified as a key regulator of Bruton's tyrosine kinase (BTK)-mediated CLL-cell retention in the lymph nodes, spleen and BM. Inhibiting BTK by ibrutinib reduced cell surface membrane expression levels of CXCR4, which resulted in the rapid redistribution of CLL cells from the spleen and lymph nodes into