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The role of CXCR4 and Lyn-kinase in stromal/leukemia interaction

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Abstract
There are ongoing investigations on key mechanisms of interaction between tumor cells and their microenvironment. Identification of these mechanisms and development of therapeutic agents targeting them brings up new opportunities for cancer therapy, including leukemia treatment. Today there are active discussions on mechanisms of interaction between tumor cells and their microenvironment mediated by CXCL12/CXCR4 and Lyn-kinase.

Keywords: microenvironment, CXCR4, imatinib, dasatinib, chronic myeloid leukemia, drug resistance, bcr-abl, Lyn

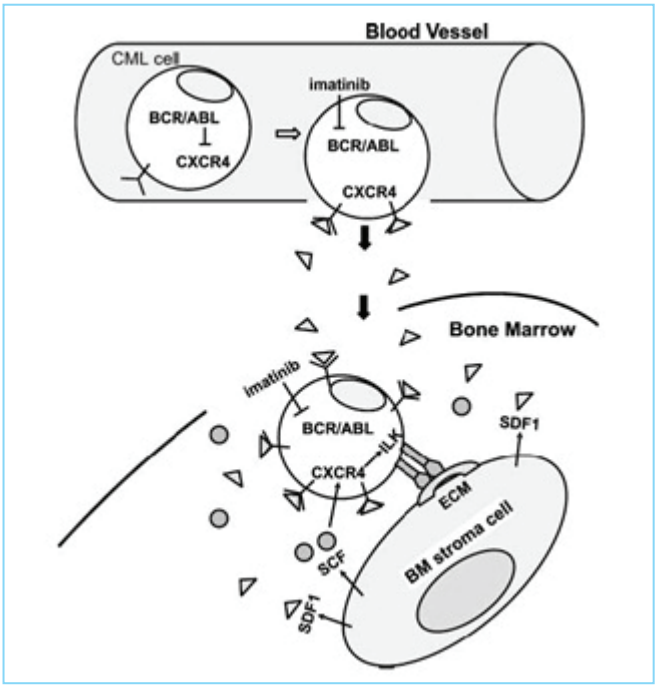
The role of CXCL12/CXCR4-mediatedchemotaxis and homing in stromal microenvironment/leukemia interaction

Animportant role in the interaction of tumor cells and stromal niches of bone marrow (BM) is played bythe chemokine CXCL12 (SDF-1, stromal cell-derived factor-1). The leukemia cells seek to infiltrate the BM's microscopic niches due to CXCL12/CXCR4–mediated chemotaxis, where stromal CXCL12 connects with CXC receptor 4 (CXCR4), located in the tumor cell membrane. As a result, tumor cells maintain self-renewal,their differentiation is blocked, and access to chemotherapeutic agents is hampered. This results in proliferation increasing and promotes survival leukemia cells. The blocking of CXCR4 stimulates the tumor cells to leave the bone marrow and to circulate in the patient's organism. As a result, the principal possibility of a new therapeutic strategy is opened on the basis of the influence ofthe CXCL12/CXCR4 system [10].

Currently, imatinib therapy is the major strategy for bcr-abl(+) CML.This therapy is based on targeted killing of the tyrosine kinase-expressing bcr-abl tumor cells. It was recently demonstrated [3] that tyrosine kinase p210BCR-ABL can inhibit CXCL12-signaling, interfering with thechemotaxis/homing process of leukemia cells. This leads to a release of immature myeloid cells from BM specific for CML and their circulation. Jin et al. [4] suggested that imatinib can act as an antagonist of bcr-abl, reconstructing CXCL12/CXCR4interaction, and it can force CML cells to migrate andconnect to the supporting BM microenvironment (Fig. 1). As result, the tumor cells are protected from the influence of therapeutic agents and this can be a potential causeof relapse. Thus, this therapeutic imatinib strategy can lead to the negative effect of indirect stromal resistance.

With the aim of verifying these hypotheses, Jin at al [4] investigated the influence of imatinib on CXCR4 expression, migration, and apoptose of leukemia cells in imatinib-sensitive and resistant CML cell lines, as well as in primary CML patient samples. They proved that imatinib blocks the influence of bcr-abl and stimulates the expression surface CXCR4 in conditions of co-cultivation of CML cells with mesenchymal stem cells (MSC). It increases the migration of CML cells to stromal cells of BM.

This proves that ITK imatinib can lead to indirect stroma resistance to therapy, associated with the CXCL12/CXCR4 system. It provides the possibility to conclude that ITK therapy should be added to any agent attenuating a CXCL12/



Cell Ther Transplant. 2011;3:e.000098.01. doi:10.3205/ctt-2011-en-000098-figure1

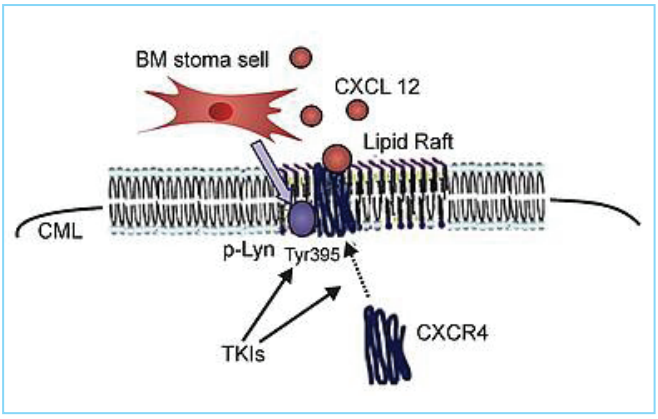
Figure 1. The influence of imatinib on CXCR4 expression, migration, and adhesion in the BM niche (see [4])

CXCR4 interaction. Research into the role of molecular inhibitors of CXCR4 in therapy CML is currently under way [1]. Dillmann et al. [1] pointed to a potential method of influencing the SDF-1/CXCR4 mechanism by using the selective CXCR4 antagonist Plerixafor, which makes bcr-abl(+) cells more sensitive to ITK therapy in the presence of BM stromal cells. In this way it was demonstrated that destroying the indirect CXCL12/CXCR4 interaction leads to target drug effectiveness rising. These investigations exhibit the high potential for the role of CXCR4 inhibitors in CML therapy.

Lyn–dependent drug resistance caused by the microenvironment

It is well known that Lyn-kinase of the Src-kinasefamily is one of the key components of indirect CXCL12/CXCR4 migration of normal and tumor hematopoietic cells. Lyn interacts with the CXCL12/CXCR4 system and is activated by tyrosine kinase p210 bcr-abl. CXCR4 and Lyn could be placed in lipid rafts, the mobile microdomains of cell membrane, enriched by glycosphingolipids, sphingomyelin, cholesterol, and signal molecules. TKI therapy helps CXCR4 reposition into the lipid raft, where it interacts with active phosphorylated Lyn (LynTyr396) in the CML cells. Recently,a new mechanism of drug resistance was discovered. It is based on lipid raft modulation and it includes changes of multivalent CXCR4 and Lyn complex compartmentalization (Fig. 2).

It was shown that mesenchymal stem cells (MSC), which produce CXCL12, stimulate Lyn-activation in the bcr-abl(-) AML cell line HL60. This Lyn-activation in the lipid rafts of membranes is a universal mechanism, andcould explaindrug resistance for all kinds of leukemia [7]. Lyn-associated CXCR4 reposition in lipid rafts of membranes can influence



Cell Ther Transplant. 2011;3:e.000098.01. doi:10.3205/ctt-2011-en-000098-figure2

Figure 2. Mechanisms of CXCR4 localization and Lyn activation in lipid rafts (see [8]). Imatinib causes CXCR4 clustering in lipid rafts with subsequent co-localization with active p-LynTyr396. It leads to the induction of cell migration to BM stromal cells, producing CXCL12

the CXCL12 / CXCR4 signal path. It activates migration and enables the survival of residual leukemia cells in BM niches.

The discovery of activated Lyn's role in the reconstitution of CXCL12/CXCR4 signal paths during bcr-abl inhibition leads to the idea that the application of Srckinase inhibitors could diminish ITK resistance, causing a microenvironment. According to this hypothesis, the double Src/abl kinase inhibitor dasatinib induces CML cell migrations to CXCL12 at a much lower level than imatinib [8]. Investigations showed that dasatinib causes an increase in the highest level of complete cytogenetic response (46% versus 28%, $p<0.0001$) in CML in comparison to imatinib [5]. Thus, possible dasatinib effectiveness and lower resistance to it, is caused most probably by blockade of Lyn-dependent resistance by the microenvironment. But this is not the complete effect. Therefore, the resistance to dasatinib is caused by another mechanism, which is different to the Lyn-activated one. Most probably it depends on the surface CXCR4 expressionincreasing [8].

Results of Fei et al. [2] showed that dasatinib therapy leads to CXCR4 expression increasing at the surface of cells in cases of p210 bcr-abl(+) acute lymphoblastic leukemia. In this case dasatinib and CXCR4-inhibitor combined therapy increases cell death. It demonstrates the usefulness of a TKI and CXCR4-inhibitor combination for CML and Ph (+)ALL therapy.

Results of Tabe et al. [8] demonstrate that lipid rafts of membranes provide key functional relays between Lyn and CXCR4. Thus, chemotaxis and homing of leukemia cells depends not only on CXCR4 inclusions in rafts, but depends on the cholesterol content in cell membranes. That is why statins, inhibiting synthesis of cholesterol, can change membrane properties, destroying rafts. Additionally, there are some data that simvastatin or lovastatin and alfa 2 β combination in CML cellsleads to inhibited cell growth [6,9]. Thus cholesterol synthesis inhibition is a potential additional approach to CML therapy.

Conclusions

The current state of the researchprovides these conclusions:

1. It is necessary to investigate the role of CXCR4 inhibitors more carefully, as they have a high potential for preventing stroma-mediated drug resistance.

2. Therapy using the double Src/abl kinase inhibitor dasatinib is more effective.

This is most probably due to the influence of Lyn-kinase, which is one of the key components of indirect CXCL12/CXCR4-migration of normal and tumor hemopoietic cells.

3. It is necessary to keep in mind the potential usefulness of statin as an additional agent in CML therapy.

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