ABSTRACT

Chronic Myeloid Leukemia (CML) is a myelo proliferative disorder in which the leukemic stem cells (LSC) give rise to abnormally high number of myeloid cells. CML is initiated by BCR-ABL fusion (9;22) (q34;q11) which is the result of reciprocal translocation between chromosome 9 and chromosome 22. BCR-ABL fusion gene codes for Bcr-Abl fusion protein which is a constitutively active tyrosine kinase. Tyrosine kinase inhibitors (TKIs) such as Imatinib mesylate (IM) is the mainline drug used for the treatment of CML. CML responds to IM treatment efficiently, especially in the chronic phase. However, in several patients, CML relapses even after few years of remission, mainly upon discontinuation of IM intake. Mutations in the BCR-ABL kinase domain have been reported to be the main cause of IM resistance. However, persistent leukemic cells in the BM in spite of IM treatment play a major role in causing relapse in CML patients. BM stromal microenvironment has been implicated to be the major cause of this persistence, where mesenchymal stromal cells (MSC) and their derivatives provide chemoprotection to CML cells through secreted factors as well as direct cell-cell contact.

In the present work, we studied the major signaling pathways involved in stroma mediated chemoprotection of CML cells. MAPK pathways (ERK, P38), NFkB, STAT pathways (STAT3/5), BMP signaling pathway through SMAD, CXCL12-CXCR-4 signaling axis, actin cytoskeleton and RhoA GTPase were examined for their role in CML-stromal interactions and CML chemoprotection. We found that actin cytoskeleton, RhoA GTPase and BMP-SMAD signaling plays an important role in establishing direct cell-cell interaction between CML and stromal cells. ERK-MAPK and BMP-SMAD signaling were found to be activated by stromal interactions in spite of IM treatment. This effect could be abrogated by use of small molecule inhibitors for ERK-MAPK and BMP-SMAD signaling as well as actin cytoskeleton disruptions in combination with IM. Moreover, it was observed that as a result of stroma mediated chemoprotection, CML cell line K562 cells could persist long-term in the presence of IM when attached to stromal cells. These persistent K562 cells acquired stroma-dependent chemoresistance which was mediated by the above mentioned signaling pathways. Interestingly, we found that after 8-12 weeks in stromal co-culture system, these K562 cells could be maintained in stroma independent suspension culture in the presence of IM, thus having acquired stroma independent chemoresistance which resembles a relapse status in the patient. The

resulting chemoresistant cells showed activation of BCR-ABL independent signaling pathways and the cells could be induced to undergo apoptosis by use of small molecule inhibitors along with IM treatment.

In conclusion, the present study showed that a reciprocal interaction exists between the CML cells and the stromal cells in their microenvironment. The stromal cells, modified by the leukemic cells, chemoprotected CML cells from IM induced cell death, which further developed into stroma independent chemoresistance which can be effectively targeted by inhibiting oncogene-independent signaling pathways.