ABSTRACT

Chronic myeloid leukemia (CML) is a bone marrow hematopoietic stem cell (HSC) disorder with the characteristic presence of Philadelphia (Ph) chromosome generated through reciprocal translocation between chromosome 9 and 22. Ph+ cells produce a constitutively active tyrosine kinase enzyme, BCR-ABL that promotes uncontrolled cell proliferation. The use of first and second generation tyrosine kinase inhibitors (TKI) like, imatinib, dasatinib, and nilotinib have been successful to an extent. However, TKIs fail to effectively eliminate CML stem cells and resistance against such drugs happens to be a recurring problem. It appears that CML cells receive active support from bone marrow microenvironment during progression. Therefore, the effective curative strategy would require identification of critical microenvironmental factors which facilitate CML cell survival despite therapeutic interventions. To that end, we have extensively studied influence of important bone marrow niche cells - such as, mesenchymal stem cell (MSC), endothelial cell (EC) and osteoblasts (OB), on the response of CML cells against imatinib. The results indicate that imatinib triggers Beclin-1 dependent autophagy in CML cells leading to apoptosis, whereas bone marrow niche cells activate Beclin-1 independent autophagy in CML cells which protects them against the drug. In addition to the cell-cell interaction, the stiffness of extracellular matrix (ECM) can also be a determinant of therapeutic response. Our study with decellularized bone marrow ECM suggests that CML cells can alter its physical arrangements and cause decondensation in response to imatinib treatment. Taken together, these data confirm cell-cell and cell-matrix interactions can have multiple consequences on CML cells following imatinib treatment. To understand the combinatorial effect of heterogeneous bone marrow niche cells and ECM on CML cells, we have fabricated a microfluidic –based platform mimicking in vivo-like conditions. The platform allows CML cells to be directly in contact with MSC, EC and OB cells in presence of a matrix protein, collagen-I. The results suggest that the reconstructed niche may promote intracellular calcium accumulation in CML cells but decrease cumulative autophagy flux in CML cells following imatinib treatment, accompanied by rapid release of calcium from CML cells affecting the polarity of plasma membrane. Towards the end of the thesis, we have addressed the challenges associated with poor intracellular drug concentration in CML using novel cell penetrating peptides derived from *Abrus agglutinin*. The two oppositely charged cell penetrating peptides, SR11 and IR15 cause perturbation in CML cell membrane and facilitate augmented intracellular imatinib concentration. Overall, this thesis provides a basis for understanding bone marrow niche mediated regulation of therapeutic response in CML cells, proposes a prognostic marker and unveils a new option for drug delivery.

Keywords: *Chronic myeloid leukemia; Bone marrow; Autophagy; Imatinib mesylate; Extracellular matrix; Matrix remodeling; Organ-on-chip; Cell penetrating peptide; Fluorescence correlation spectroscopy*.