Abstract

Chronic myeloid leukemia (CML) is a myeloproliferative stem cell disease characterized by the expression of BCR-ABL oncoprotein with constitutive tyrosine kinase activity. Although the development of tyrosine kinase inhibitors (TKI) such as imatinib dramatically improved the treatment of CML, a certain subset of patients develops resistance to TKI drugs. The most common cause of TKI resistance are point mutations in the *BCR-ABL1* gene, followed by other mutation-independent mechanisms. Survival and proliferation of CML cells in the presence of TKI drugs are accompanied by adaptive changes in their metabolism. Drug resistance can be maintained by extrinsic signals, among which exosomes, small vesicles released by (drug-resistant) cells, have been shown to play an important role.

The aim of this thesis was to characterize two CML cell lines sensitive and resistant to imatinib, as well as the exosomes derived from imatinib-resistant CML cells by proteomic approaches. Identification of metabolic vulnerabilities in drug-resistant cells enables their targeting by clinically available drugs, thus offering potential therapeutic targets for their selective elimination. Analysis of exosomes derived from imatinib-resistant cells can identify specific membrane surface proteins exploitable as clinically relevant diagnostic markers associated with imatinib resistance.

Two imatinib resistant CML cell lines were established for this study. In imatinib-resistant CML-T1^{IR} cells, upregulation of Na^+/H^+ exchanger regulatory factor 1 (NHERF1) was found, which could influence cytosolic pH, Ca^{2+} concentration, or the WNT signaling pathway,. Testing selective toxicity of several compounds revealed that modulators of calcium homeostasis, calcium channel blockers, and calcium signaling inhibitors were selectively toxic to CML-T1^{IR} cells. In a model of imatinib-resistant K562^{IR} cells, upregulation of signal transducer and activator of transcription 3 (STAT3) was detected. STAT3 and the insulin-like growth factor 1 receptor/insulin receptor substrate 1 (IGF1R/IRS-1) signaling pathway were suggested as potential therapeutic targets in imatinib-resistant K562^{IR} cells. This work also shows that exosomes from donor K562^{IR} cells can be internalized by recipient imatinib-sensitive K562 cells and increase their survival in imatinib. Proteomic analysis of the exosomes from K562^{IR} cells revealed strong enrichment of three membrane proteins IFITM3, CD146, and CD36. The flow cytometry confirmed their enrichment also on the cell surface of the donor K562^{IR} cells. The results suggest that proteomic analysis is a powerful tool in designing potential therapeutic targets and clinically valuable diagnostic markers in TKI resistant CML cells.