Ústav hematologie a krevní transfuze

Institute of Hematology and Blood Transfusion: Department of Proteomics

Screening of therapeutic targets in chronic myeloid leukemia cells resistant to general treatment

One of the main reasons for the failure of antitumor therapy is the development of resistance to commonly used chemotherapeutics. In case of chronic myeloid leukemia (CML) the resistance develops primarily to tyrosine kinase inhibitors such as imatinib (IMA).

The origin of CML is preceded by translocation of chromosomes 9 and 22 resulting in so-called Philadelphia chromosome, which encodes the BCR-ABL fusion oncogene. The product of this gene is tyrosine kinase Bcr-Abl with constitutive activity potentiating signaling and proliferative pathways essential for CML cells. The prognosis of patients with CML has significantly improved after the introduction of IMA, a Bcr-Abl inhibitor, into clinical practice. However, almost 40 % of patients are resistant to this treatment. The resistance is usually caused by point mutations in the BCR-ABL gene encoding the part of the protein responsible for chemotherapeutic binding or by other mechanisms involving, for example, amplification of the BCR-ABL gene. By a process analogous to what happens in the patient's body, we derived several cell lines with mutations in BCR-ABL (CML-T1) and without mutations (K562) resistant to tyrosine kinase inhibitors (CML-T1 IR and K562 IR).

We compared these cells in terms of their protein composition with the original treatment-sensitive cells using techniques such as separation by electrophoresis and chromatography, mass spectrometry, immunochemistry, fluorescence microscopy or flow cytometry. Our main goal is to identify proteins, alternatively signaling pathways or surface protein markers, which could serve as an indicator of disease prognosis or specific future therapeutic target for efficient and specific elimination of resistant CML cells.

In our laboratory, we used this approach to reveal a significantly increased level of NHERF1 protein in CML-T1 IR compared to CML-T1. The biological role of NHERF1 indicated the calcium homeostasis and Wnt signalization as a therapeutic target. Based on the increased concentration of calcium ions in CML-T1, we applied clinically used drugs acting as modulators of calcium homeostasis and demonstrated their selective toxic effect on CML-T1 IR (Fig. 1). This study confirms that modern proteomic techniques represent a suitable tool to elucidate the origin of resistance or identify novel therapeutic targets. The application of proven procedures has the potential to discover putative

mechanisms, new drugs and enables the way to individualized treatment of CML patients with resistance to commonly used chemotherapeutics [1].

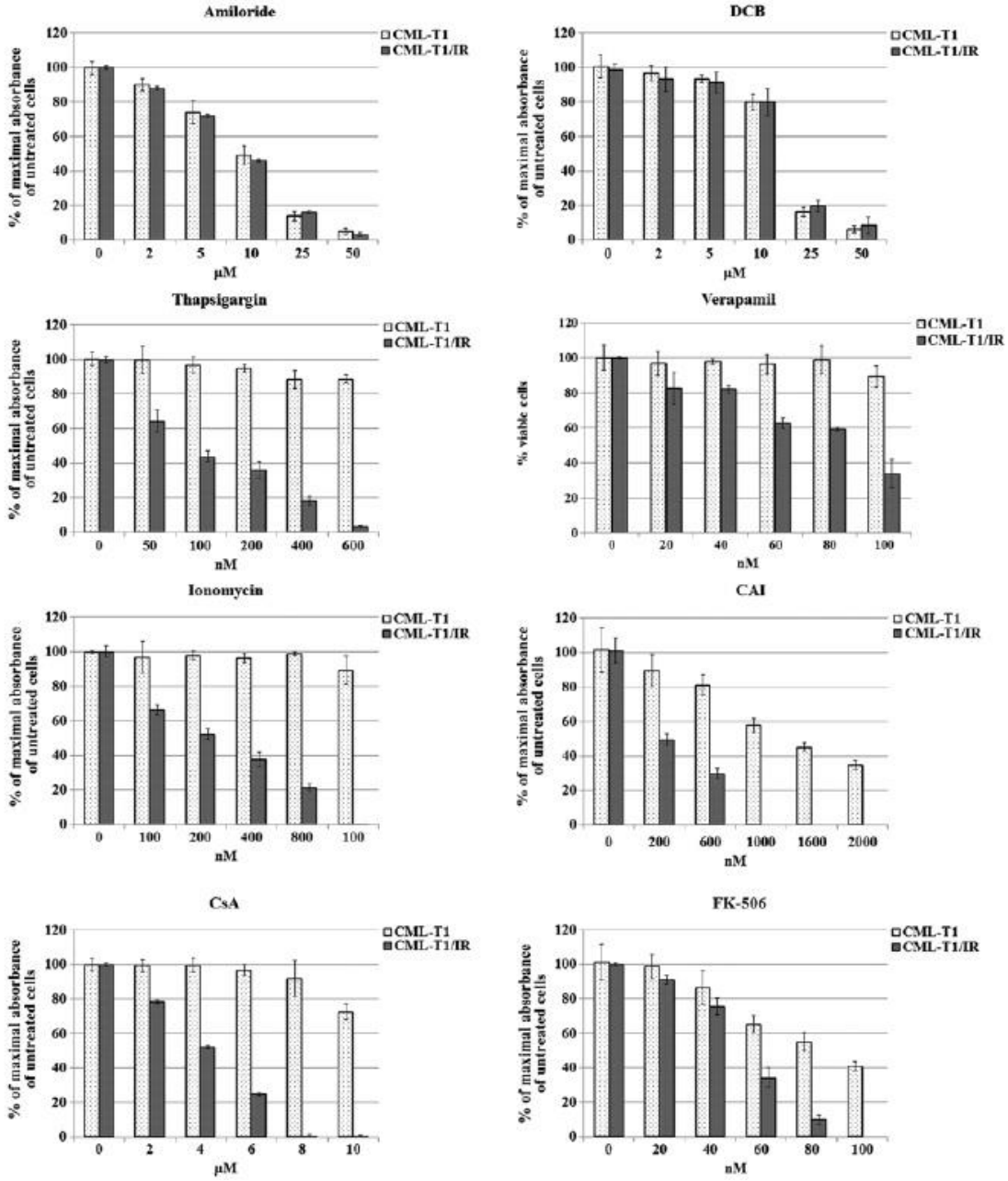

Fig. 1.: Effect of selective inhibitors on the viability of CML-T1 and CML-T1 IR cells. Cell viability was determined spectrophotometrically 3 days after inhibitor administration using an MTT assay. The absorbance of control CML-T1 sample was defined as 100 %. The data were collected on three biological samples in technical triplicates.

Publication:

[1] Toman O, Kabickova T, Vit O, Fiser R, Polakova KM, Zach J, Linhartova J, Vyoral D, Petrak J. Proteomic analysis of imatinib-resistant CML-T1 cells reveals calcium homeostasis as a potential therapeutic

target. Oncol Rep. 2016 Sep;36(3):1258-68. doi: 10.3892/or.2016.4945. Epub 2016 Jul 18. PMID: 27430982; PMCID: PMC4968618.