

Translocation t(12;21) with the presence of the fusion gene ETV6-RUNX1 (TEL-AML1) is the most common chromosomal aberration found in acute lymphoblastic leukemia in childhood. The occurrence of the ETV6-RUNX1 is associated with excellent prognosis and high sensitivity to the treatment with the enzyme L-asparaginase (ASNase). Resistance to the drug aggravates the outlook of the patient and increases the risk of treatment failure, therefore, the CLIP working group has been for a long time involved in the identification of the mechanism of action of ASNase and the origin of the resistance to it. This thesis follows previous findings of the group and is devoted to the analysis of the importance of ETV6-RUNX1 and signalization and metabolic changes accompanying shifts in the L-asparaginase resistance.

In the first part of the thesis, the knockout clones with stable increased resistance to ASNase have been established thanks to the CRISPR/Cas9 system, which created frameshift in the fusion gene. The accomplishment in this regard and removal of the fusion protein was confirmed on the level of DNA, mRNA and protein expression. The presence of other significant chromosomal aberrations affecting the sensitivity to ASNase was ruled out by the means of SNP analysis.

In the second part of the project, the signalization reaction to the treatment with L-asparaginase was observed, in particular with regards to the signalization complex mTORC1. The differences in the responses of original REH cell lines and obtained knockout mutants deficient in the fusion protein ETV6-RUNX1 was also tracked. Apart from the mTOR, autophagy markers and proteins affecting cellular metabolism were also detected.

In the last part of the investigation was paid close attention to the analysis of the metabolic profile of the cells in the context of the ASNase treatment and the presence or absence of the ETV6-RUNX1 protein. With the employment of the Seahorse analyzer, the oxygen consumption rate was measured, which is an indicator of the respiratory chain activity. Utilizing the radiolabelled metabolites, we were able to determine the intensity of the mitochondrial oxidation of fatty acids and glucose uptake from the media.

The research demonstrated an alteration in the mTORC1 signalization in response to the ASNase treatment. The absence of the fusion protein manifested itself in the changes of ACC, Akt and mTOR signalization and the autophagy markers Atg5 and Beclin1 concentration decrease. The fusion protein also influenced the character of the metabolic changes induced by the ASNase, which could explain the observed increased chemoresistance of the knockout cells.