Abstract

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Title of diploma thesis: Interindividual variability in expression of selected membrane transporters; their impact on prognosis and therapy of patients with acute myeloid leukaemia.

Acute myeloid leukaemia (AML) is a malignant disease of hematopoietic system. Available treatment does not produce suitable results, as the 5-year survival is only about 30%. The primary induction therapy has remained the same for many years – combination of cytarabine and anthracycline, known as ”7+3“. By karyotyping and immunophenotyping of patients in the last few years, heterogeneity of the disease was confirmed, which also led to the development of targeted drugs. Other factors such as transporters that play a role in drug transport across plasma membranes may affect the treatment outcome. In my diploma thesis, I therefore focused on the effect of selected membrane transporters OCTN1, OCTN2 and ABCC4 on the prognosis and therapy of AML patients.

First, we specified the number of transcripts of studied genes using the RT-PCR and ddPCR methods in samples isolated from mononuclear cells of the blood of *de novo* diagnosed AML patients. The significant interindividual variability was found for the OCTN1 and ABCC4 transporters, which represent influx and efflux transporters for cytarabine. Based on the results from PCR, we generated the Kaplan-Meier survival curves and discovered that the OCTN1 transporter appears to be predictive marker of survival. Using the values from PCR method and clinical data from University Hospital Hradec Králové, we took a closer look at possible context to the patients’ characteristics. The most interesting fact, which had not appeared in other studies yet, was that the most common FLT3 mutation was related to the significant downregulation of predictive OCTN1 transporter. On the contrary, we did not observe any changes for NPM1 mutation. Regarding the most recent and widely used risk classification ELN, we noticed that the patients in adverse risk category had significantly increased OCTN2 expression. It could be explained as a

defence mechanism of cells that could very quickly adapt to their surroundings and high need of L-carnitine for fatty acid oxidation and energy production.

Based on new knowledge of pathophysiology of the disease, the development of new targeted drugs accelerated rapidly. From FLT3 inhibitors category, two compounds have been approved in the Czech Republic so far – midostaurin and gilteritinib. Because of the identified relation between OCTN1 and FLT3 mutation, we hypothesized about influencing OCTN1 transporter by selective FLT3 inhibitors from the perspective of changes in both function and expression. Using model MDCKII-OCTN1 cells, we found out that none of approved target drugs functionally affected OCTN1 transporter. After we exposed MV4-11 cell line, that carries FLT3 mutation, to tested drugs, we observed significant increase of OCTN2 expression and also significant moderate increase of OCTN1 expression.

The results of our experimental work confirmed role of OCTN1 transporter for survival of AML patients treated by cytarabine. They also suggest possible modification of the therapeutic scheme of AML patients with FLT3 mutation. The selective inhibitor of FLT3 could be added into the induction chemotherapy to increase the level of OCTN1 transporter to improve the cytarabine therapy outcome. However, further study of this hypothesis is necessary to verify its clinical significance.