Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent type of childhood cancer. The key component in the therapy, L-asparaginase (ASNase), hydrolyzes plasma asparagine and glutamine. Leukemic cells are sensitive to the depletion due to low activity of asparagine synthetase. Although the treatment is very effective, resistance and side effects remain a serious problem in some cases and its mechanism of action is not well understood.

In this study, we wanted to elucidate the effect of *ASNS* expression level on the sensitivity of ALL cells to ASNase treatment. Our aim was also to clarify the intracellular consequences of the amino acid depletion to define the reason of different patients´ response.

We used four ALL cell lines (NALM-6, RS4;11, REH, and UOCB-6) and 30 diagnostic bone marrow samples of ALL patients to study the relationship between *ASNS* expression and sensitivity to ASNase using MTS proliferation assay. RNA interference was used to study the effect of a range of *ASNS* levels on the response to ASNase treatment. Using a cell line model with a gradually knocked-down *ASNS* gene, we defined a cutoff level below which *ASNS* gene expression does not correlate with sensitivity to ASNase. Importantly, *ASNS* gene expression in patients' ALL blasts is below this level. We confirmed that there was no correlation between *ASNS* gene expression and sensitivity to ASNase in ALL blasts. In addition, we show that cells with low *ASNS* expression level do not respond to asparagine deprivation by upregulation of *ASNS* gene expression.

To study further the effect of ASNase on leukemic cells, we have generated ASNase-resistant subclones through chronic exposure to the enzyme. Pathway analysis of gene expression profiles of the cell lines (REH, NALM-6 and their resistant counterparts) and primary samples (sensitive and resistant to ASNase; Holleman *et al*. (NEJM, 2004)) revealed that ASNase affects the translation machinery and metabolism of leukemic cells. Therefore, we studied the impact of ASNase on the main metabolic pathways in leukemic cells. Treating leukemic cells with ASNase increased fatty acid oxidation (FAO) and cell respiration and inhibited glycolysis. FAO, together with the decrease in protein translation and pyrimidine synthesis, was regulated through inhibition of the RagB-mTORC1 pathway, whereas the effect on glycolysis was RagBmTORC1 independent. Since FAO has been suggested to have a pro-survival

function in leukemic cells, we tested its contribution to cell survival following ASNase treatment. Pharmacological inhibition of FAO significantly increased the sensitivity of ALL cells to ASNase. Moreover, constitutive activation of the mTOR pathway increased apoptosis in leukemic cells treated with ASNase, but did not increase FAO.

In conclusion, the *ASNS* expression level does not predict sensitivity to ASNase in leukemic blasts. Moreover, our study uncovers a novel therapeutic option based on the combination of ASNase and FAO inhibitors.