IDENTIFICATION THE KEY PLAYERS OF METABOLIC REPROGRAMMING OF LEUKEMIC CELLS UPON L-ASPARAGINASE TREATMENT.

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L-asparaginase (ASNase) is the key component in the treatment of acute lymphoblastic leukemia (ALL) in children. ASNase depletes extracellular asparagine (Asn) and glutamine (Gln). In our previous findings, we had demonstrated that ASNase treatment leads to metabolic reprogramming of leukemic cells in order to compensate the lack of mentioned amino acids and can turn on pro-survival processes which could interfere with the effectiveness of the treatment. We aim to describe the mechanism of action of ASNase in more detail and interactions between bioenergetics processes. In order to complete our aims, we used pre-B ALL leukemia cells lines (REH and NALM6) treated with ASNase in two different time points. The project was contemplated in two different ways. First, we measured the intracellular metabolites of the central carbon metabolism and amino acids (AAs) by employing Liquid Chromatography - Mass Spectrometry (LC/MS). Then extracellular amino acids were measured from cultured media with LC/MS and High-performance LC. Next, using Label-Free Proteome MS Quantification we detected changes in proteins involved in metabolic processes. Results were confirmed on mRNA and protein level. Treatment with ASNase led to significant decrease of extracellular levels of Asn and Gln followed by increase of aspartate and glutamate. Intracellular Asn and Gln were decreased as well whereas aspartate and glutamate were not affected. Interestingly, levels of non-essential AAs (alanine, serine, and glycine) and also essential AAs (histidine, isoleucine/leucine, methionine, phenylalanine, valine, tryptophan and threonine) were increased. Proteome analysis identified 16 metabolic-related proteins. After validation, we selected arginosuccinate synthase (ASS1), aspartate aminotransferase (GOT1) and phosphoserine aminotransferase (PSAT1) as possible key effectors of the metabolic reprograming. Our results show that ASNase disturbs AAs metabolism. We hypothesize that activation of AAs biosynthetic pathways could allow leukemia cells maintain normal levels of aspartate and glutamate necessary for energy and biomass maintenance and also compensate for depletion of Asn and Gln by synthesis of other AAs. Supported by the Charles University Grant agency (794218) and AZV grant 15-28848A.