L-ASPARAGINASE BY-PRODUCT GLUTAMATE IMPEDES THE EFFECT OF CHEMOTHERAPY BY AUGMENTING GLUTATHIONE BIOSYNTHESIS

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L-asparaginase (ASNase) is one of the crucial components of acute lymphoblastic leukemia (ALL) therapy. ASNase transforms asparagine (Asn) and glutamine (Gln) to aspartate (Asp) and glutamate (Glu), respectively. High Asp and Glu extracellular concentrations are considered to be a by-product of ASNase treatment without any known consequences. However, our results show that even though TCA cycle is diminished after ASNase treatment, leukemic cells are able to maintain intracellular Asp and Glu levels. We evaluated the role of Asp and Glu in the resistance mechanism of leukemic cells to ASNase.

Using stable isotope tracing with $U^{13}C$-Asp and $U^{13}C$-Glu we discovered that leukemia cell lines were able to import Asp and Glu from the culture media. Noteworthy, leukemia cell lines and also primary leukemia cells expressed the genes coding for Asp/Glu transporters. Moreover, primary leukemia cells were able to import Glu from the media.

Next, we discovered that high Glu doses, but not high Asp doses, helped leukemia cell lines to survive in Asn-/Gln-depleted conditions. Furthermore, in high Glu conditions, using flow cytometry, we detected elevated intracellular ROS compared to Asn-/Gln-depleted media. Since GSH is the main antioxidant molecule in the cells and Glu is one of its three amino acids, we pursued the idea that cells under Asn-/Gln-depleted conditions used Glu to synthesize GSH and by that overcame the nutrient stress. Indeed, we discovered that leukemia cells used imported Glu to biosynthesize GSH. Together with the fact that imported Glu also fueled TCA cycle, GSH biosynthesis probably partially rescued the cells from oxidative stress caused by respiration. When we used GSH synthesis inhibitor BSO, intracellular ROS levels were more elevated in high Glu conditions compared to media without Glu, indicating that *de novo* GSH biosynthesis is crucial for the cells in high Glu conditions.

Overall, this is the first study describing the transport of Glu into leukemic cells, their survival advantage after ASNase treatment and, hence, a novel mechanism of leukemia cell resistance to ASNase.

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