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GLUTAMINE DEPRIVATION AFFECTS THE EXPRESSION OF GENES WHICH CONTROL PYRUVATE DEHYDROGENASE ACTIVITY: THE IMPACT OF ERN1 KNOCKDOWN

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Pyruvate dehydrogenase (PDH) is a mitochondrial multienzyme complex that catalyzes the oxidative decarboxylation of pyruvate and is one of the major enzymes responsible for the regulation of homeostasis of carbohydrate fuels in mammals [1-2]. It is known that pyruvate dehydrogenase kinase inhibits pyruvate dehydrogenase activity via phosphorylation of its subunits PDHA1 and PDHA2 and thereby regulates metabolite flux through the tricarboxylic acid cycle, down-regulates aerobic respiration and inhibits the formation of acetyl-coenzyme A from pyruvate. Glutamine is a substrate for glycolysis and thereby is important for the development and a more aggressive behavior of malignant tumors, especially gliomas, which are highly aggressive tumors with very poor prognosis. It is well known that glutamine supply and endoplasmic reticulum stress are very important and complementary factors for the malignant tumor growth. Furthermore, inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1), a major signaling pathway of endoplasmic reticulum stress, significantly modifies the effects of glutamine deprivation on the expression of numerous genes [3]. At the same time, the comprehensive molecular mechanisms of the interaction of stress signaling pathway mediated ERN1 with glutamine supply are complex yet and warrant additional study. Moreover, there is also data that nutrient starvation is a very important factor of the resistance of cancer cells to chemotherapy. It is well known that activation of ERN1 branch of the endoplasmic reticulum stress response is tightly linked to apoptosis and cell death and that inhibition of its function has been demonstrated to result in a significant anti-proliferative effect in glioblastoma growth.

The aim of the current investigation was to study the expression of genes encoded pyruvate dehydrogenase subunits (PDHA1, PDHB, PDHX, DLAT, and DLD) in U87 glioma cells in response to glutamine deprivation in U87 glioma cells in relation to knockdown of ERN1 for evaluation of a possible dependence of the expression of these important regulatory genes from glutamine supply and ERN1 signaling.

Methods. The expression of *PDHA1, PDHB, PDHX, DLAT*, and *DLD* genes was studied by real-time qPCR in control U87 glioma cells (transfected by vector) and cells with knockdown of ERN1 (transfected by dnERN1) after exposure to glutamine deprivation condition. Total RNA was extracted from glioma cells using TRIZOL reagent. An RNA quantity as well as spectral characteristics was measured using NanoDrop One. For reverse transcription of mRNAs we used Thermo Scientific Verso cDNA Synthesis Kit (Germany). The values of mRNA expressions were normalized to the level of ACTB mRNA and represented as percent of control (100%).

Results. It was shown that the expression level of *PDH1, PDHB, DLAT*, and *DLD* genes was down-regulated in control glioma cells treated by glutamine deprivation. At the same time, ERN1 knockdown is suppressed the effect of glutamine deprivation on *PDHB* and *DLD* gene expressions in glioma cells, but did not change significantly the impact of glutamine deprivation on the expression of *PDHA1, DLAT*, and *PDHX* genes (Figure).

Discussion. These results are important for the evaluation of possible significance of glutamine deprivation in ERN1 dependent control of glioma cell proliferation because there are data indicating

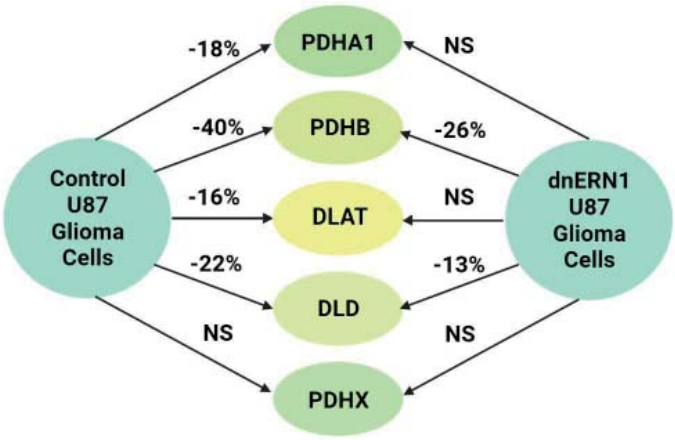

Fig. **The impact of glucose deprivation on the expression level of genes encoding pyruvate dehydrogenase subunits PDHA1, PDHB, DLAT, DLD, PDHX in control and ERN1 knockdown U87 glioma cells**

that the endoplasmic reticulum stress signaling mediated by ERN1 is involved in numerous metabolic pathways and ERN1 knockdown has clear anti-tumor effect [3]. Results of this study clarify possible mechanisms of glutamine deprivation on the proliferation/surviving of ERN1 knockdown glioma cells through specific changes in the expression profile of genes encoding subunits of PDH.

Conclusions. The results of this investigation demonstrated that the expression of *PDH1, PDHB, PDHX, DLAT,* and *DLD* genes was significantly affected by exposure of U87 glioma cells under glutamine deprivation condition and that the effect of glutamine deprivation on the expression of most these genes was modified in cells with knockdown of ERN1, a major signaling pathway of the endoplasmic reticulum stress.

Key words: pyruvate dehydrogenase, mRNA expression, ERN1 knockdown, glutamine deprivation, U87 glioma cells.

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