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ERN1 KNOCKDOWN AFFECTS THE EXPRESSION OF *PDHA1*, *PDHB*, *PDHX*, *DLD*, AND *DLAT* GENES AND MODIFIES THEIR HYPOXIC REGULATION

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Hypoxia and endoplasmic reticulum (ER) stress are important factors affecting tumor growth, and inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1) protein, which is responsible for one of the main pathways for ER stress signaling, has the ability to suppress glioma cell proliferation and growth. Pyruvate dehydrogenase complex is a critical factor for glioma cell growth because they have an increased rate of glucose consumption [1].

Aim. The purpose of this study was to investigate the role of ERN1 in the regulation of the expression of pyruvate dehydrogenase complex genes in U87MG glioma cells.

Methods. Three sublines of U87MG glioma cells were used in the experiment, which Prof. O. Minchenko created in collaboration with Prof. M. Moenner: stably transfected with an empty vector and two different dominant/negative ERN1 constructs (dnERN1 and dnrERN1) [1]. The subline with dnERN1 has suppressed both ERN1 endoribonuclease and protein kinase while subline with dnrERN1 has inhibited only ERN1 endoribonuclease. Cells were cultured as described previously [1]. Hypoxic conditions were created using 0.5 mM dimethyloxalylglycine (4 hours) as described previously [2]. We also treated the glioblastoma cells with inhibited ERN1 endoribonuclease by tunicamycin (500 ng/ml) to clarify the role of other signaling pathways of ER stress al in the control of *PDH* gene expressions. The expression levels of *PDHA1* (pyruvate dehydrogenase E1 alpha 1 subunit), *PDHB* (pyruvate dehydrogenase E1 beta subunit), *PDHX* (pyruvate dehydrogenase complex component X), *DLAT* (dihydrolipoamide S-acetyltransferase), and *DLD* (dihydrolipoamide dehydrogenase) genes were analyzed using real-time qPCR (quantitative polymerase chain reaction) using "QuantStudio 5 Real-Time PCR System" (Applied Biosystems) and normalized to the expression level of beta-actin (*ACTB*).

Results and Discussion. The results of this study demonstrated that hypoxia decreased the expression of *PDHA1*, *PDHB*, *DLAT*, and *DLD* genes in control glioblastoma cells and that inhibition of ERN1 endoribonuclease and protein kinase significantly modified the impact of hypoxia on the expression of *DLD* and *PDHX* genes. We also showed that the expression of all *PDH* genes was down-regulated by suppression of ERN1 endoribonuclease and protein kinase in control glioblastoma cells being more significant for *DLAT* and *DLD* genes. At the same time, the inhibition of ERN1 endoribonuclease only led to down-regulation the expression of *PDHA1*, *PDHB*, and *DLAT* genes but had no significant effect on the *PDHX* and *DLD* gene expressions in glioblastoma cells. Furthermore, the exposure of glioblastoma cells with inhibited ERN1 endoribonuclease to tunicamycin led to down-regulation of the expression of *PDHA1* (-10%), *PDHB* (-28%), *PDHX* (-36%), *DLAT* (-24%) and *DLD* (-50%) genes. These results demonstrated that other signaling pathways of ER stress also participate in the control of *PDH* gene expressions. The schematic representation of the research results is shown in the Figure.

The results of this study demonstrated that the ERN1, a major endoplasmic reticulum stressed sensor signal pathway, controlled PDH gene expressions through different mechanisms: via ERN1 endoribonuclease (*PDHA1* and *PDHB*) or ERN1 protein kinase (*PDHX* and *DLD*). These results are fundamentally new for genes of the pyruvate dehydrogenase complex. They are in good agreement with previously published data about the role of protein kinase in the regulation of gene

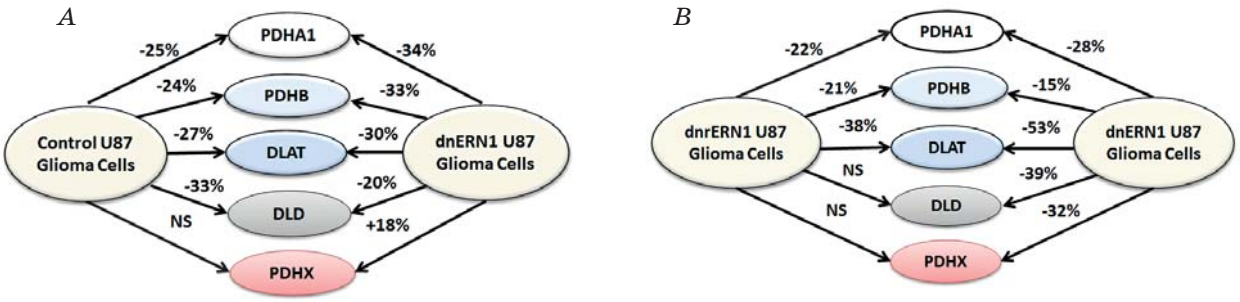

Fig. **Schematic representation of the research results:**
A — the effects of the suppression of ERN1 endoribonuclease (dnrERN1) and both ERN1 endoribonuclease and protein kinase (dnERN1) constructs on the expression of *PDHA1*, *PDHB*, *PDHX*, *DLAT*, and *DLD* genes in U87MG glioma cells; *B* — the impact of hypoxia on PDH gene expressions in control and ERN1 knockdown (dnERN1) glioma cell

expression [2, 4, 5]. It has been shown that PDH gene expression is controlled by hypoxia and that hypoxic regulation of *DLD* and *PDHX* genes is ERN1-dependent, which is consistent with the existing data [4, 6].

Conclusions. It was found that hypoxia affected the expression level of most genes of the pyruvate dehydrogenase complex, and PDHX and DLD genes hypoxic regulation depended on the activity of the ERN1 protein (ERN1 protein level and activity were studied previously [2]. It has been shown that the expression level of pyruvate dehydrogenase complex genes is controlled by ERN1, an endoplasmic reticulum stress signal protein, through its endoribonuclease and/or protein kinase activity, and in different ways for individual genes: *PDHX* and *DLD* — through the protein kinase activity of *ERN1*, *PDHA1* and *PDHB* genes — through the endoribonuclease *ERN1*, and *DLAT* gene — through both of its enzymatic activities.

Key words: endoplasmic reticulum stress, ERN1, hypoxia, glioma cells, pyruvate dehydrogenase complex.

Authors' contribution. YPK was engaged in PCR analysis, nucleic acid gel electrophoresis and presentation of the results, OVH carried out cDNA synthesis and analysis of the results, YMV worked with cell culture and mRNA extraction, OHM is responsible for conceptualization, formal analysis, editing, and supervision.

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