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ERN1 dependent impact of glutamine and glucose deprivations on the pyruvate dehydrogenase genes expression in glioma cells

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Objective. The aim of the present study was to investigate the expression of pyruvate dehydrogenase genes such as PDHA1, PDHB, DLAT, DLD, and PDHX in U87 glioma cells in response to glutamine and glucose deprivations in control glioma cells and endoplasmic reticulum to nucleus signaling 1 (ERN1) knockdown cells, the major endoplasmic reticulum (ER) stress signaling pathway, to find out whether there exists a possible dependence of these important regulatory genes expression on both glutamine and glucose supply as well as ERN1 signaling.

Methods. The expression level of PDHA1, PDHB, DLAT, DLD, and PDHX genes was studied by real-time quantitative polymerase chain reaction in control U87 glioma cells (transfected by empty vector) and cells with inhibition of ERN1(transfected by dnERN1) after cells exposure to glucose and glutamine deprivations.

Results. The data showed that the expression level of PDHA1, PDHB, DLAT, and DLD genes was down-regulated (more profound in PDHB gene) in control glioma cells treated with glutamine deprivation. At the same time, ERN1 knockdown modified the impact of glutamine deprivation on the expression level of all these genes in glioma cells: suppressed the sensitivity of PDHB and DLD genes expression and removed the impact of glutamine deprivation on the expression of PDHA1 and DLAT genes. Glucose deprivation did not significantly change the expression level of all studied genes in control glioma cells, but ERN1 knockdown is suppressed the impact of glucose deprivation on PDHX and DLD genes expression and significantly enhanced the expression of PDHA1 and PDHB genes. No significant changes were observed in the sensitivity of PDHX gene expression to glutamine deprivation neither in control nor ERN1 knock-down glioma cells. The knock-down of ERN1 removed the sensitivity of DLAT gene expression to glucose deprivation.

Conclusion. The results of this investigation demonstrate that the exposure of control U87 glioma cells under glutamine deprivation significantly affected the expression of PDHA1, PDHB, DLAT, and DLD genes in a gene specific manner and that impact of glutamine deprivation was modified by inhibition of the ER stress signaling mediated by ERN1. At the same time, glucose deprivation affected the expression of PDHA1, PDHB, PDHX, and DLD genes in ERN1 knockdown glioma cells only. Thus, the expression of pyruvate dehydrogenase genes under glutamine and glucose deprivation conditions appears to be controlled by the ER stress signaling through ERN1.

Key words: U87 glioma cells, ERN1 knockdown, glutamine and glucose deprivations, pyruvate dehydrogenase, gene expression

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Many tumor cells including glioma cells have elevated rates of glucose uptake, but reduced rates of oxidative phosphorylation. This altered metabolic phenotype is essential for tumorigenesis. There are data indicating that HIF transcription factor induces the glycolytic genes expression and also actively represses the oxygen consumption by inducing pyruvate dehydrogenase kinase 1, which phosphorylates and inhibits pyruvate dehydrogenase and promotes cancer metastasis and therapy resistance (Jin et al. 2016; Cai et al. 2020).

The pyruvate dehydrogenase (PDH) complex is a mitochondrial multi-enzyme complex that catalyzes the overall conversion of pyruvate to acetyl coenzyme A (acetyl-CoA) and carbon dioxide and provides the primary link between glycolysis and the tricarboxylic acid cycle. This complex is composed of multiple copies of three enzymatic components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and lipoamide dehydrogenase (E3). The E1 enzyme is a heterotetramer of two alpha (PDHA1) and two beta (PDHB) subunits. Pyruvate dehydrogenase E1A (PDHA1) is a crucial regulator of pyruvate metabolic pathways that determines pyruvate entry into the citric acid cycle or glycolysis (Zaher et al. 2021). The silencing of PDHA1 expression prevents of HUVECs proliferation and inhibits angiogenesis *in vitro* as well as *in vivo* (Yetkin-Arik et al. 2019). The PDH activity depends on the stability of a multi-protein PDH complex. Down-regulation of PDH-E1beta (PDHB) subunit of this complex inhibits PDH activity under hypoxia. Thus, PDH acts as an oncogenic factor by tuning glycolytic metabolism in cancer cells. Down-regulation of PDH is critical for the aberrant preferential activation of glycolysis in cancer cells under normoxic conditions (Yonashiro et al. 2018).

Dihydrolipoamide S-acetyltransferase (DLAT) represents an E2 component of PDH complex and catalyzes the conversion of pyruvate to acetyl-CoA and CO_2 and thereby links the glycolysis to the tricarboxylic cycle. PDH is regulated by phosphorylation of its E1 component, but SIRT4 enzymatically hydrolyzes the lipoamide cofactors from the DLAT diminishing PDH activity (Mathias et al. 2014). The activity of the PDH has been found to be affected by the expression level of mitochondrial C1QBP protein, which interacts with DLAT (Chen et al. 2016). Mitochondrial protein dihydrolipoamide dehydrogenase (DLD) represents an E3 component of PDH complex and functions in a homodimeric form. Recently, it has been shown that cysteine deprivation induced cancer cell death over inducing the ferroptosis via glutaminolysis, which was significantly decreased by short-interfering RNA (siRNA) targeting DLD (Shin et al. 2020). This protein is also present in several multi-enzyme complexes that regulate energy metabolism, but in monomeric form can function as a protease (Babady et al. 2007). Component X of PDH complex (PDHX) is an E3 binding protein subunit, which is essential for a functional activity of a PDH complex. PDHX is required for anchoring DLD to the dihydrolipoamide transacetylase core of the PDH complex and defects in this gene are a cause of PDH deficiency. The silencing of PDHX by miR-27b leads to an altered metabolic configuration that is better to cell proliferation, thereby promoting breast cancer progression (Eastlack et al. 2018). Therefore, PDHX is a metabolically essential gene for the cell growth because its expression is required for the maintenance of PDH activity and the production of ATP and its knockdown inhibited the proliferation of cancer cells and *in vivo* tumor growth (Inoue et al. 2021).

Glutamine as well as glucose, as a substrate for glycolysis, is an important for the development and a more aggressive behavior of gliomas, which are highly aggressive tumors with very poor prognosis (Colombo et al. 2011; Zhao et al. 2017; Alimohammadi et al. 2020). Transformed cells exhibit a high rate of glutamine consumption and Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to a glutamine addiction (DeBerardinis et al. 2007; Wise et al. 2008; Gao et al. 2009). Xu et al. (2015) have shown that tumor suppressor NDRG2 (N-Myc Downstream-Regulated Gene 2) function is an essential regulator in glycolysis and glutaminolysis via repression of c-Myc. Glutamine and glucose supply as well as endoplasmic reticulum (ER) stress are very important and complementary factors for the tumor growth. This is also confirmed by the fact that inhibition of endoplasmic reticulum to nucleus signaling 1 (ERN1)/inositol requiring enzyme 1 (IRE1) modifies the effect of glutamine and glucose deprivations on the expression of numerous genes (Minchenko et al. 2013, 2015a, 2020, 2021; Tsymbal et al. 2016, 2020; Riabovol et al. 2019; Krasnytska et al. 2022). Bioinformatics profiling identifies a glucose-related risk signature for the malignancy of glioma (Zhao et al. 2017). There are also data indicating that nutrient starvation is very important factor for the suppression of cancer cells chemoresistance possibly by inhibiting the unfolded protein response (Awale et al. 2006; Kim et al. 2010; He et al. 2018).

The ER stress and its signaling pathways adapt and enhance tumor cells proliferation and suppress

apoptosis under stressful conditions (Manie et al. 2014; Doultsinos et al. 2017; Obacz et al. 2017; Papaioannou and Chevet 2018; Almanza et al. 2019). There are data indicating that the suppression of ER stress signaling results in a significant anti-proliferative effect in the glioma growth (Auf et al. 2010; Minchenko et al. 2014; Hetz et al. 2019), but inhibition of ERN1 endoribonuclease has more strong anti-proliferative effect on glioma cells and leads to specific changes in the expression of genes related to ERN1 signaling pathway (Auf et al. 2013; Minchenko et al. 2015b).

The aim of the current study was to investigate the expression of PDH genes, such as *PDHA1*, *PDHB*, *DLAT*, *DLD*, and *PDHX* in U87 glioma cells in response to glutamine as well as glucose deprivation in control glioma cells and cells with knockdown of ERN1, the major pathway of the ER stress signaling to reveal a possible dependence of these important regulatory genes on both glutamine and glucose supply as well as ERN1 signaling.

Materials and Methods

Cell lines and culture conditions. The glioma cell line U87 was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (DMEM; Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco) and streptomycin (0.1 mg/ml; Gibco) at 37°C in incubator with 5% CO_2. In this work, we used two sublines of these cells. One subline was obtained by selection of stable transfected clones with overexpression of vector pcDNA3.1, which was used for creation of dnERN1. These untreated glioma cells were used as control 1 (control glioma cells) in the study of the effect of glucose and glutamine deprivations on the level of gene expressions. Second subline was obtained by selection of stable transfected clones with overexpression of ERN1 dominant/negative construct (dnERN1), having suppression of both the protein kinase and endoribonuclease activities of this signaling enzyme (Auf et al. 2010). It has been shown that cells with dnERN1 have a lower proliferation rate, do not express spliced variant of XBP1, a key transcription factor in ERN1 signaling, and they do not have the phosphorylated isoform of ERN1 after induction of ER stress by tunicamycin (Auf et al. 2010, 2013; Minchenko et al. 2015b). The impact of glutamine deprivation on the expression of *PDHA1*, *PDHB*, *DLAT*, *DLD*, and *PDHX* genes in the cells with ERN1 deficiency introduced by dnERN1 was compared with cells transfected with the previously mentioned empty vector pcDNA3.1 (control glioma cells). Both sublines of glioma cells used in this study are grown in the presence of geneticin (G418), while these cells carrying empty vector pcDNA3.1 or dnERN1 construct. Glutamine and glucose deprivations were created by changing the complete DMEM medium into culture plates on DMEM medium without glutamine or glucose, correspondingly, and plates were exposed to this condition for 16 h.

RNA isolation. Total RNA was extracted from glioma cells using the Trizol reagent according to manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The RNA pellets was washed with 75% ethanol and dissolved in nuclease-free water. For additional purification RNA samples were re-precipitated with 95% ethanol and re-dissolved again in nuclease-free water. RNA concentration and spectral characteristics

Table 1
Characteristics of the primers used for quantitative real-time polymerase chain reaction.

Gene symbol	Gene name	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
PDHA1	Pyruvate dehydrogenase E1 alpha 1 subunit	F: 5'-tgtgtgatggtcaggaagct R: 5'-acatgtgcatcgatcctcct	389–408 577–558	NM_000284.4
PDHB	Pyruvate dehydrogenase E1 beta subunit	F: 5'-tcgagggctgtggaagaaat R: 5'-agtaggtcttggcagctgag	253–272 437–418	NM_000925.4
DLAT	Dihydrolipoamide S-acetyltransferase	F: 5'-tccaactccccagcctttag R: 5'-ccatctggtcctgtcccttt	1055–1074 1210–1191	NM_001931.5
DLD	Dihydrolipoamide dehydrogenase	F: 5'-cggaggtgaaagtattggcg R: 5'-tgcagactgtcttgaagcct	27–46 276–257	NM_000108.5
PDHX	Pyruvate dehydrogenase complex component X	F: 5'-ccctcaccagaaccacagat R: 5'-gcccgtttgtttcaactgga	519–538 716–697	NM_003477.3
ACTB	beta-Actin	F: 5'-ggacttcgagcaagagatgg R: 5'-agcactgtgttggcgtacag	747–766 980–961	NM_001101

were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described (Minchenko et al. 2018). The impact of glutamine and glucose deprivations on the expression levels of PDH mRNAs were measured in control U87 glioma cells and cells with a deficiency of ERN1, introduced by dnERN1, by quantitative polymerase chain reaction (qPCR) using SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). The PCR was performed in triplicate. The expression of beta-actin (ACTB) mRNA was used as control of analyzed RNA quantity. The pair of primers specific for each studied gene was received from Sigma-Aldrich (St. Louis, MO, U.S.A.) and Metabion Int. AG (Martinstried, Germany) and used for qPCR (Table 1).

Quantitative PCR analysis. This analysis was performed using a special computer program "Differential expression calculator" and statistical analysis using Excel program and OriginPro 7.5 software as described previously (Minchenko et al. 2015b). Comparison of two means was performed by the use of two-tailed Student's t-test. The $p<0.05$ was considered significant in all cases. The values of studied gene expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100%). All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments. The amplified DNA fragments were also analyzed on a 2% agarose gel and that visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

Results

To reveal a possible role of glutamine and glucose deprivations as well as ER stress signaling mediated by ERN1 protein in the expression level of genes encoding PDH complex, we studied the effect of these nutrient deprivation conditions on the expression of *PDHA1*, *PDHB*, *DLAT*, *DLD*, and *PDHX* genes in U87 glioma cells with and without ERN1 functional activity. As shown in Figure 1, the expression of PDHA1 subunit mRNA in control glioma cells, transfected by empty

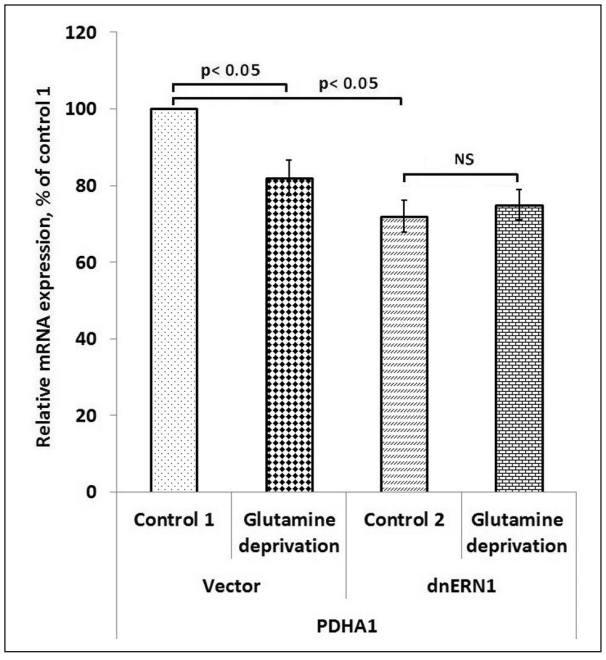

Figure 1. The impact of glutamine deprivation on the expression level of pyruvate dehydrogenase E1 alpha 1 subunit (*PDHA1*) gene in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of PDHA1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

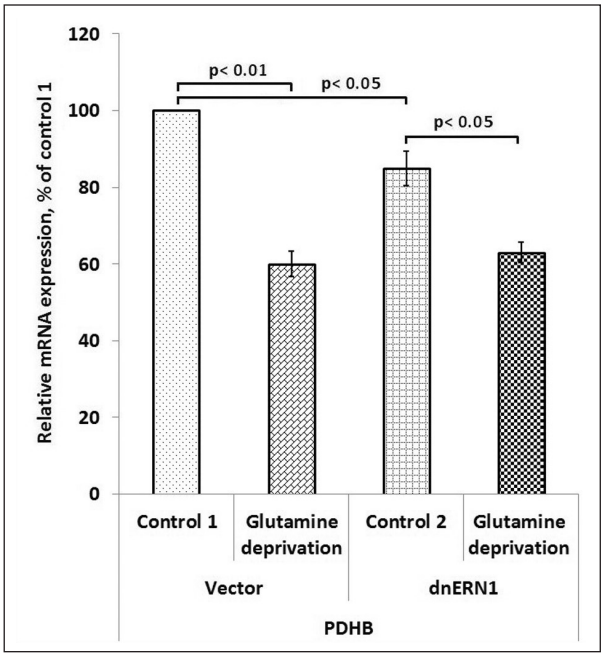

Figure 2. The impact of glutamine deprivation on the expression level of pyruvate dehydrogenase E1 beta subunit (*PDHB*) gene in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of PDHB mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

vector pcDNA3.1, is decreased after exposure to glutamine deprivation (–18%) in comparison with the cells growing in complete DMEM medium. Inhibition of ERN1 signaling enzyme function by dnERN1 eliminates the sensitivity of *PDHA1* gene expression to this experimental condition in comparison with corresponding control (Figure 1). Next, the effect of glutamine deprivation on the expression of gene encoding PDHB subunit in relation to inhibition of ERN1 function is shown in Figure 2. Glutamine deprivation resulted in a significant down-regulation of this PDH gene expression (–40%) in comparison with control glioma cells, transfected by empty vector (Figure 2). Inhibition of ERN1 signaling protein function led to lesser changes in the expression level of this gene (–26%; Figure 2).

The effect of glutamine deprivation on the expression of gene encoding DLAT protein in glioma cells in relation to complete inhibition of ERN1 signaling enzyme function was evaluated. It was shown that the expression level of *DLAT* gene is down-regulated (+16%) in control glioma cells (transfected by empty vector) under glutamine deprivation in comparison with the cells growing in regular medium (Figure 3). At the same time, inhibition of ERN1 signaling enzyme function removes the effect of glutamine deprivation on this gene expression as compared to corresponding control cells (transfected by dnERN1; Figure 3).

As shown in Figure 4, exposure of control glioma cells to glutamine deprivation led to a down-regulation of DLD mRNA expressions (–22%) in comparison with cells growing with glutamine in the medium. Inhibition of both enzymatic activities of ERN1 had significantly lesser impact on glutamine deprivation of this gene expression (–13%). At the same time, the expression of PDHX gene was resistant to glutamine deprivation both in control and ERN1 knockdown glioma cells in comparison with corresponding controls (Figure 5).

As shown in Figures 6–10, the expression of all studied genes of PDH complex is resistant to glucose deprivation in control glioma cells, but inhibition of ERN1 signaling significantly modifies the sensitivity of most these genes to this experimental condition. Thus, the expression level of PDHA1 and PDHB

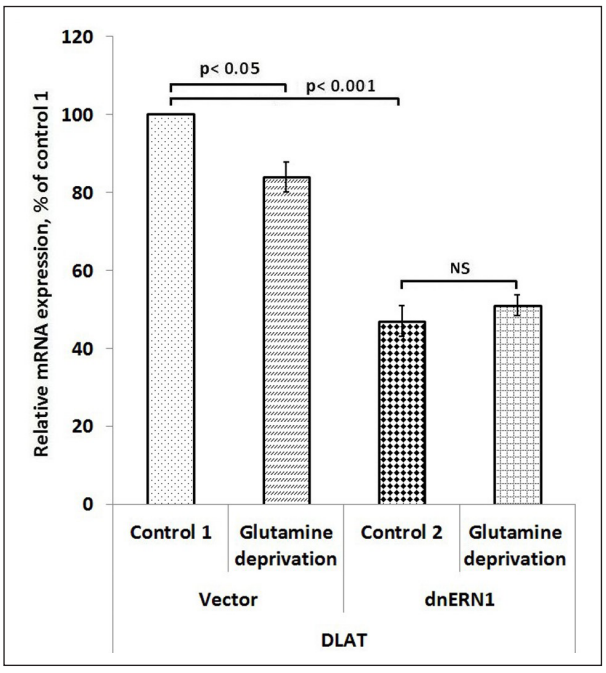

Figure 3. The impact of glutamine deprivation on the expression level of dihydrolipoamide S-acetyltransferase (*DLAT*) gene, encoding E2 component of pyruvate dehydrogenase complex, in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of DLAT mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

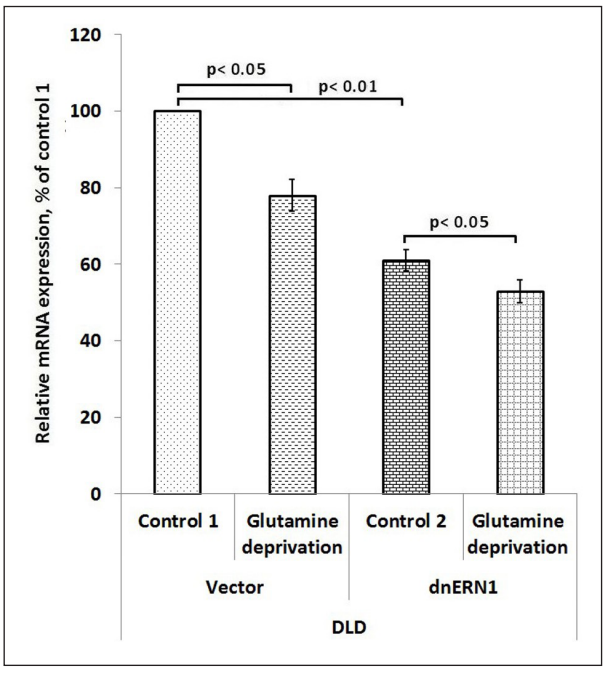

Figure 4. The impact of glutamine deprivation on the expression level of dihydrolipoamide dehydrogenase (*DLD*) gene, encoding E3 component of pyruvate dehydrogenase complex in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of DLD mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

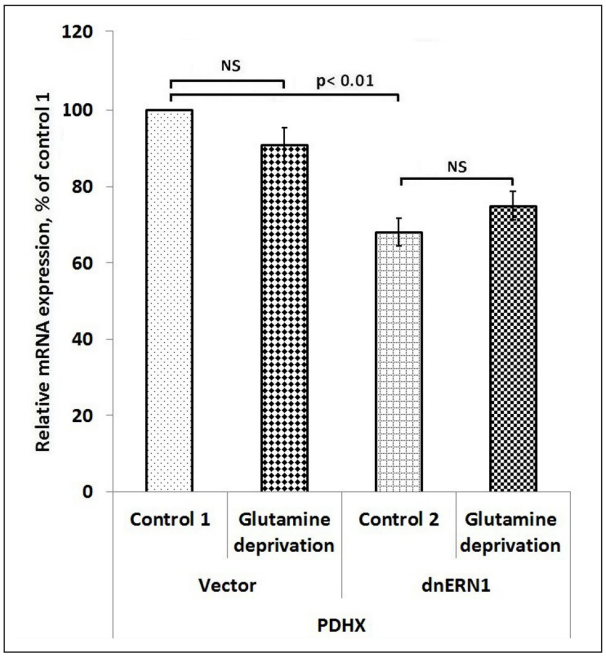

Figure 5. The impact of glutamine deprivation on the expression level of pyruvate dehydrogenase complex component X (*PDHX*) gene in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of PDHX mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

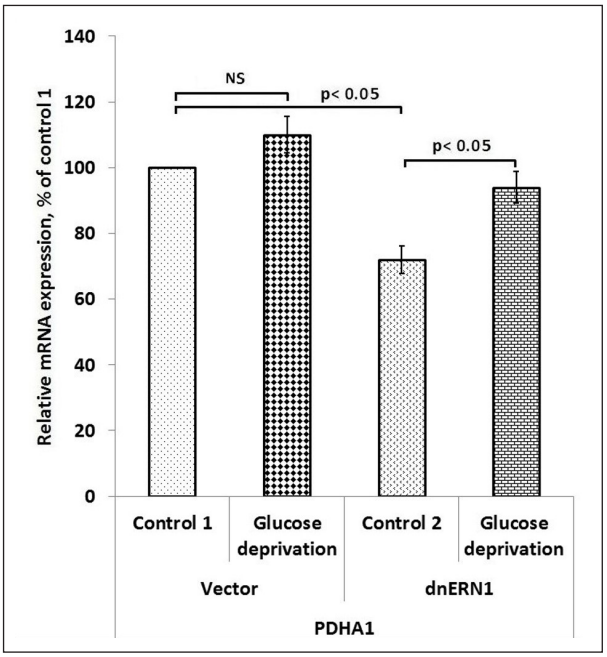

Figure 6. The impact of glucose deprivation on the expression level of pyruvate dehydrogenase E1 alpha 1 subunit (*PDHA1*) gene in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of PDHA1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

subunits is increased (+30 and +32%, respectively) in ERN1 knock-down glioma cells under glucose deprivation (Figures 6 and 7). At the same time, the *DLD* and *PDHX* genes expression are decreased (–14 and –21%, respectively) in glioma cells with inhibited the ERN1 signaling growing under glucose starvation condition (Figures 9 and 10). Thus, knock-down of ERN1 has the ability to eliminate the tolerance of *PDHA1*, *PDHB*, *DLD*, and *PDHX* genes in glioma cells to glucose starvation.

Glutamine deprivation affects the expression of most genes of PDH complex, but glucose deprivation does not change the expression level of all studied genes in control glioma cells. Inhibition of ERN1 signaling significantly modified the sensitivity of most genes encoding enzymes of PDH complex to both glucose and glutamine deprivations. Results of this investigation are summarized in Figure 11, which clearly demonstrates the gene-specific character of changes in the expression profile of most studied PDH genes in glioma cells under glutamine and glucose deprivations in ERN1 dependent manner.

Discussion

In this work, the effect of glutamine and glucose deprivations on the expression of genes encoding different enzymes of PDH complex in U87 glioma cells in relation to inhibition of ERN1, the major signaling pathway of the unfolded protein response was studied. Control glioma cells transfected by empty vector pcDNA3.1, and cells with full ERN1 deficiency introduced by dnERN1 were used. This is important for the evaluation of possible significance of glutamine as well as glucose deprivation in ERN1 dependent control of glioma cell proliferation because there are data indicating that the ER stress signaling mediated by ERN1 is involved in numerous metabolic pathways and ERN1 knockdown has clear anti-tumor effect (Auf et al. 2010, 2013; Bravo et al. 2013; Manie et al. 2014; Minchenko et al. 2014, 2015a,b; Logue et al. 2018).

Results of our study clarify possible mechanisms of glutamine and glucose deprivations on the proliferation/surviving of ERN1 knockdown glioma cells through specific changes in the expression profile of

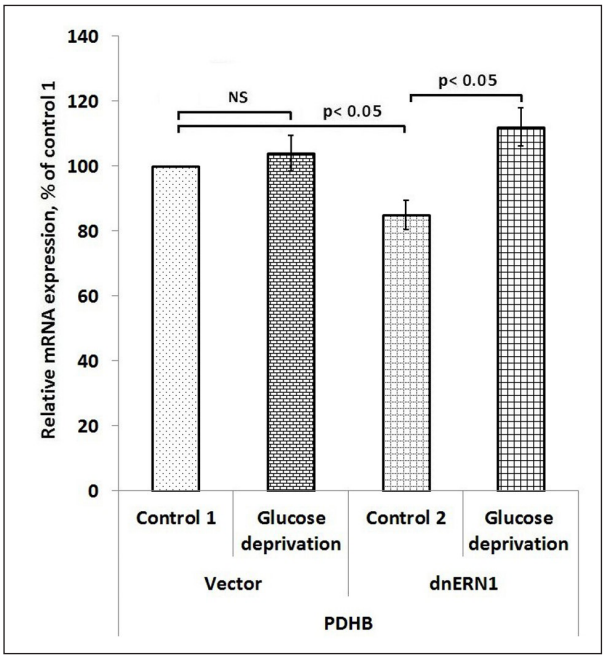

Figure 7. The impact of glucose deprivation on the expression level of pyruvate dehydrogenase E1 beta subunit (*PDHB*) gene in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of PDHB mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

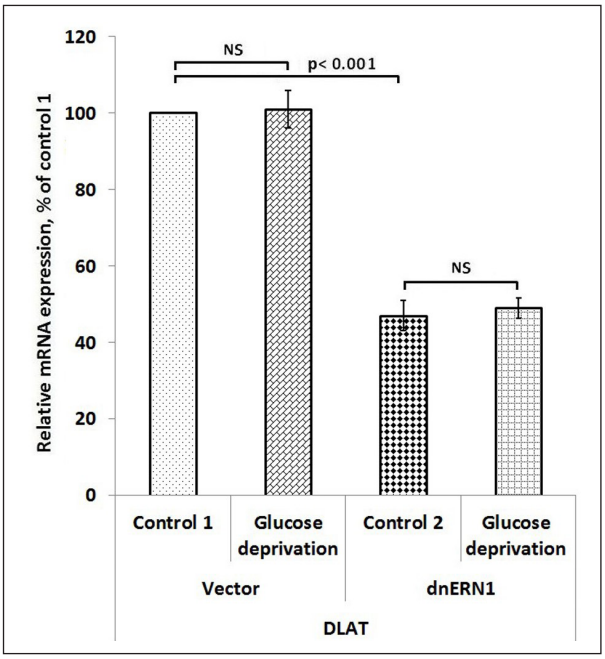

Figure 8. The impact of glucose deprivation on the expression level of dihydrolipoamide S-acetyltransferase (*DLAT*) gene, encoding E2 component of pyruvate dehydrogenase complex in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of DLAT mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

genes encoding important enzymes of PDH complex (Babady et al. 2007; Mathias et al. 2014; Eastlack et al. 2018; Yonashiro et al. 2018; Jin et al. 2016; Yetkin-Arik et al. 2019; Cai et al. 2020; Shin et al. 2020; Inoue et al. 2021; Zaher et al. 2021).

We showed that glutamine deprivation leads to significant down-regulation of the expression of *PDHA1*, *PDHB*, *DLAT*, and *DLD* genes in control glioma cells and that ERN1 knock-down decreases the impact of glutamine deprivation condition on *PDHB* and *DLD* gene expressions or introduces the tolerance of *PDHA1* and *DLAT* gene expressions to glutamine starvation (Figure 11). These results clearly demonstrated that the expression of *PDHA1*, *PDHB*, *DLAT*, and *DLD* genes is controlled by glutamine supply and that this control is ER stress dependent particularly through ERN1 signaling. This is in accordance with the data our previous studies (Minchenko et al. 2013, 2020, 2021; Tsymbal et al. 2016; Krasnytska et al. 2022) as well as studies of other authors (Drogat et al. 2007). The changes in *PDHA1* and *PDHB* genes expression under glutamine deprivation are in agreement with data of Yetkin-Arik et al. (2019) indicating that silencing of PDHA1 expression prevents the proliferation of HUVECs, because this enzyme of PDH complex is a crucial regulator of pyruvate metabolic pathways (Zaher et al. 2021).

We also showed that PDHX is resistant to glutamine deprivation and these results also agree well with its essential role in the functional activity of a PDH complex (Eastlack et al. 2018). Recently, it was shown that PDHX is a metabolically essential gene for the cell growth because its expression is required for the maintenance of PDH activity and the production of ATP and its knock-down inhibited the proliferation of cancer cells and *in vivo* the tumor growth (Inoue et al. 2021).

Results of this study also demonstrate that the exposure of control glioma cells under glucose deprivation condition does not affect the expression level of all studied genes of PDH complex, but inhibition of ERN1 signaling significantly modifies the sensitivity of most of these genes to glucose deprivation. It is possible that this tolerance to glucose starvation is mediated by ER stress particularly through ERN1 signaling (Awale et al. 2006; Kim

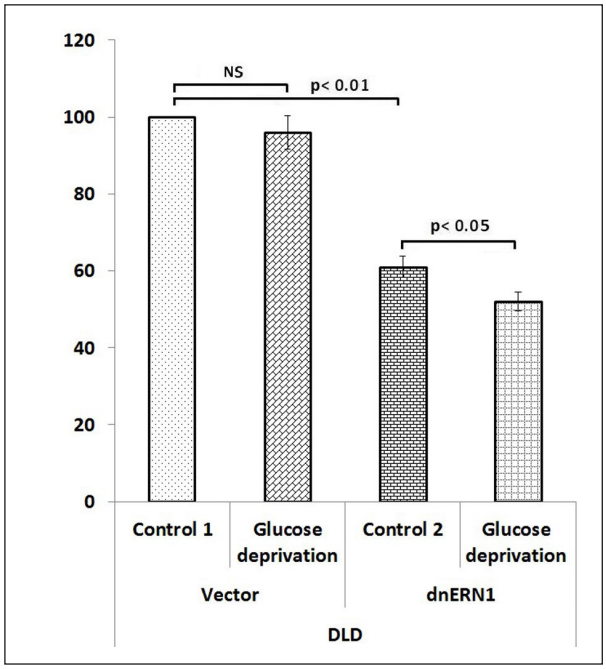

Figure 9. The impact of glucose deprivation on the expression level of dihydrolipoamide dehydrogenase (*DLD*) gene, encoding E3 component of pyruvate dehydrogenase complex in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of DLD mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

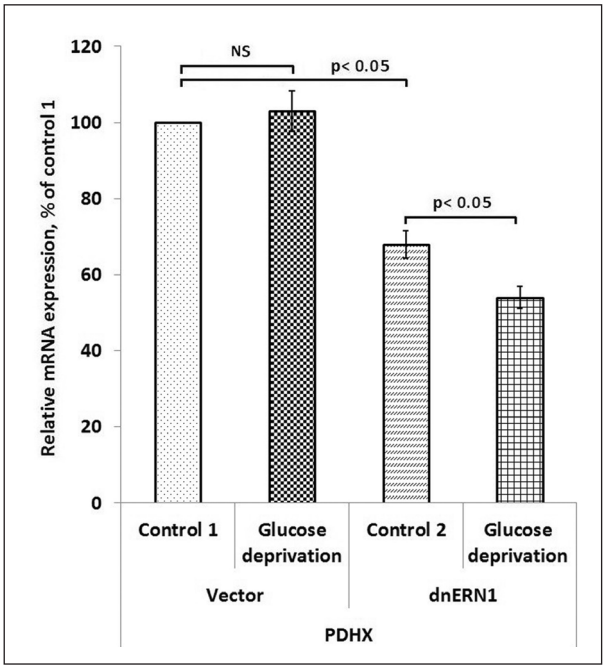

Figure 10. The impact of glucose deprivation on the expression level of pyruvate dehydrogenase complex component X (*PDHX*) gene in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 (dnERN1) measured by qPCR. Values of PDHX mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

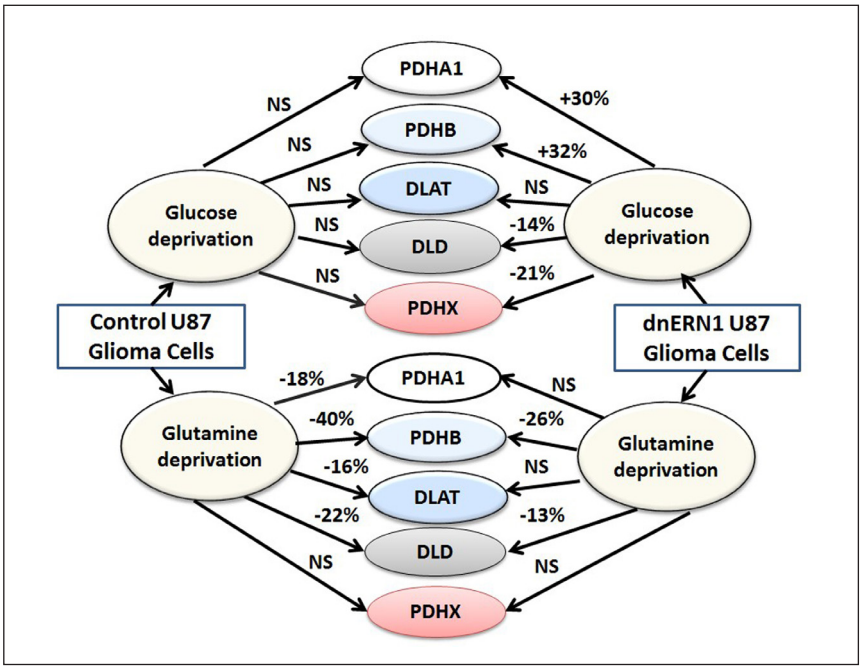

Figure 11. Schematic demonstration of changes in the expression profile of *PDHA1*, *PDHB*, *DLAT*, *DLD*, and *PDHX* genes in the control and ERN1 knock-down U87 glioma cells under glucose and glutamine deprivations.

et al. 2010; He et al. 2018). This also well agrees with our data indicating that the knock-down of ERN1 has the ability to eliminate the tolerance of *PDHA1*, *PDHB*, *DLD*, and *PDHX* genes in glioma cells to glucose starvation. It is possible that the up-regulation of *PDHA1* and *PDHB* genes expression under glucose deprivation in ERN1 knock-down glioma cells reflects a compensatory mechanism connected with decreased proliferation rate of glioma cells under inhibition of ERN1 and glucose starvation (Awale et al. 2006; Kim et al. 2010; Colombo et al. 2011; He et al. 2018).

Conclusion

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding enzymes of PDH complex in glioma cells in response to glutamine and glucose deprivations and their correlation with inhibition of ERN1 signaling and reduced cell proliferation in cells harboring dnERN1, attesting to the fact that ER stress as well as glutamine and glucose supply are necessary components of the glioma growth and cell survival.

Our results validate the interaction of ER stress signaling pathway ERN1 with glutamine and glucose deprivations in gene-specific manner. However, the detailed molecular mechanisms of this regulation have not been yet clearly defined and warrant further investigation.

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Conflict of Interest: *The authors declare no conflict of interest.*

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