EXPERIMENTAL ARTICLES

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GLUTAMINE DEPRIVATION EFFECT ON *DEK*, *TPD52*, *BRCA1*, *ADGRE5*, *LIF*, *GNPDA1*, AND *COL6A1* GENE EXPRESSIONS IN IRE1 KNOCKDOWN U87 GLIOMA CELLS

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To study effect of glutamine deprivation on the expression of genes encoding the key proliferation associated factors on a relation to inhibition of inositol requiring enzyme-1 IRE1 in U87 glioma cells was the aim of the research. It was shown that glutamine deprivation down-regulated the expression of *DEK*, *BRCA1*, *LIF*, and *COL6A1* genes in control glioma cells (transfected by empty vector), up-regulated *ADGRE5* gene expression, and did not significantly change the expression of *TPD52* and *GNPDA1*. Inhibition of IRE1 signaling enzyme activity modified the effect of glutamine deprivation on the expression of *TPD52*, *BRCA1*, *LIF*, *DEK*, *ADGRE5*, and *COL6A1* genes: introduces the effect of glutamine deprivation on *TPD52* and *GNPDA1*, reduced — on *COL6A1*, and enhanced — on *ADGRE5*, *DEK*, and *BRCA1* in U87 glioma cells. Therefore, glutamine deprivation affect the expression level of most studied genes in U87 glioma cells in relation to the functional activity of IRE1 signaling enzyme, which is responsible for control of cell proliferation and glioma growth.

Key words: mRNA expression, *DEK*, *BRCA1*, *COL6A1*, *ADGRE5*, *GNPDA1* genes, glutamine deprivation, IRE1 knockdown, U87 glioma cells.

Glioblastoma multiforme is the most hostile type of brain cancer, which is highly aggressive tumor with extremely poor prognosis and to date there is no efficient treatment available [1–3]. Its aggressiveness is due to increased invasion, migration, proliferation, angiogenesis, and a decreased apoptosis [4]. The endoplasmic reticulum stress is an important component of tumor growth, including glioblastoma multiforme [5–8]. IRE1 (inositol requiring enzyme-1) signaling pathway of endoplasmic reticulum stress is a central mediator of the unfolded protein response and inhibition of this signaling pathway leads to a suppression of glioma growth through down-regulation of angiogenesis and proliferation processes as a result of metabolic reprogramming of cancer cells [5, 9–12]. The endoplasmic reticulum stress controls the expression of numerous regulatory and proliferation related genes, which are responsible for glioma growth [9, 13–16]. Glutamine is an important factor of glioma development and a more agressive behaviour [17, 18]. Moreover, tumor cells have high-energetic and anabolic needs and are known to adapt their metabolism to be able to survive and keep proliferating under conditions of nutrient stress. It is interesting to note that PRKCE (protein kinase C epsilon) is a critical metabolic tumor suppressor and its deficiency promotes the plasticity necessary for cancer cells to reprogram their metabolism to utilize glutamine through the serine

biosynthetic pathway in the absence of glucose [19]. Polet et al. [20] found that glutamine deprivation inhibited leukemia cell growth but also led to a glucose-independent adaptation maintaining cell survival through the up-regulation of phosphoglycerate dehydrogenase (PHGDH) and phosphoserine aminotransferase (PSAT1), two enzymes of the serine pathway. Moreover, serine is a key pro-survival actor that needs to be handled to sensitize leukemia cells to glutamine-targeting modalities, because it contributed to cell regrowth following glutamine deprivation [20]. A better knowledge of tumor responses to glutamine deprivation condition is required to elaborate therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms. Therefore, glutamine deprivation affected the expression of numerous tumor growth related genes and the effect of glutamine deprivation on most of these genes expression is dependent on IRE1signaling enzyme function [21–25].

Numerous genes have already been correlated with glioblastoma multiforme growth, but mechanisms of the regulation of most of these genes by IRE1 signaling pathway and glutamine deprivation not to be clarified yet. Among them tumor protein D52 (*TPD52*), breast cancer 1, early onset (*BRCA1*), adhesion G protein-coupled receptor E5 (*ADGRE5*), leukemia inhibitory factor (*LIF*), DEK oncogene (*DEK*), glucosamine-6-phosphate deaminase 1 (*GNPDA1*), and collagen, type VI, alpha 1 (*COL6A1),* which encode important proteins for control of tumor growth [26–30]. Expression of *ADGRE5* gene may play a role in the progression of several types of cancer [27, 31]. BRCA1 is a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumor suppressor [28]. A multifunctional protein, TPD52, preferentially inhibits proliferation and migration/invasion in renal carcinoma cells, at least in part, through the PI3K/Akt signaling pathway [29, 32]. Recently was shown that a transcription factor B-Myb is overexpressed and plays an oncogenic role in several types of human cancers and that its overexpression causes up-regulation of various downstream genes, including *COL6A1,* involved in cell proliferation, tumorigenesis, and metastasis [30, 33]. The leukemia inhibitory factor is a multifunctional highly glycosylated protein, which downregulates tumor suppressor p53 protein level and function in human colorectal cancer cells [34, 35]. LIF is frequently overexpressed in many types of human tumors and promotes the progression and metastasis of tumors [36]. It induces the expression of microRNA-21, which in turn mediates the promoting effect of LIF on epithelial-mesenchymal transition. Furthermore, blocking miR-21 function greatly abolished the promoting effect of LIF on epithelial-mesenchymal transition and the migration ability of cancer cells [36].

The aim of this study was investigation the effect of glutamine deprivation on the expression of *DEK, BRCA1, ADGRE5, LIF, GNPDA1,* and *COL6A1*, and *TPD52* genes in glioma cells on a relation to inhibition of IRE1, a major signaling enzyme of endoplasmic reticulum stress, with hopes of elucidating its mechanistic part in the development and progression of glioma trough endoplasmic reticulum stress.

Materials and Methods

Cell Lines and Culture Conditions. In this study we used two sublines of U87 glioma cells (U87 MG, ATCC HTB-14), which are growing at 37 °C in high glutamine (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (DMEM; Gibco, Invitrogen, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), streptomycin (0.1 mg/ml; Gibco) and penicillin (100 units/ml; Gibco) in a 5% CO_2 incubator. One subline was obtained by selection of stable transfected clones with overexpression of empty vector (pcDNA3.1), which was used for creation of dominant-negative constructs of IRE1 (dnIRE1). This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of effects of glutamine deprivation on the expression level of DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1, and COL6A1 mRNAs. Other subline of U87 glioma cells was obtained by selection of stable transfected clones with overexpression of dnIRE1 and has suppressed both protein kinase and endoribonuclease activities of IRE1, signaling enzyme of endoplasmic reticulum stress. The expression level of all studied genes in these cells was compared with cells, transfected by vector (control 1). The subline, which overexpress dnIRE1, was also used as control 2 for investigation of the effect of glutamine deprivation condition on the expression level of studied genes in cells with inhibited function of IRE1 signaling enzyme. For selection of these clones geneticin (G418, 0.8 mg/ml) was used. Glutamine deprivation condition were created by changing the complete DMEM medium into culture plates

on the medium without glutamine (from Gibco) and plates were exposed to this condition for 16 h.

The suppression level of IRE1 both enzymatic activity in glioma cells that overexpress a dominant-negative construct of inositol requiring enzyme-1 was estimated previously [9, 15] by determining the expression level of XBP1 alternative splice variant (XBP1s), a key transcription factor in IRE1 signaling, using cells treated by tunicamycin (0.01 mg/ml during 2 hrs). Efficiency of XBP1s inhibition was 95%. Moreover, the proliferation rate of glioma cells with mutated IRE1 is decreased more than in 2 fold [15]. Thus, the blockade of both kinase and endoribonuclease activity of signaling enzyme IRE1 has significant effect on proliferation rate of glioma cells.

RNA isolation. Total RNA was extracted from glioma cells as previously described [37]. The RNA pellets were 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 as well as spectral characteristics was measured using NanoDrop Spectrophotometer.

Reverse transcription and quantitative PCR analysis. QuaniTect Reverse Transcription Kit (QIAGEN, Hilden, Germany) was used for cDNA synthesis as described previously [38]. The expression level of DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1, COL6A1, and ACTB mRNAs were measured in glioma cell line U87 and its subline (clone 1C5) by real-time quantitative polymerase chain reaction using "QuantStudio 5 Real-Time PCR System" (Applied Biosystems) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, AB gene House, Epsom, Surrey, UK). Polymerase chain reaction was performed in triplicate.

For amplification of the adhesion G protein-coupled receptor E5 (ADGRE5), also known as CD97 antigen, cDNA we used next primers: forward 5′– CCTCAGAACTCCTCG-TGTGT –3′ and reverse 5′– TGTTCCAGCAGT-CCGAGAAT –3′. The nucleotide sequences of these primers correspond to sequences 471–490 and 633–614 of human ADGRE5 cDNA (GenBank accession number NM_001784). The size of amplified fragment is 160 bp.

The amplification of cDNA of the leukemia inhibitory factor (LIF) was performed using forward primer (5′– CCCTGGTCCCTACTC-AACAA –3′) and reverse primer (5′– CTGGACCCTGACACCCTAAA –3′). These oligonucleotides correspond to sequences 1850–1869 and 2083–2064 of human LIF cDNA (GenBank accession number NM_002309). The size of amplified fragment is 234 bp.

For amplification of the DEK oncogene (DEK) cDNA we used next primers: forward 5′– GGTCAGTTCAGTGGCTTTCC –3′ and reverse 5′– CCTTGCCATTCCAGAACTGT –3′. The nucleotide sequences of these primers correspond to sequences 575–594 and 838–819 of human DEK cDNA (GenBank accession number NM_003472). The size of amplified fragment is 264 bp.

The amplification of cDNA of the breast cancer 1, early onset (BRCA1) was performed using forward primer (5′– TGAAGAAAG-AGGAACGGGCT –3′) and reverse primer (5′– TGGCTCCCATGCTGTTCTAA –3′). These oligonucleotides correspond to sequences 4264–4283 and 4503–4484 of human BRCA1 cDNA (GenBank accession number NM_007294). The size of amplified fragment is 240 bp.

For amplification of the tumor protein 52 (TPD52) cDNA we used next primers: forward 5′– CACAGAGACCCTCTCGGAAG –3′ and reverse 5′– CCCTTTGGCAATGTTCTGTT –3′. The nucleotide sequences of these primers correspond to sequences 89–108 and 266–247 of human TPD52 cDNA (GenBank accession number NM_005079). The size of amplified fragment is 178 bp.

The amplification of cDNA for the glucosamine-6-phosphate deaminase 1 (GNPDA1) was performed using forward primer (5′– TTAACCCAGGGCCAGAGAAG –3′) and reverse primer (5′– TGGGTGTTTTCT-GGGTGGAT –3′). These oligonucleotides correspond to sequences 147–166 and 381–362 of human GNPDA1 cDNA (GenBank accession number NM_005471). The size of amplified fragment is 235 bp.

For amplification of the collagen, type VI, alpha 1 (COL6A1) cDNA we used next primers: forward 5′– CTGGGCGTCAAAGTCTTCTC –3′ and reverse 5′– ATTCGAAGGAGCAGCACACT –3′. The nucleotide sequences of these primers correspond to sequences 646 — 665 and 856 — 837 of human CTSF cDNA (GenBank accession number NM_001848). The size of amplified fragment is 211 bp.

The amplification of the beta-actin (ACTB) cDNA was performed using forward — 5′– GGACTTCGAGCAAGAGATGG –3′ and reverse — 5′– AGCACTGTGTTGGCGTACAG –3′primers. These primers nucleotide

sequences correspond to 747–766 and 980–961 of human ACTB cDNA (GenBank accession number NM_001101). The size of amplified fragment is 234 bp. The expression of beta-actin mRNA was used as control of analyzed RNA quantity.

The primers were received from Sigma-Aldrich (St. Louis, MO, USA). The quality of amplification products was analyzed by melting curves and by electrophoresis using 2% agarose gel. An analysis of quantitative PCR was performed using special computer program "Differential Expression Calculator". The values of DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1, and COL6A1 mRNA expressions were normalized to the expression of beta-actin mRNA and represented as percent of control 1 (100%).

Statistical analysis. All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments. Statistical analysis was performed according to Student's *t*-test using Excel program as described previously [38].

Results and Discussion

To determine if glutamine deprivation affects the expression of genes of interest through the IRE1 branch of endoplasmic reticulum stress response, we investigated the effect of glutamine deprivation condition on the expression of genes, encoding DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1, and COL6A1, in U87 glioma cells in relation to knockdown of IRE1 signaling enzyme, which is a major component of the unfolded protein response/endoplasmic reticulum stress. As shown in Fig. 1, the exposure of control glioma cells (transfected by empty vector) upon glutamine deprivation condition leads to up-regulation of ADGRE5 mRNA expression (+18%) as compared to cells growing with glutamine, but in cells with IRE1 knockdown the level of this gene expression is more significantly increased upon glutamine deprivation condition (+77%). Furthermore, inhibition of IRE1 signaling down-regulates the expression level of *ADGRE5* gene in glioma cells growing with glutamine (Fig. 1). Next, we have shown that glutaminede privation-significantly down-regulates the expression level of BRCA1 mRNA in control glioma cells (–48%), but in cells without functional activity of IRE1 signaling enzyme effect of the absence of glutamine was much stronger (–57%; Fig. 2). Thus, IRE1 knockdown enhances the effect of glutamine deprivation on the expression of *BRCA1* gene in glioma cells. However, in glioma cells growing with glutamine the inhibition of IRE1 signaling leads to strong up-regulation of this gene expression (Fig. 2).

As shown in Fig. 3 and 4, glutamine deprivation significantly down-regulates the expression level of COL6A1 and DEK mRNAs in control glioma cells (–39 and –24%, correspondingly), but inhibition of IRE1 signaling enzyme function modified the effect of the absence of glutamine on these gene expressions in U87 glioma cells: decreased effect of glutamine deprivation on *COL6A1* expression (–15%) and increased — on the expression level of *DEK* gene (–33%) in glioma cells. We have also shown that inhibition of IRE1 signaling leads to down-regulation of both *COL6A1* and *DEK* gene expressions in glioma cells growing with glutamine, but effect IRE1 knockdown was significantly stronger on *COL6A1* gene: –73% for *COL6A1* gene and –18% for *DEK* gene (Fig. 3 and 4).

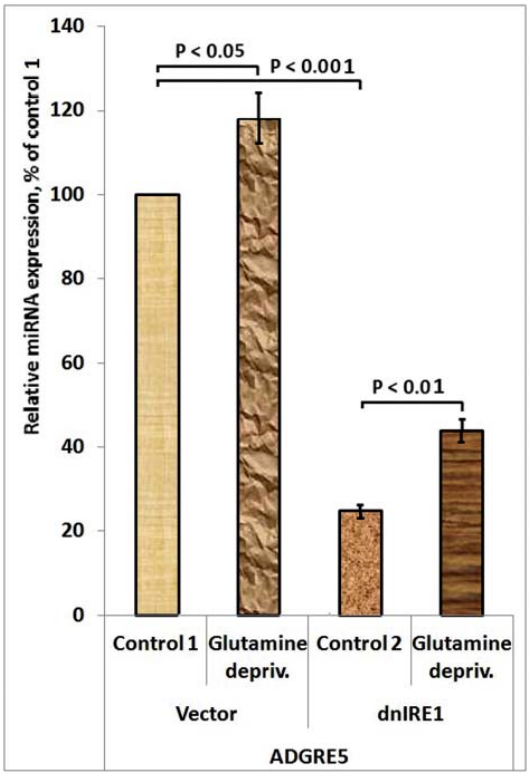

Fig. 1. **Effect of glutamine deprivations on the expression of mRNA level of adhesion G protein-coupled receptor E5 (ADGRE5), also known as CD97 antigen, in control U87 glioma cells stable transfected with vector (Vector) and cells with IRE1 knockdown (dnIRE1) measured by qPCR**

Hereinafter: values of this mRNA expressions were normalized to beta-actin mRNA and represented as percent of control 1 (100%); mean ± SEM

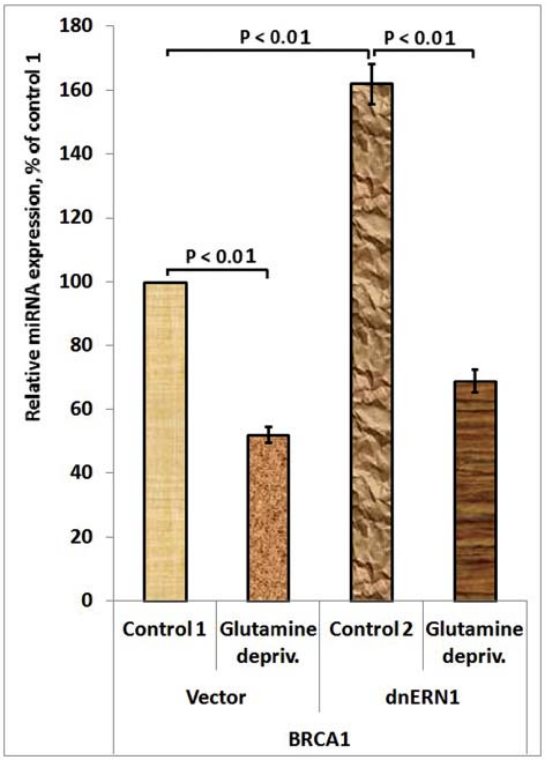

Fig. 2. **Effect of glutamine deprivation on the expression level of breast cancer 1, early onset (BRCA1) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

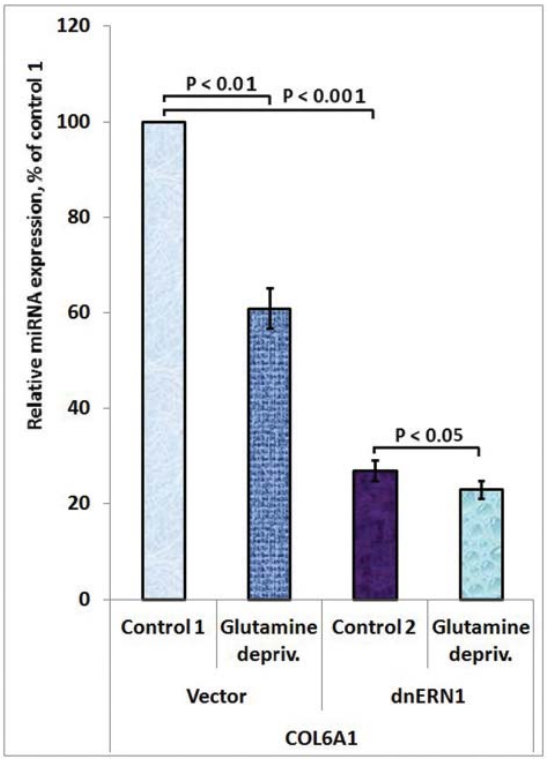

Fig. 3. **Relative effect of glutamine deprivation on the expression level of collagen, type VI, alpha 1 (COL6A1) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

At the same time, the expression level of *GNPDA1* gene does not change significantly upon glutamine deprivation in control glioma cells, but IRE1 knockdown introduces the sensitivity of this gene expression to glutamine deprivation (Fig. 5). Thus, in glioma cells without functional activity of signaling enzyme IRE1 the expression of *GNPDA1* gene is increased (+18%). Furthermore, in glioma cells growing with glutamine the expression of this gene was strongly suppressed (–56%) upon inhibition of IRE1 signaling enzyme function (Fig. 5). Results, presented in Fig. 6, demonstrate that exposure of control glioma cells upon the absence of glutamine leads to small but statistically significant down-regulation of leukemia inhibitory factor mRNA expression (–15%) as compared to cells growing with glutamine. At the same time, the expression of this gene was resistant to glutamine deprivation in cells with knockdown of IRE1 signaling enzyme (Fig. 6). Investigation of the level of leukemia inhibitory factor mRNA expression in glioma cells growing with glutamine demonstrated that inhibition of IRE1 signaling leads to strong suppression (in 9 fold) of this gene expression (Fig. 6). As shown in Fig. 7, the expression of gene encoding for TPD52 is resistant to glutamine deprivation condition in control U87 glioma cells, but inhibition of IRE1 signaling enzyme function by dnIRE1 introduces the sensitivity of this gene expression to glutamine deprivation (–54%). Thus, IRE1 knockdown significantly modifies the effect of glutamine deprivation on the expression of *TPD52* gene in U87 glioma cells. Additionally, we found that the expression of this gene is strongly up-regulated (+135%) in glioma cells with functional IRE1 when growing with glutamine (Fig. 7).

Therefore, this study has demonstrated that IRE1 knockdown affects the expression of all studied genes in glioma cells growing with glutamine: up-regulates the expression of genes encoding factors with antitumor properties — BRCA1 and TPD52 [28, 29, 32] and down-regulates all other studied genes,

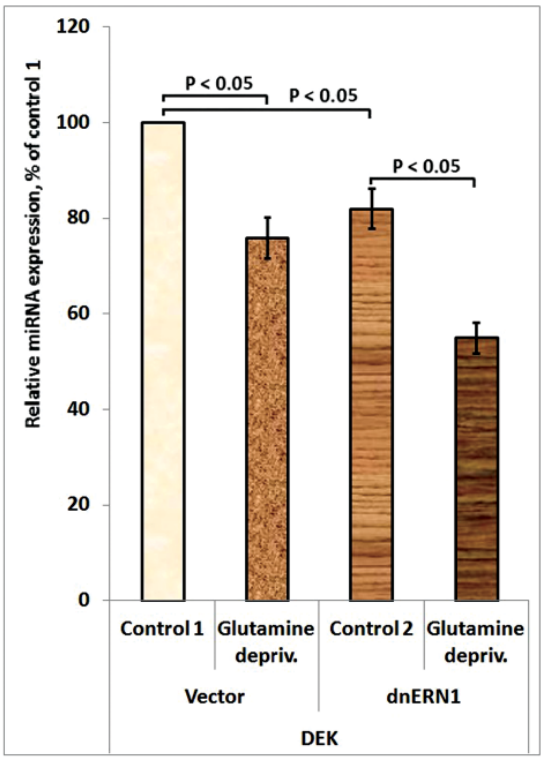

Fig. 4. **Relative effect of glutamine deprivation on the expression level of DEK oncogene (DEK) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

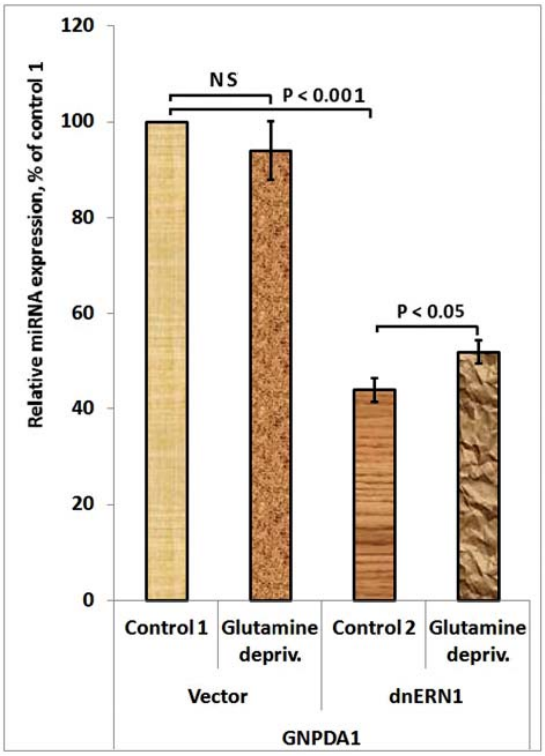

Fig. 5. **Effect of glutamine deprivation on the expression level of glucosamine-6-phosphate deaminase 1 (GNPDA1) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**
Hereinafter: NS — no significant changes.

which involved in cell proliferation, tumorigenesis, and metastasis [26, 27, 30, 31, 33–36]. Thus, our results of this investigation correlate well with numerous results of other scientists as well as with the important role of IRE1 signaling in tumor growth [10, 12, 14, 39]. The endoplasmic reticulum stress is responsible for enhanced cancer cell proliferation and knockdown of IRE1, a major signaling pathway of endoplasmic reticulum stress, resulted in a significant anti-proliferative effect on glioma cell proliferation and tumor growth [9, 11, 13, 40].

We have also shown that glutamine deprivation affects the expression of the majority of the studied genes encoding important proliferation related factors preferentially in the IRE1-dependent manner and that these changes potentially contribute to suppression of glioma cell proliferation upon glutamine deprivation [20, 41]. Moreover, glutamine deprivation or inhibition of mitochondrial aspartate transaminase (GOT2) results in a profound induction of senescence and a suppression of pancreatic ductal adenocarcinoma cells growth [42]. Thus, we have shown that the expression level of pro-proliferative *COL6A1*, *LIF*, and *DEK* genes is down-regulated in control glioma cells in the absence of glutamine and this data correlate well with an important role of glutamine in the malignant progression of cancer cells [19, 40, 42, 43]. At the same time, glutamine deprivation leads to up-regulation of anti-proliferative *ADGRE5* gene in control glioma cells and stronger up-regulation of this gene expression in cells with knockdown of IRE1 signaling enzyme and possibly contributes to suppression of proliferation of these glioma cells [9, 10, 14, 15].

In conclusion, our results demonstrate that the majority of the genes studied are stress responsive through IRE1 signaling pathway and sensitive to glutamine deprivation in IRE1 dependent manner; thus, potentially contribute to regulation of cell proliferation and metastasis through various signaling pathways and stress related transcription, but the mechanisms and

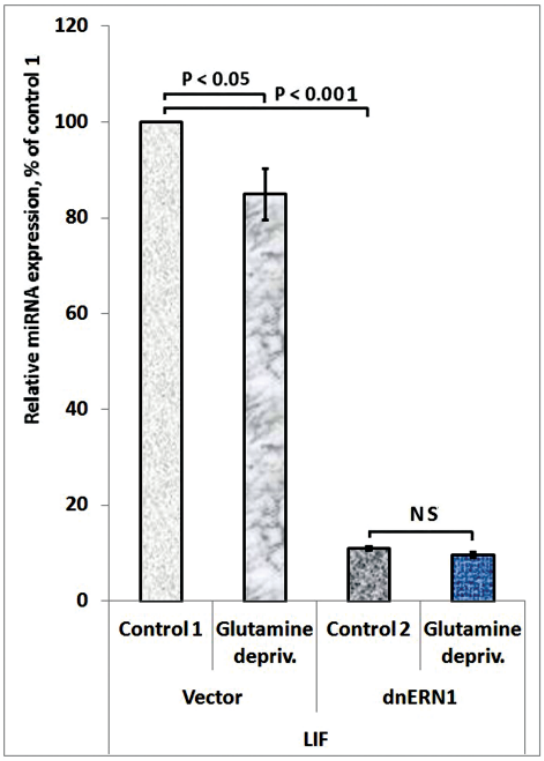

Fig. 6. **Effect of glutamine deprivation on the expression level of leukemia inhibitory factor (LIF) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

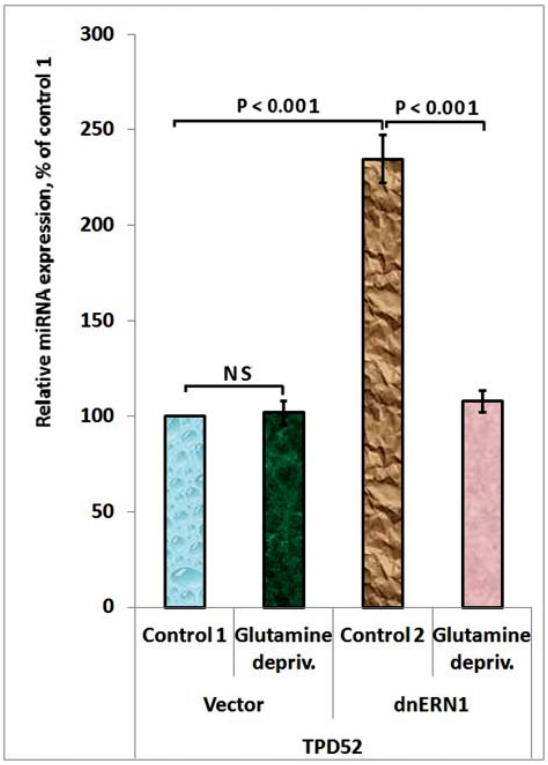

Fig. 7. **Effect of glutamine deprivation on the expression level of tumor protein D52 (TPD52) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

functional significance of suppression or activation of their expression through IRE1 inhibition as well as glutamine deprivation are different and warrant further investigation. Thus, the changes observed in the studied genes expression preferentially agree with slower proliferation rate of glioma cells harboring dnIRE1, attesting to the fact that targeting the unfolded protein response is viable, perspective approach in the development of cancer therapeutics [10, 14, 40].

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ВПЛИВ ДЕФІЦИТУ ГЛУТАМІНУ НА ЕКСПРЕСІЮ ГЕНІВ *DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1* ТА *COL6A1* У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87 З НОКАУТНИМ IRE1

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Метою роботи було вивчити вплив дефіциту глутаміну на експресію декількох генів, що кодують ключові фактори регуляції проліферації, у клітинах гліоми лінії U87 за умов пригнічення inositol requiring enzyme-1 IRE1. Показано, що відсутність глутаміну знижувала експресію генів *DEK, BRCA1, LIF* та *COL6A1* у контрольних (трансфікованих пустим вектором) клітинах гліоми, збільшувала експресію *ADGRE5*, але істотно не змінювала експресію *TPD52* та *GNPDA1*. Пригнічення функції сигнального ензиму IRE1 у клітинах гліоми лінії U87 змінювало ефект дефіциту глутаміну на експресію генів *TPD52, BRCA1, LIF, DEK, ADGRE5* та *COL6A1*: індукувало ефект дефіциту глутаміну на *TPD52* та *GNPDA1*, зменшувало — на *COL6A1* і посилювало — на *ADGRE5, DEK* та *BRCA1*. Таким чином, дефіцит глутаміну змінював рівень експресії більшості досліджених генів у клітинах гліоми лінії U87 залежно від функціональної активності сигнального ензиму IRE1, який відповідає за контроль проліферації клітин та росту гліом.

Ключові слова: експресія мРНК, гени *DEK, BRCA1, COL6A1, ADGRE5, GNPDA1*, дефіцит глутаміну, пригнічення IRE1, клітини гліоми лінії U87.

ВЛИЯНИЕ ДЕФИЦИТА ГЛУТАМІНА НА ЭКСПРЕССИЮ ГЕНОВ *DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1* И *COL6A1* В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87 С НОКАУТНЫМ IRE1

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Целью работы было изучить влияние дефицита глутамина на экспрессию нескольких генов, кодирующих ключевые факторы регуляции пролиферации в клетках глиомы линии U87 при угнетении inositol requiring enzyme-1 IRE1. Показано, что отсутствие глутамина снижало экспрессию генов *DEK, BRCA1, LIF* и *COL6A1* в контрольных (трансфецированных пустым вектором) клетках глиомы, увеличивало экспрессию *ADGRE5*, но существенно не изменяло экспрессию *TPD52* и *GNPDA1*. Угнетение функции сигнального энзима IRE1 в клетках глиомы линии U87 изменяло эффект дефицита глутамина на экспрессию генов *TPD52, BRCA1, LIF, DEK, ADGRE5* и *COL6A1*: индуцировало эффект дефицита глутамина на гены *TPD52* и *GNPDA1*, уменьшало — на *COL6A1* и усиливало — на *ADGRE5, DEK* и *BRCA1*. Таким образом, дефицит глутамина изменял уровень экспрессии большинства изученных генов в клетках глиомы линии U87 в зависимости от функциональной активности сигнального энзима IRE1, который отвечает за контроль пролиферации клеток и роста глиом.

Ключевые слова: экспрессия мРНК, гены *DEK, BRCA1, COL6A1, ADGRE5, GNPDA1*, дефицит глутамина, угнетение IRE1, клетки глиомы линии U87.