EXPERIMENTAL ARTICLES

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IRE1 KNOCKDOWN MODIFIES GLUCOSE AND GLUTAMINE DEPRIVATION EFFECTS ON THE EXPRESSION OF PROLIFERATION RELATED GENES IN U87 GLIOMA CELLS

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We have studied the expression of genes encoding proliferation related factors and enzymes such as *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* in U87 glioma cells upon glucose and glutamine deprivation in relation to inhibition of inositol requiring enzyme 1, a central mediator of endoplasmic reticulum stress. It was shown that glutamine deprivation leads to up-regulation of the expression of *BET1*, *MYL9*, and *MTIF2* genes and down-regulation of *CD24*, *ING2*, *ENDOG*, *POLG*, and *TSFM* genes in control (with native IRE1) glioma cells. At the same time, glucose deprivation enhances the expression of *MYL9* gene only and decreases – *ING1*, *ING2*, and *MTIF2* genes in control glioma cells. Thus, effect of glucose and glutamine deprivation on gene expressions in glioma cells is gene-specific. Inhibition of inositol requiring enzyme 1 by dnIRE1 significantly modifies the effect of both glutamine and glucose deprivation on the expression of most studied genes with different direction and magnitude, especially for *ING2*, *CD24*, and *MTIF2* genes. Present study demonstrates that IRE1 knockdown modifies glucose and glutamine deprivation effects on the expression of proliferation related genes and possibly contributes to slower tumor growth of these glioma cells after inhibition of IRE1 signaling enzyme.

Key words: proliferation related genes expression, IRE1 inhibition, glucose and glutamine deprivation, glioma cells.

Plentiful studies have proven the connection between endoplasmic reticulum stress and malignant growth [1–4]. Neoplasms use signaling pathways of endoplasmic reticulum stress response to adapt and to enhance tumor cells proliferation under unfavorable environmental conditions [5–7]. It is well known that activation of IRE1/ERN1 (inositol requiring enzyme 1/endoplasmic reticulum to nucleus signaling 1) branch of the endoplasmic reticulum stress response under certain circumstances may lead to apoptosis and to cell death, and suppression of its function significantly inhibits glioma growth [8–11]. Glucose and glutamine are substrates for glycolysis and glutaminolysis. These key metabolic pathways participate in cell cycle control at multiple points and thus are of importance for tumor progression [12, 13]. The activation of glycolysis and glutaminolysis in cancer cells depends on two ubiquitin ligases, which control the transient appearance and metabolic activity of the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) and glutaminase 1 (GLS1), the first enzyme of glutaminolysis pathway [12].

Gliomas constitute one of the most aggressive groups of malignant neoplasms with poor survival prognosis and scarce therapeutic options. Aberrant vascularization, common in expanding solid tumors, results in deficiency of oxygen and nutrients. Glucose shortage associated with malignant progression triggers apoptosis through the endoplasmic reticulum unfolded protein response [14].

To some extend, a reduced glucose flux may be causative to endoplasmic reticulum stress [14]. Thus, a better understanding of effects of glucose and glutamine deprivations on genes expression especially in relation to endoplasmic reticulum stress is required to elaborate therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms [4, 14–18].

The endoplasmic reticulum is a key cellular compartment, extremely sensitive to perturbations of its homeostasis. Such perturbations, which affect the ER folding capacity, activate a set of signaling pathways, named the unfolded protein response. The latter plays an important role in metabolic integration [5, 6, 19]. In mammals this adaptive response is mediated by three membrane-spanning sensory proteins, among which IRE1 is the most evolutionary conversed and an indispensable [1, 6, 7, 11]. The IRE1 enzyme possesses two enzymatic activities: protein kinase and endoribonuclease. Recently it was demonstrated, that protein kinase of IRE1 is responsible not only for autophosphorylation but also indirectly controls expression of certain genes through phosphorylation of heterologous substrates [7, 20]. The IRE1 endoribonuclease performs a unique cytosolic splicing of the pre-XBP1 (X-box binding protein 1) mRNA whose mature transcript encodes a transcription factor, which stimulates the expression of numerous unfolded protein response specific genes, and also is involved in the degradation of a specific subset of mRNA and miRNA [21–26].

Tumor growth is tightly associated with the endoplasmic reticulum stress response signaling pathways, which are linked to the cell proliferation and death processes [2, 3, 8]. It is well known that *IL13RA2* (interleukin 13 receptor, alpha 2), *CD24* (signal transducer CD24 molecule), *KRT18* (keratin 18), *ING1* (inhibitor of growth family, member 1), *ING2*, *ENDOG* (endonuclease G) and many other genes play an important role in the regulation of numerous metabolic and proliferative processes as well as control of tumorigenesis [27–31]. The *IL13RA2* gene is often overexpressed in glioma and other tumors [27]. CD24 encodes a sialoglycoprotein that modulates growth and differentiation signals [29]. The *ING1* and *ING2* genes encode tumor suppressor proteins that can induce cell growth arrest and apoptosis [30, 32]. Furthermore, the ING1 is a nuclear protein that physically interacts with the tumor suppressor protein TP53 and is a component of its signaling pathway. Reduced expression and rearrangement of this gene have been detected in various cancers [33, 34]. Moreover, ING1 protein stabilizes TP53 by inhibiting its polyubiquitination [35].

Recently was shown that KRT18 (keratin 18) also contributes to decreased malignancy of non-small cell lung carcinoma and is directly regulated by EGR1 (early growth response 1) [36]. There is data that the decreased expression of MYL9 (myosin, light chain 9, regulatory) may play an important role in tumor progression of prostate cancer [31]. It is possible that membrane trafficking proteins BET1 (Bet1 Golgi vesicular membrane trafficking protein) and BET3/TRAPPC3 (trafficking protein particle complex 3), which participate in vesicular transport from the endoplasmic reticulum to the Golgi complex, also involved in the endoplasmic reticulum stress responsible transport of unfolded proteins and tumorigenesis [37]. The *ENDOG, POLG* (DNA directed polymerase gamma), *TSFM* (Ts mitochondrial translational elongation factor), and *MTIF2* (mitochondrial translational initiation factor 2) genes encode mitochondrial proteins, which are related to the control of mitochondrial genome function as well as to cell proliferation [38–42]. Moreover, ENDOG regulates an integral network of apoptotic endonucleases, which appear to act simultaneously before and after cell death by destroying the host cell DNA [38, 43].

The aim of this study was to investigate the effects of glucose and glutamine deprivation on the expression of a subset of genes encoding factors and enzymes (*IL13RA2, KRT18, CD24, ING1, ING2, MYL9, BET1, BET3/TRAPPC3, ENDOG, POLG, TSFM*, and *MTIF2*), which participate in the regulation of cell proliferation and apoptosis, in U87 glioma cell line and its subline with IRE1 loss of function for evaluation of possible significance of these genes in the control of tumor growth through IRE1-mediated endoplasmic reticulum stress signaling.

Materials and Methods

Cell Lines and Culture Conditions

The glioma cell line U87 (HTB-14) 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, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco,

USA) and streptomycin (0.1 mg/ml; Gibco) at 37 °C in a 5% CO_2 incubator. Glucose and glutamine deprivation conditions were created by exchanging the complete DMEM medium in culture plates with DMEM medium lacking glucose or glutamine. Plates were exposed to these conditions for 16 h.

In this work we used two sublines of U87 glioma cells. One subline was obtained by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dominant-negative construct (dnIRE1). This untreated subline of glioma cells was used as control (control glioma cells) in the study of the effects of glutamine and glucose deprivations on the expression level of *IL13RA2, KRT18, CD24, ING1, ING2, MYL9, BET1, BET3/TRAPPC3, ENDOG, POLG, TSFM*, and *MTIF2* genes. Second subline was obtained by selection of stable transfected clones with overexpression of dnIRE1 and has suppressed both protein kinase and endoribonuclease activities of this bifunctional signaling enzyme of endoplasmic reticulum stress [20]. Previously was shown that these cells have low proliferation rate and do not express XBP1 alternative splice variant, a key transcription factor in IRE1 signaling, after induction endoplasmic reticulum stress by tunicamycin [20, 44]. The expression levels of the studied genes in these cells upon glutamine and glucose deprivations were compared with cells, transfected with vector (control glioma cells).

RNA isolation

Total RNA was extracted from glioma cells using Trizol reagent according to manufacturer protocols (Invitrogen, USA). 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 and spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis

QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis according to manufacturer protocol. The expression levels of *E2F8, EPAS1, HOXC6, TBX3, TBX2, GTF2F2, GTF2B, MAZ, SNAI2, TCF3, TCF8,* and ACTB mRNA were measured in U87 glioma cells by real-time quantitative polymerase chain reaction (qPCR) using Mx 3000P QPCR (Stratagene, USA) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, ABgene House, UK). Polymerase chain reaction was performed in triplicate using specific primers, which were received from Sigma-Aldrich (USA).

For amplification of *IL13RA2* (interleukin 13 receptor, alpha 2) cDNA we used forward (5′–TCTTGGAAACCTGGCATAGG–3′ and reverse (5′–TCTGATGCCTCCAAATAGGG–3′) primers. The nucleotide sequences of these primers correspond to sequences 591 – 610 and 742–723 of human *IL13RA2* cDNA (NM_000640). The size of amplified fragment is 152 bp. The amplification of *KRT18* (keratin 18, type I) cDNA was performed using two oligonucleotides primers: forward — 5′–CACAGTCTGCTGAGGTTGGA–3′ and reverse — 5′–GAGCTGCTCCATCTGTAGGG–3′. The nucleotide sequences of these primers correspond to sequences 966 – 985 and 1129 – 1110 of human *KRT18* cDNA (NM_000224). The size of amplified fragment is 164 bp.

The amplification of *CD24* (CD24 molecule; Signal transducer CD24) cDNA was performed using two oligonucleotides primers: forward — 5′–AACTAATGCCACCACCAAGG–3′ and reverse — 5′–CCTGTTTTTCCTTGCCACAT–3′. The nucleotide sequences of these primers correspond to sequences 590–609 and 777–758 of human *CD24* cDNA (NM_013230). The size of amplified fragment is 169 bp. For amplification of *MYL9* (myosin, light chain 9, regulatory), also known as *MYRL2* (myosin regulatory light chain 2) cDNA we used forward (5′–ACCCCACAGACGAATACCTG –3′ and reverse (5′–CCGGTACATCTCGTCCACTT–3′) primers. The nucleotide sequences of these primers correspond to sequences 285–304 and 526–507 of human *MYL9* cDNA (NM_006097). The size of amplified fragment is 242 bp.

For amplification of *ING1* (inhibitor of growth family, member 1) cDNA we used forward (5′–CCAAGGGCAAGTGGTACTGT–3′ and reverse (5′–CTGCCATCCCTA-TGAAAGGA–3′) primers. The nucleotide sequences of these primers correspond to sequences 1601–1620 and 1845–1826 of human *ING1* cDNA (NM_005537). The size of amplified fragment is 245 bp. The amplification of *ING2* (inhibitor of growth family, member 2) cDNA was performed using two oligonucleotides primers: forward — 5′– ACGTCTACAGCAGCTTCTCC–3′ and reverse — 5′– TGCGGGGTCTTCTTGAAGAT –3′. The nucleotide sequences of these primers correspond to sequences 369–388 and 589–570

of human *ING2* cDNA (NM_001564). The size of amplified fragment is 221 bp.

For amplification of *BET1* (Bet1 Golgi vesicular membrane trafficking protein), cDNA we used forward (5′–AGAAGTTGGTGTTTCGCTGG–3′ and reverse (5′–AGTTCCCATAGTTGCCAGGA–3′) primers. The nucleotide sequences of these primers correspond to sequences 49–68 and 214–195 of human *BET1* cDNA (NM_005868). The size of amplified fragment is 166 bp. The amplification of *TRAPPC3* (trafficking protein particle complex 3), also known as *BET3*, cDNA was performed using two oligonucleotides primers: forward — 5′–GGCACCGAGAGCAAGAAAAT–3′and reverse — 5′–CCAACATTTGACCGAGCCAA–3′. The nucleotide sequences of these primers correspond to sequences 158–177 and 333–314 of human *TRAPPC3* cDNA (NM_014408). The size of amplified fragment is 176 bp.

The amplification of *ENDOG* (endonuclease G) cDNA was performed using two oligonucleotides primers: forward — 5′– GTTCTACCTGAGCAACGTCG–3′ and reverse — 5′–TGCCGATGACCTGGTACTTT–3′. The nucleotide sequences of these primers correspond to sequences 659–678 and 843–824 of human *ENDOG* cDNA (NM_004435). The size of amplified fragment is 185 bp. For amplification of *POLG* (DNA directed polymerase gamma) cDNA we used forward (5′–GATCTGGCCAATGATGCCTG–3′ and reverse (5′–AAACTCCTCCTCCTCACTGC–3′) primers. The nucleotide sequences of these primers correspond to sequences 1675 – 1694 and 1899 – 1880 of human *POLG* cDNA (NM_002693). The size of amplified fragment is 225 bp.

The amplification of TSFM (Ts Mitochondrial Translational Elongation Factor) cDNA was performed using two oligonucleotides primers: forward — 5′–AAACCTTGAAGACGTTGGCC–3′ and reverse — 5′–CTGCCTCTTCACCTTCTCCA –3′. The nucleotide sequences of these primers correspond to sequences 798–817 and 1021–1002 of human *TSFM* cDNA (NM_005726). The size of amplified fragment is 224 bp. For amplification of *MTIF2* (mitochondrial translational initiation factor 2) cDNA we used forward (5′–TGTGGAAGAGCACCCAGTAG–3′ and reverse (5′–AGACCACAATCCATTCCCGT –3′) primers. The nucleotide sequences of these primers correspond to sequences 2167–2186 and 2397–2378 of human *MTIF2* cDNA (NM_002453). The size of amplified fragment is 231 bp.

The amplification of beta-actin (ACTB) cDNA was performed using forward — 5′– GGACTTCGAGCAAGAGATGG –3′ and reverse — 5′–AGCACTGTGTTGGCGTACAG–3′ primers. These primer nucleotide sequences correspond to 747–766 and 980–961 of human *ACTB* cDNA (NM_001101). The size of amplified fragment is 234 bp. The expression of beta-actin mRNA was used as control of analyzed RNA quantity.

Quantitative PCR analysis was performed using "Differential expression calculator" software. The values of *IL13RA2, KRT18, CD24, ING1, ING2, MYRL2, BET1, TRAPPC3, ENDOG, POLG, TSFM, MTIF2*, and *ACTB* gene expressions were normalized to the expression of beta-actin mRNA and represented 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 visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA). Statistical analysis was performed according to Student's test using Excel program as described previously [45]. All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments.

Results and Discussion

To test the effect of glutamine and glucose deprivations on expression levels of different genes, strongly related to both positive and negative control of cell proliferation in relation to IRE1 signaling enzyme function, we used the U87 glioma cell sublines, which constitutively express vector pcDNA3.1 (control cells) or dnIRE1 [10, 20]. Fig. 1 demonstrates that glutamine deprivation affects the expression of studied genes at mRNA level: up-regulates the expression of *BET1* (+30%), *MYL9* (+13%), and *MTIF2* (+18%) genes and down-regulates of *CD24* (–39%), *ING2* (–35%), *ENDOG* (–36%), *POLG* (–24%), and *TSFM (*–18%) gene expressions as compared to control glioma cells growing with glutamine. It is interesting to note that more prominent changes in the expression levels were shown for *CD24, ING2,* and *ENDOG* genes as compared to other studied genes. At the same time, the expression of *IL13RA2, KRT18, ING1,* and *BET3/TRAPPC3* genes at mRNA level was resistant to glutamine deprivation in control glioma cells.

We also analyzed the expression levels of genes encoded different proliferation

and apoptosis related factors and enzymes in glioma cells upon glucose deprivation. As shown in Fig. 2, glucose deprivation up-regulates the expression level of *MYL9* (+55%) gene only and down-regulates of *ING1* (−14%), *ING2* (−20%), and *MTIF2* (−13%) gene expressions in control glioma cells as compared to cells growing with glucose. More significant changes in the expression level of studied genes were shown for *MYL9* gene only. At the same time, the expression of *IL13RA2*, *KRT18*, *CD24*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, and *TSFM* genes at mRNA level was resistant to glucose deprivation in control glioma cells.

We next studied how inhibition of IRE1 modulates the effect of glutamine and glucose deprivation on the expression of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* genes. As shown in Fig. 3, the expression of *IL13RA2*, *KRT18*, *CD24*, *MYL9*, and *POLG* genes is resistant to glutamine deprivation condition in glioma cells with knockdown of signaling enzyme IRE1. However, the expression of genes encoding ING1, ING2, ENDOG, and POLG upon glutamine deprivation is significantly down-regulated in glioma cells with IRE1knockdown: for *ING1* in 2.4 fold, for *ING2* in 2.0 fold, and for *ENDOG* (−16%). At the same time, the expression of four studied genes is up-regulated in U87 glioma cells upon this experimental condition (*BET1*, *TRAPPC3*, *TSFM*, and *MTIF2*): +60%, +23%, +26%, and +96%, respectively (Fig. 3). Therefore, inhibition of IRE1 completely abolishes the effect of glutamine deprivation on the expression *CD24*, *MYL9*, and *POLG* genes.

Results presented in Fig. 4 demonstrate that inhibition of signaling enzyme IRE1 leads to significant up-regulation of the expression of *MTIF2* (+62%), *MYL9* (+27%), *IL13RA2* (+24%), *BET1* (+23%), and *TRAPPC3* (+19%) genes in glioma cells upon glucose deprivation condition. At the same time, the expression of four other genes is down-regulated in U87 glioma cells with IRE1 knockdown upon glucose deprivation: *KRT18* (−13%), *CD24* (−37%), *ING1* (−48%), and *ING2* (−27%). However, the expression of genes encoded ENDOG, TSFM, and POLG is resistant to glucose deprivation condition in glioma cells with IRE1 knockdown (Fig. 4).

Fig. 5 contains the results of comparative study of the sensitivity of *IL13RA2*, *CD24*, *ING1*, *BET1*, *MTIF2*, and *BET3* (*TRAPPC3*) gene expressions to glutamine deprivation in two types of glioma cells: control cells and cells without IRE1 signaling enzyme function. It was shown that inhibition of IRE1 signaling enzyme enhances the sensitivity of *IL13RA2*, *CD24*, *ING1*, *BET1*, *TRAPPC3*, and *MTIF2* genes expression to glutamine deprivation condition in glioma cells. At the same time, the sensitivity of *MYL9* gene expression is eliminated upon glucose deprivation. As shown in Fig. 6, the sensitivity of *ENDOG* and *POLG*

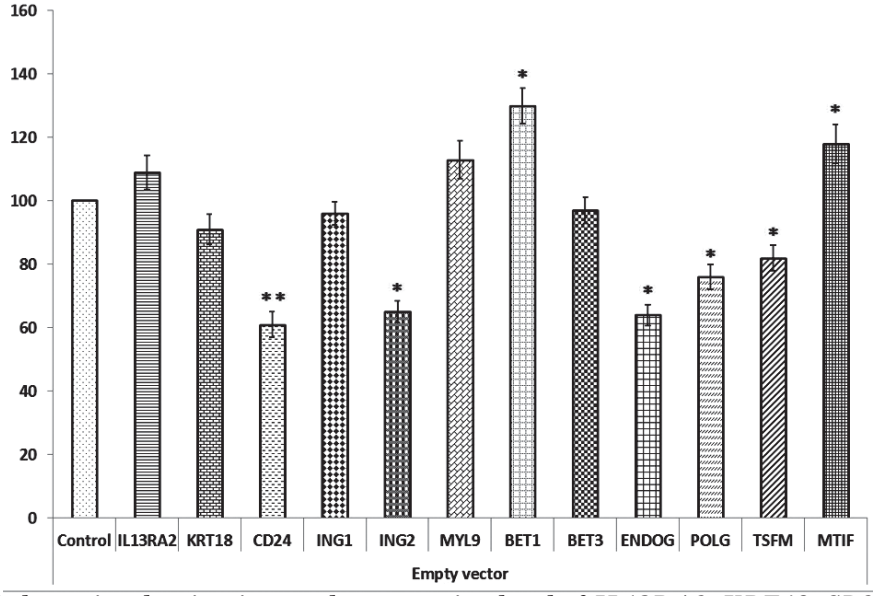

Fig. 1. **Effect of glutamine deprivation on the expression level of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* genes in control glioma cell line U87 measured by quantitative PCR:**
hereinafter — values of these gene expressions were normalized to beta-actin expression and represented as percent of control (vector, 100%); mean ± SEM;
$n = 4$; * — $P < 0.05$ as compared to control

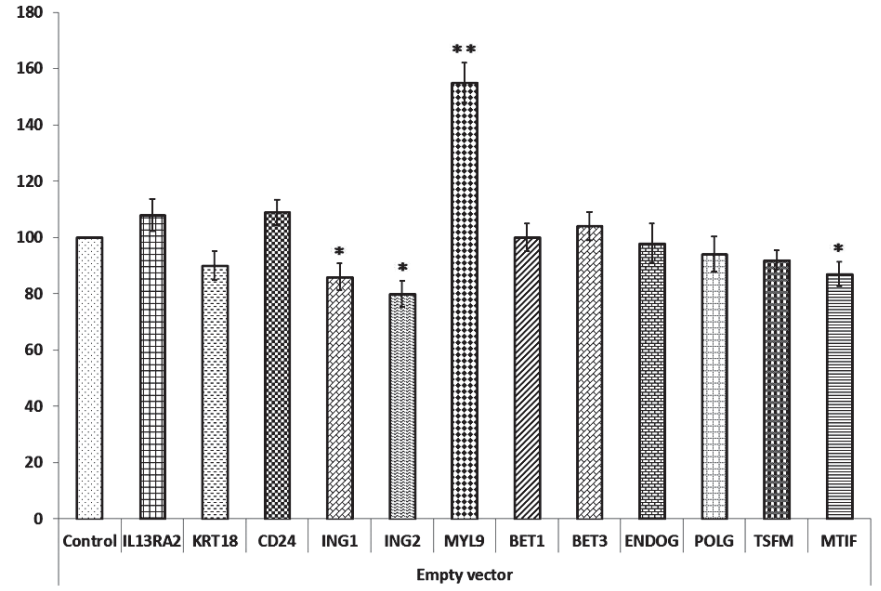

Fig. 2. **Effect of glucose deprivation on the expression level of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* genes in control glioma cell line U87 measured by quantitative PCR**

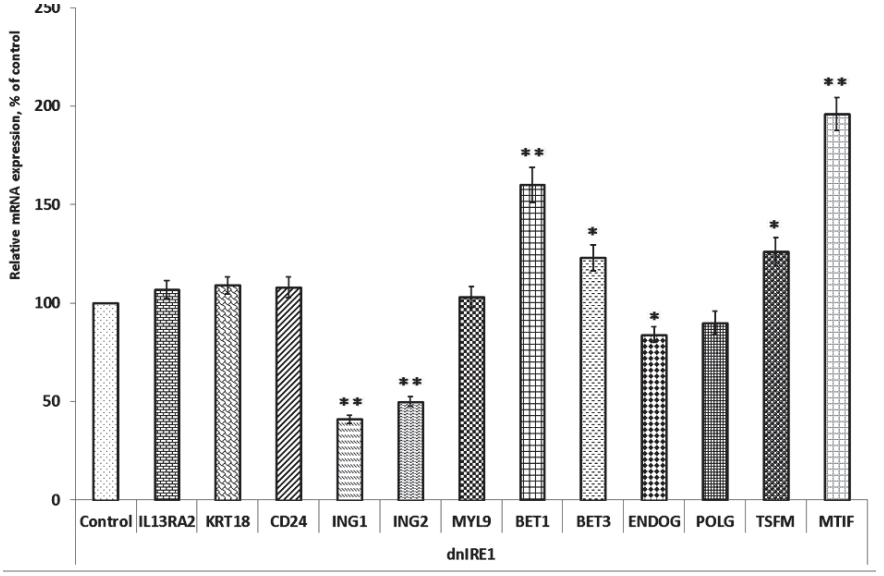

Fig. 3. **Effect of glutamine deprivation on the expression level of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* genes in glioma cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR:**
hereinafter — both controls is accepted as 100%; NS — no significant changes

genes expression to glutamine deprivation condition is significantly decreased upon inhibition of IRE1 enzyme function. At the same time, inhibition of IRE1 signaling enzyme significantly enhances the sensitivity of *TSFM* and *MTIF* genes expression in glioma cells to glutamine deprivation condition (Fig. 6).

We have also investigated the effect of inhibition of IRE1 signaling enzyme on sensitivity of *IL13RA2*, *CD24*, *ING1*, *MYL9*, *BET1*, and *MTIF2* genes expression in glioma cells to glucose deprivation. As shown in Fig. 7, the sensitivity of *IL13RA2*, *CD24*, *ING1*, *BET1*, and *MTIF2* genes expression to glucose deprivation condition is significantly increased by inhibition of IRE1 enzyme function, but the sensitivity of *MYL9* gene expression is decreased. Furthermore, inhibition of IRE1 signaling enzyme does not change the effect of glutamine deprivation on the expression *IL13RA2* gene (Fig. 1 and 3) and the effect of glucose deprivation on the expression *KRT18*, *ENDOG*, and *POLG* genes (Fig. 2 and 4) in glioma cells.

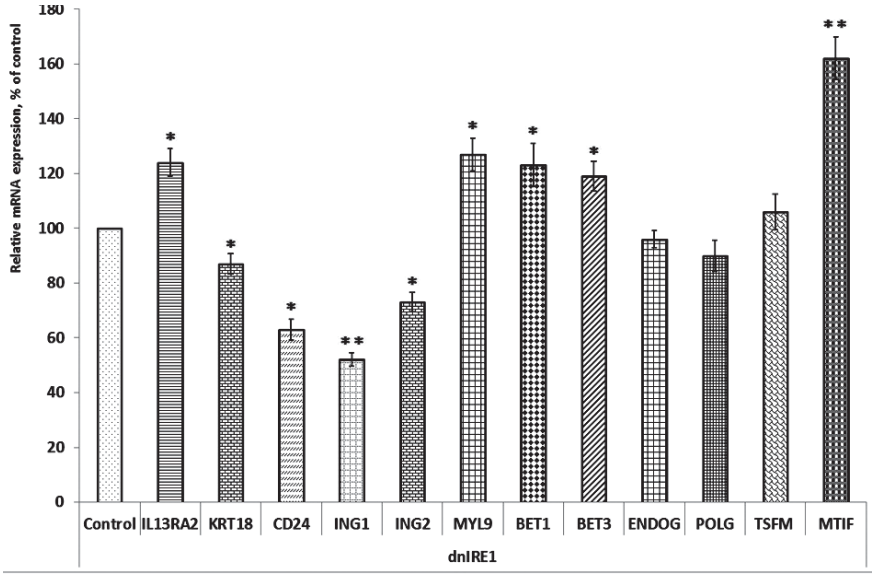

Fig. 4. **Effect of glucose deprivation on the expression level of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* genes in glioma cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR**

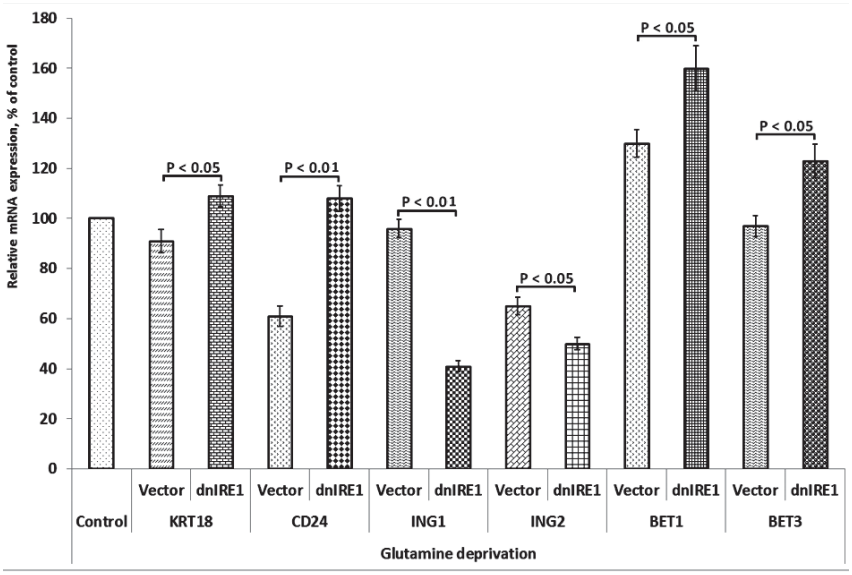

Fig. 5. **Comparative effect of glutamine deprivation on the expression of level of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *BET1*, and *BET3* (*TRAPPC3*) genes in two types of glioma cells: control cells transfected by vector (Vector) and cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR**

Results of this study clearly demonstrated that the expression levels of almost all studied genes encoding proliferation-related factors and enzymes are affected by glutamine and glucose deprivation and inhibition of IRE1 modified sensitivity of these gene expressions to both glutamine and glucose deprivations. Our results are consistent with data that glycolysis and glutaminolysis are related to the control of cell proliferation through regulation of cell cycle and tumor suppressor genes [12, 46, 47]. Recently, we have shown that genes encoded insulin-like growth factor binding proteins are strongly dependent on the endoplasmic reticulum stress and particularly on its IRE1 signaling pathway, because inhibition of IRE1, especially its endoribonuclease activity, significantly affects expression of these genes, and that inhibition of IRE1 modifies sensitivity of insulin-like growth factor binding protein genes expression to glucose deprivation [48, 49].

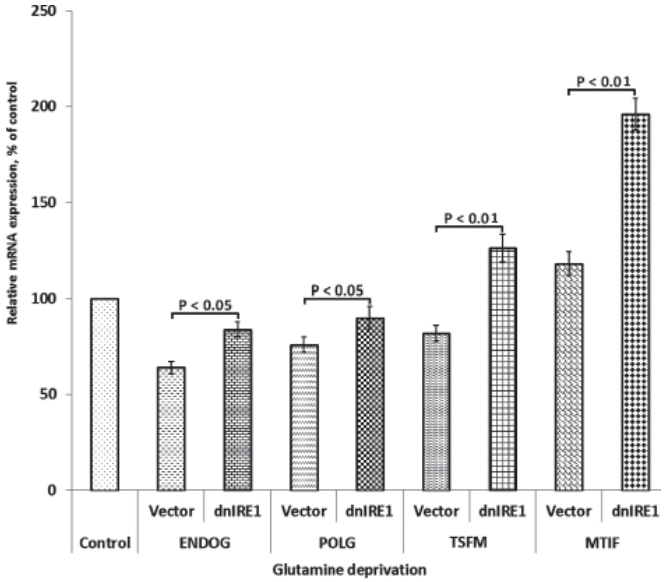

Fig. 6. **Comparative effect of glutamine deprivation on the expression level of *ENDOG, POLG, TSFM*, and *MTIF2* genes in two types of glioma cells: control cells transfected by vector (Vector) and cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR**

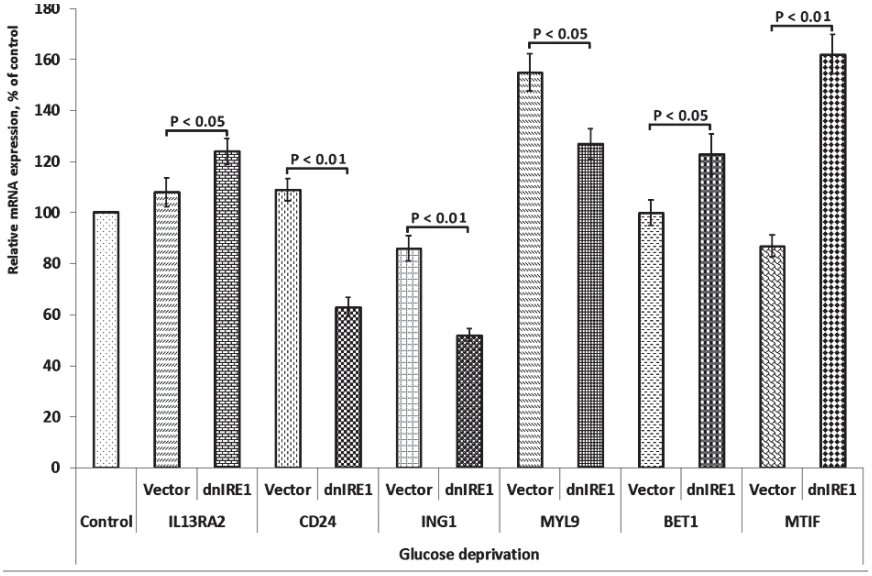

Fig. 7. **Comparative effect of glucose deprivation on the expression level of *IL13RA2*, *CD24, ING1, MYL9, BET1*, and *MTIF2* genes in two types of glioma cells: control cells transfected by vector (Vector) and cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR**

In this study we have shown that glutamine deprivation down-regulates the expression level of an anti-proliferative *CD24* gene and that inhibition of IRE1 signaling enzyme function in U87 glioma cells eliminates this effect and that these results correlate with a suppression of cell proliferation by IRE1 inhibition [10]. At the same time, the expression level of this gene is resistant to glucose deprivation in control glioma cells, but inhibition of IRE1 introduces sensitivity of CD24 gene expression to glucose deprivation. Thus, our results argue with data from Colombo et al. [12], who show that glucose and glutamine are required for tumor progression through cell cycle control and that deprivation of these substrates of glycolysis and glutaminolysis have opposite effect. Moreover, effects of glucose and glutamine deprivation conditions on the expression of genes studied in this work are gene-specific and are similar for *ING2* and *MYL9* genes and different for many other genes (*CD24, ING1, BET1, ENDOG, POLG, TSFM*, and *MTIF2*.

In general, our results are consistent with data that glycolysis and glutaminolysis are related to the control of cell proliferation through regulation of genes controlling cell proliferation and tumor suppressor genes. Inhibition of IRE1, a central mediator of endoplasmic reticulum stress response, preferentially modified sensitivity of these gene expressions to glutamine and glucose deprivations and possibly contributes to slower glioma growth. However, molecular mechanisms of the regulation of these genes by glutamine and glucose deprivation through the endoplasmic reticulum stress response pathways are complex and warrant further investigation for clarification the role of glycolysis and glutaminolysis in cancer progression as well as development new strategies of anti-tumor therapy.

Results of this study clearly demonstrated that the expression levels of almost all studied genes (*IL13RA2*, *KRT18, CD24, ING1, ING2, MYL9, BET1, TRAPPC3, ENDOG, POLG, TSFM* and *MTIF2*), which encode key factors and enzymes responsible for control of cell proliferation and apoptosis, are affected by glucose and glutamine deprivations in glioma cells in gene-specific manner and the sensitivity of their expression levels to the deficiency of essential nutrients is modified by IRE1 signaling branch of endoplasmic reticulum stress.

REFERENCES

1. *Clarke H. J., Chambers J. E., Liniker E., Marciniak S. J.* Endoplasmic reticulum stress in malignancy. *Cancer Cell*. 2014, 25 (5), 563–573. doi: 10.1016/j.ccr.2014.03.015.
2. *Wang S., Kaufman R. J.* The impact of the unfolded protein response on human disease. *J. Cell. Biol.* 2012, V. 197, P. 857–867. doi: 10.1083/jcb.201110131.
3. *Yadav R. K., Chae S. W., Kim H. R., Chae H. J.* Endoplasmic reticulum stress and cancer. *J. Cancer Prev*. 2014, 19 (2), 75–88. doi: 10.15430/JCP.2014.19.2.75.
4. *Cao S. S., Kaufman R. J.* Targeting endoplasmic reticulum stress in metabolic disease. *Expert Opin. Ther. Targets*. 2013, 17 (4), 437–448. doi: 10.1517/14728222.2013.756471.
5. *Bravo R. 1., Parra V., Gatica D., Rodriguez A. E., Torrealba N., Paredes F., Wang Z. V., Zorzano A., Hill J. A., Jaimovich E., Quest A. F., Lavandero S.* Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration. *Int. Rev. Cell. Mol. Biol.* 2013, V. 301, P. 215–290. doi: 10.1016/B978-0-12-407704-1.00005-1.
6. *Manié S. N., Lebeau J., Chevet E.* Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 3. Orchestrating the unfolded protein response in oncogenesis: an update. *Am. J. Physiol. Cell. Physiol*. 2014, 307 (10), 901–907. doi: 10.1152/ajpcell.00292.2014.
7. *Minchenko O. H., Kharkova A. P., Bakalets T. V., Kryvdiuk I. V.* Endoplasmic reticulum stress, its sensor and signaling systems and the role in the regulation of gene expressions in malignant tumor growth and hypoxia. *Ukr Biokhim. Zh.* 2013, 85 (5), 5–16. (In Ukrainian).
8. *Moenner M., Pluquet O., Bouchecareilh M., Chevet E.* Integrated endoplasmic reticulum stress responses in cancer. *Cancer Res*. 2007, 67 (22), 10631–10634. doi: 10.1158/0008-5472.CAN-07-1705.
9. *Drogat B., Auguste P., Nguyen D. T., Bouchecareilh M., Pineau R., Nalbantoglu J., Kaufman R. J., Chevet E., Bikfalvi A., Moenner M.* IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. *Cancer Res*. 2007, 67 (14), 6700–6707. doi: 10.1158/0008-5472. CAN-06-3235.
10. *Auf G., Jabouille A., Guerit S., Pineau R., North S., Platonova N., Maitre M., Favereaux A., Vajkoczy P., Seno M., Bikfalvi A., Minchenko D., Minchenko O., Moenner M.* Inositol-requiring enzyme 1alpha is a key regulator of angiogenesis and invasion in malignant glioma. *Proc. Natl. Acad. Sci. U.S.A.* 2010, 107 (35), 15553–15558. doi: 10.1073/pnas.0914072107.
11. *Minchenko O. H., Kubaichuk K. I., Minchenko D. O., Kovalevska O. V., Kulinich A. O., Lypova N. M.* Molecular mechanisms of IRE-1-mediated angiogenesis. *Int. J. Physiol. Pathophysiol*. 2014, 5 (1), 1–22.
12. *Colombo S. L., Palacios-Callender M., Frakich N., Carcamo S., Kovacs I., Tudzarova S., Moncada S.* Molecular basis for the differential use of glucose and glutamine in cell proliferation as revealed by synchronized HeLa cells. *Proc. Natl. Acad. Sci. U.S.A.* 2011, 108 (52), 21069–21074. doi: 10.1073/pnas. 1117500108.
13. *Yalcin A., Clem B. F., Imbert-Fernandez Y., Ozcan S. C., Peker S., O'Neal J., Klarer A. C., Clem A. L., Telang S., Chesney J.* 6-Phosphofructo-2-kinase (PFKFB3) promotes cell cycle progression and suppresses apoptosis via Cdk1-mediated phosphorylation of p27. *Cell Death and Disease.* 2014, V. 5, P. e1337. doi: 10.1038/cddis.2014.292.
14. *Huber A. L., Lebeau J., Guillaumot P., Pétrilli V., Malek M., Chilloux J., Fauvet F., Payen L., Kfoury A., Renno T., Chevet E., Manié S. N.* p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows

malignant progression upon low glucose. *Mol. Cell.* 2013, 49 (6), 1049–1059. doi: 10.1016/j.molcel.2013.01.009.
15. *Lenihan C. R., Taylor C. T.* The impact of hypoxia on cell death pathways. *Biochem. Soc. Trans.* 2013, V. 41, P. 657–663. doi: 10.1042/BST20120345.
16. *Han J., Back S. H., Hur J., Lin Y. H., Gildersleeve R., Shan J., Yuan C. L., Krokowski D., Wang S., Hatzoglou M., Kilberg M. S., Sartor M. A., Kaufman R. J.* ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat. Cell. Biol.* 2013, 15 (5), 481–490. doi: 10.1038/ncb2738.
17. *Maurel M., Chevet E., Tavernier J., Gerlo S.* Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* 2014, 39 (5), 245–254. doi: 10.1016/j.tibs. 2014.02.008.
18. *Malhotra J. D., Kaufman R. J.* ER stress and its functional link to mitochondria: role in cell survival and death. *Cold Spring Harb. Perspect. Biol.* 2011, V. 3, P. a004424. doi: 10.1101/cshperspect.a004424.
19. *Pluquet O., Dejeans N., Chevet E.* Watching the clock: endoplasmic reticulum-mediated control of circadian rhythms in cancer. *Ann. Med.* 2014, 46 (4), 233–243. doi: 10.3109/07853890.2013.874664.
20. *Auf G., Jabouille A., Delugin M., Guérit S., Pineau R., North S., Platonova N., Maitre M., Favereaux A., Vajkoczy P., Seno M., Bikfalvi A., Minchenko D., Minchenko O., Moenner M.* High epiregulin expression in human U87 glioma cells relies on IRE1alpha and promotes autocrine growth through EGF receptor. *BMC Cancer.* 2013, V. 13, P. 597. doi: 10.1186/1471-2407-13-597.
21. *Minchenko D. O., Kubaichuk K. I., Hubenia O. V., Kryvdiuk I. V., Kharkova A. P., Herasymenko R. M., Sulik R. V., Karbovskyi L. L., Minchenko O. H.* Endoplasmic reticulum stress and angiogenesis in cancer. *Int. J. Physiol. Pathophysiol.* 2014, 5 (3), 261–281.
22. *Pluquet O., Dejeans N., Bouchecareilh M., Lhomond S., Pineau R., Higa A., Delugin M., Combe C., Loriot S., Cubel G., Dugot-Senant N., Vital A., Loiseau H., Gosline S. J., Taouji S., Hallett M., Sarkaria J. N., Anderson K., Wu W., Rodriguez F. J., Rosenbaum J., Saltel F., Fernandez-Zapico M. E., Chevet E.* Posttranscriptional regulation of PER1 underlies the oncogenic function of IREα. *Cancer Res.* 2013, 73 (15), 4732–4743. doi: 10.1158/0008-5472.CAN-12-3989.
23. *Acosta-Alvear D., Zhou Y., Blais A., Tsikitis M., Lents N. H., Arias C., Lennon C. J., Kluger Y., Dynlacht D. D.* XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. *Mol. Cell.* 2007, V. 27, P. 53–66. doi:10.1016/j.molcel.2007.06.011.
24. *Hollien J., Lin J. H., Li H., Stevens N., Walter P., Weissman J. S.* Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell. Biol.* 2009, 186 (3), 323–331. doi: 10.1083/jcb.200903014.
25. *Aragón T., van Anken E., Pincus D., Serafimova I. M., Korennykh A. V., Rubio C. A., Walter P.* Messenger RNA targeting to endoplasmic reticulum stress signalling sites. *Nature.* 2009, 457 (7230), 736–740. doi: 10.1038/nature07641.
26. *Hassler J., Cao S. S., Kaufman R. J.* IRE1, a double-edged sword in pre-miRNA slicing and cell death. *Dev. Cell.* 2012, 23 (5), 921–923. doi: 10.1016/j.devcel.2012.10.025.
27. *Barderas R., Bartolome R. A., Fernandez-Acenero M. J., Torres S., Casal J. I.* High expression of IL-13 receptor alpha2 in colorectal cancer is associated with invasion, liver metastasis, and poor prognosis. *Cancer Res.* 2012, 72 (11), 2780–2790. doi: 10.1158/0008-5472.CAN-11-4090.
28. *Taguchi A., Taylor A. D., Rodriguez J., Celiktaş M., Liu H., Ma X., Zhang Q., Wong C. H., Chin A., Girard L., Behrens C., Lam W. L., Lam S., Minna J. D., Wistuba I. I., Gazdar A. F., Hanash S. M.* A search for novel cancer/testis antigens in lung cancer identifies VCX/Y genes, expanding the repertoire of potential immunotherapeutic targets. *Cancer Res.* 2014, 74 (17), 4694–4705. doi: 10.1158/0008-5472.CAN-13-3725.
29. *Parlato M., Souza-Fonseca-Guimaraes F., Philippart F., Misset B., Adib-Conquy M., Cavaillon J. M.* CD24-triggered caspase-dependent apoptosis via mitochondrial membrane depolarization and reactive oxygen species production of human neutrophils is impaired in sepsis. *J. Immunol.* 2014, 192 (5), 2449–2459. doi: 10.4049/jimmunol.1301055.
30. *Chen J., Tran U. M., Rajarajacholan U., Thalappilly S., Riabowol K.* ING1b-inducible microRNA203 inhibits cell proliferation. *Br. J. Cancer.* 2013, 108 (5), 1143–1148. doi: 10.1038/bjc.2013.50.
31. *Huang Y. Q., Han Z. D., Liang Y. X., Lin Z. Y., Ling X. H., Fu X., Cai C., Bi X. C., Dai Q. S., Chen J. H., He H. C., Chen Y. R., Jiang F. N., Zhong W. D.* Decreased expression of myosin light chain MYL9 in stroma predicts malignant progression and poor biochemical recurrence-free survival in prostate cancer. *Med. Oncol.* 2014, 31 (1), 820. doi: 10.1007/s12032-013-0820-4.
32. *Bose P., Thakur S., Thalappilly S., Ahn B. Y., Satpathy S., Feng X., Suzuki K., Kim S. W., Riabowol K.* ING1 induces apoptosis through direct effects at the mitochondria. *Cell Death Dis.* 2013, V. 4, P. E788. doi: 10.1038/cddis.2013.321.
33. *Liu J., Lin Y., Yang H., Deng Q., Chen G., He J.* The expression of p33(ING1), p53, and

autophagy-related gene Beclin1 in patients with non-small cell lung cancer. *Tumour Biol*. 2011, 32 (6), 1113–1121. doi: 10.1007/s13277-011-0211-4.

34. *Guo X. B., Jing C. Q., Li L. P., Zhang L., Shi Y. L., Wang J. S., Liu J. L., Li C. S.* Down-regulation of miR-622 in gastric cancer promotes cellular invasion and tumor metastasis by targeting ING1 gene. *World J. Gastroenterol.* 2011, 17 (14), 1895–1902. doi: 10.3748/wjg.v17.i14.1895.
35. *Thalappilly S., Feng X., Pastyryeva S., Suzuki K., Muruve D., Larocque D., Richard S., Truss M., von Deimling A., Riabowol K., Tallen G.* The p53 tumor suppressor is stabilized by inhibitor of growth 1 (ING1) by blocking polyubiquitination. *PLoS ONE*. 2011, 6 (6), E21065. doi: 10.1371/journal. pone.0021065.
36. *Zhang H., Chen X., Wang J., Guang W., Han W., Zhang H., Tan X., Gu Y.* EGR1 decreases the malignancy of human non-small cell lung carcinoma by regulating KRT18 expression. *Sci. Rep*. 2014, V. 4, P. 5416. doi: 10.1038/srep05416.
37. *Zhang T., Hong W*. Ykt6 forms a SNARE complex with syntaxin 5, GS28, and Bet1 and participates in a late stage in endoplasmic reticulum-Golgi transport. *J. Biol. Chem*. 2001, 276 (29), 27480–27487. doi: 10.1074/jbc.M102786200.
38. *Zhdanov D. D., Fahmi T., Wang X., Apostolov E. O., Sokolov N. N., Javadov S., Basnakian A. G.* Regulation of Apoptotic Endonucleases by EndoG. *DNA Cell Biol*. 2015, 34 (5), 316–326. doi: 10.1089/dna.2014.2772.
39. *Linkowska K., Jawień A., Marszałek A., Malyarchuk B. A., Tońska K., Bartnik E., Skonieczna K., Grzybowski T*. Mitochondrial DNA Polymerase γ Mutations and Their Implications in mtDNA Alterations in Colorectal Cancer. *Ann. Hum. Genet*. 2015, Apr 7. doi: 10.1111/ahg.12111.
40. *Lee W. T., St John J*. The control of mitochondrial DNA replication during development and tumorigenesis. *Ann. N. Y. Acad. Sci*. 2015, V. 1350, P. 95–106. doi: 10.1111/nyas.12873.
41. *Le Roy F., Silhol M, Salehzada T., Bisbal C.* Regulation of mitochondrial mRNA stability by RNase L is translation-dependent and controls IFNalpha-induced apoptosis. *Cell Death Differ*. 2007, 14 (8), 1406–1413. doi:10.1038/sj.cdd. 4402130.
42. *Swan E. J., Maxwell A. P., McKnight A. J*. Distinct methylation patterns in genes that affect mitochondrial function are associated with kidney disease in blood-derived DNA from individuals with Type 1 diabetes. *Diabet. Med*. 2015, 32 (8), 1110–1115. doi: 10.1111/dme.12775.
43. *Zhang X., Bian X., Kong J*. The proapoptotic protein BNIP3 interacts with VDAC to induce mitochondrial release of endonuclease G. *PLoS One*. 2014, 9 (12), e113642. doi: 10.1371/journal.pone.0113642.
44. *Minchenko D. O., Karbovskyi L. L., Danilovskyi S. V., Moenner M., Minchenko O. H*. Effect of hypoxia and glutamine or glucose deprivation on the expression of retinoblastoma and retinoblastoma-related genes in ERN1 knockdown glioma U87 cell line. *Am. J. Mol. Biol.* 2012, 2 (1), 21–31. doi:10.4236/ajmb.2012.21003.
45. *Bochkov V. N., Philippova M., Oskolkova O., Kadl A., Furnkranz A., Karabeg E., Breuss J., Minchenko O. H., Mechtcheriakova D., Hohensinner P., Rychli K., Wojta J., Resink T., Binder B. R., Leitinger N*. Oxidized phospholipids stimulate angiogenesis via induction of VEGF, IL-8, COX-2 and ADAMTS-1 metalloprotease, implicating a novel role for lipid oxidation in progression and destabilization of atherosclerotic lesions. *Circ. Res.* 2006, 99 (8), 900–908. doi: 10.1161/01.RES.0000245485.04489.ee.
46. *Minchenko D. O., Danilovskyi S. V., Kryvdiuk I. V., Bakalets T. V., Yavorskyi V. V., Sulik R. V., Hubenia O. V., Minchenko O. H*. Effect of glutamine and glucose deprivation on the expression of *TP53*, *MDM2*, *USP7* and *PERP* genes in glioma U87 cells with IRE-1 knockdown. *Int. J. Adv. Med. Sci.* 2014, 2 (2), 13–8.
47. *Minchenko D. O., Danilovskyi S. V., Kryvdiuk I. V., Hlushchak N. A., Kovalevska O. V., Karbovskyi L. L., Minchenko O. H*. Acute L-glutamine deprivation affects the expression of TP53-related protein genes in U87 glioma cells. *Fiziol Zh*. 2014, 60 (4), 11–21.
48. *Minchenko D. O., Kharkova A. P., Karbovskyi L. L., Minchenko O. H*. Expression of insulin-like growth factor binding protein genes and its hypoxic regulation in U87 glioma cells depends on ERN1 mediated signaling pathway of endoplasmic reticulum stress. *Endocr. Regul*. 2015, 49 (2), 73–83. doi:10.4149/endo_2015_02_73.
49. *Minchenko D. O., Kharkova A. P., Tsymbal D. O., Karbovskyi L. L., Minchenko O. H*. Inhibition of IRE1 affects the expression of insulin-like growth factor binding protein genes and modifies its sensitivity to glucose deprivation in U87 glioma cells. *Endocr. Regul*. 2015, 49 (4), 185–197. doi:10.4149/endo_2015_04_185.

ПРИГНІЧЕННЯ ЗАЛЕЖНОГО ВІД ІНОЗИТОЛУ ЕНЗИМУ-1 ЗМІНЮЄ ВПЛИВ ДЕФІЦИТУ ГЛЮКОЗИ ТА ГЛУТАМІНУ НА РІВЕНЬ ЕКСПРЕСІЇ БІЛЬШОСТІ ГЕНІВ, ЩО КОНТРОЛЮЮТЬ ПРОЦЕСИ ПРОЛІФЕРАЦІЇ, У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87

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Метою роботи було вивчення експресії генів, що кодують фактори та ензими, які стосуються проліферації, зокрема *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM* та *MTIF2*, у клітинах гліоми лінії U87 за умов дефіциту глюкози та глутаміну залежно від пригнічення IRE1 — центрального медіатора стресу ендоплазматичного ретикулуму. Встановлено, що за умов дефіциту глутаміну спостерігається посилення експресії генів *BET1*, *MYL9* та *MTIF* і зниження експресії генів *CD24*, *ING2*, *ENDOG*, *POLG* та *TSFM* у контрольних клітинах гліоми (з нативним IRE1). Водночас за умов дефіциту глюкози зростає рівень експресії гена *MYL9* і зменшується рівень генів *ING1*, *ING2* та *MTIF* у контрольних клітинах гліоми. Таким чином, ефект дефіциту глюкози і глутаміну на експресію генів у клітинах гліоми є геноспецифічним. Пригнічення IRE1 за допомогою dnIRE1 суттєво змінює вплив дефіциту глюкози і глутаміну на експресію більшості досліджених генів, однак по-різному за напрямком і величиною, особливо для генів *ING2*, *CD24* та *MTIF*. Продемонстровано, що експресія генів, які стосуються проліферації, змінюється за умов дефіциту глюкози та глутаміну залежно від функції IRE1 і, можливо, зумовлює зниження інтенсивності росту пухлин після пригнічення цього ензиму.

Ключові слова: експресія генів, пов'язаних з проліферацією, пригнічення IRE1, дефіцит глюкози та глутаміну, клітини гліоми.

УГНЕТЕНИЕ ЗАВИСИМОГО ОТ ИНОЗИТОЛА ЭНЗИМА-1 ИЗМЕНЯЕТ ВЛИЯНИЕ ДЕФИЦИТА ГЛЮТАМИНА И ГЛЮКОЗЫ НА УРОВЕНЬ ЭКСПРЕССИИ БОЛЬШИНСТВА ГЕНОВ, КОТОРЫЕ КОНТРОЛИРУЮТ ПРОЦЕССЫ ПРОЛИФЕРАЦИИ В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87

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Целью работы было изучение экспрессии генов, кодирующих факторы и энзимы, имеющие отношение к пролиферации, в частности *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM* и *MTIF2*, в клетках глиомы линии U87 при дефиците глюкозы и глютамина в зависимости от угнетения IRE1 — центрального медиатора стресса эндоплазматического ретикулума. Установлено, что при дефиците глютамина отмечается усиление экспрессии генов *CD24*, *ING2*, *ENDOG*, *POLG* и *TSFM* и снижение экспрессии генов *CD24*, *ING2*, *ENDOG*, *POLG* и *TSFM* в контрольных клетках глиомы (с нативным IRE1). В то же время при дефиците глюкозы происходит увеличение уровня экспрессии гена *MYL9* и снижается уровень экспрессии генов *ING1*, *ING2* и *MTIF* в контрольных клетках глиомы. Таким образом, эффект дефицита глюкозы и глютамина на экспрессию генов в клетках глиомы является геноспецифическим. Угнетение IRE1 с помощью dnIRE1 существенно изменяет влияние дефицита глюкозы и глютамина на экспрессию большинства исследованных генов, но по-разному по направлению и величине, особенно для генов *ING2*, *CD24* и *MTIF*. Продемонстрировано, что экспрессия генов, имеющих отношение к пролиферации, изменяется при дефиците глюкозы и глютамина в зависимости от функции IRE1 и, возможно, обусловливает в снижение интенсивности роста опухолей после угнетения IRE1.

Ключевые слова: экспрессия генов, связанных с пролиферацией, угнетение IRE1, дефицит глюкозы, дефицит глютамина, клетки глиомы.