EXPERIMENTAL ARTICLES

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EXPRESSION OF UBIQUITIN SPECIFIC PEPTIDASE GENES IN IRE1 KNOCKDOWN U87 GLIOMA CELLS UPON GLUCOSE DEPRIVATION

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We have studied the effect of glucose deprivation on the expression of genes encoding for ubiquitin specific peptidases (USP) and autophagy related 7 (ATG7) in U87 glioma cells in relation to inhibition of inositol requiring enzyme-1 (IRE1). It was shown that glucose deprivation was down-regulated the expression of *USP1* and *USP10* genes and up-regulated *USP4* and *USP25* genes in control (transfected by empty vector) glioma cells. At the same time, the expression level of *USP14, USP22,* and ATG7 genes in these cells did not significantly change upon glucose deprivation condition. Inhibition of IRE1 signaling enzyme function in U87 glioma cells modified effect of glucose deprivation on the expression of most studied genes. Therefore, glucose deprivation affected the expression level of most ubiquitin specific peptidases genes in relation to the functional activity of IRE1 enzyme, which controls cell proliferation and tumor growth as a central mediator of endoplasmic reticulum stress.

Key words: mRNA expression, USP genes, IRE1 inhibition, glucose deprivation, U87 glioma cells.

E3 ubiquitin ligases and deubiquitylases play an important role in cancer [1–3]. Ubiquitin is a highly conserved protein involved in regulation of intracellular protein breakdown, cell cycle regulation, chromatin remodeling, and stress response. It is released from degraded proteins by disassembly of the polyubiquitin chains, which is mediated by ubiquitin-specific proteases, members of the ubiquitin-specific processing family of proteases for deubiquitination of proteins [1, 4, 5]. Our previous results demonstrated possible interaction/cross-talk between unfolding protein response signaling and ubiquitin system during adjustment to episodes of hypoxia during tumor development [6]. Ubiquitin specific peptidases (*USP*) and ubiquitin activating enzyme E1-like protein/autophagy related 7 (*GSA7*/*ATG7*) are involved in cancer cells survival and progression. *USP1* and *USP7* are responsible for deubiquitination of mono-ubiquitinated PCNA (proliferating cell nuclear antigen), which activates error-prone DNA polymerases and controls an oxidative-stress-induced mutagenesis in human cells [7]. Decreased levels of *USP1* in cancer cells have been implicated in lung and glioblastoma tumors growth and progression [8, 9]. There is data that serine phosphorylation is critical for the activity of *USP1* and its interaction with WD40-repeat protein *UAF1*; while two nuclear localization signals in *USP1* mediate nuclear import of the *USP1*/*UAF1* complex [10, 11]. Ubiquitin specific peptidase 4 function is important during tumorigenesis because this deubiquitinating enzyme has a key role in the regulation of TP53 and TGFβ signaling and is also a positive regulator of the WNT/β-catenin signaling [12–14]. Deubiquitinating enzyme UPS10 suppresses the proliferation and growth of cancer cells through stabilization of p53 protein [15]. Additional anti-tumorigenic effect of USP10 achieved by antagonizing

c-MYC activity through stabilization of a tumor suppressor SIRT6 [16]. In agreement, microRNA-191 mediated lower protein level of USP10 has been demonstrated to promote pancreatic cancer progression [15]. It was shown that USP14 is a tumor promoting peptidase, its phosphorylation and activation by Akt not only regulates the ubiquitin-proteasome system, but also promotes tumor progression through regulation of cellular proliferation and apoptosis of cancer cells [17, 18]. Inhibition of USP14 could be used as potential anti-cancer therapeutic strategy [19]. USP22 protease has been demonstrated to participate in regulation of the cell cycle progression in many cancer cell types [20, 21]. This enzyme removes ubiquitin from histones, thus regulating gene transcription [22]. It is interesting to note that deubiquitinating enzyme USP25 is involved in endoplasmic reticulum (ER)-associated degradation (ERAD) of misfolded/anomalous proteins [23]. USP25 counteracts ubiquitination of ERAD substrates by the ubiquitin ligase HRD1, rescuing them from degradation by the proteasome [23]. USP25 is a novel TRiC interacting protein that is catalyzed deubiquitination of the TRiC protein and stabilized this chaperonin, thereby reducing accumulation of misfolded protein aggregates [24].

The ubiquitin activating enzyme E1-like protein (GSA7), which is also known as autophagy related 7 (ATG7), is an essential component of autophagic machinery and a multifunctional protein, which mediates inhibition of cell proliferation and activation of apoptosis through induction of cellular senescence [25].

The endoplasmic reticulum stress is responsible for enhanced cancer cell proliferation and IRE1 knockdown by a dominant-negative construct of IRE1 (dnIRE) resulted in a significant anti-proliferative effect on glioma growth [26–29]. The rapid growth of solid tumors generates micro-environmental changes in association to hypoxia, nutrient deprivation and acidosis, which promote neovascularisation, cell survival and proliferation [30–32]. The activation of endoplasmic reticulum stress is indispensable for tumor growth as it facilitates adaptation to stressful environmental conditions. IRE1 is the most evolutionary conserved sensor that responds to protein misfolding with a highly tuned program aimed to either resolve the stress or direct the cell towards apoptosis in case stress becomes too severe, which makes it a key regulator of cell life and death processes [27, 33]. Recently, we have shown that glucose deprivation affects the expression of proliferation related genes in U87 glioma cells and that IRE1 knockdown modifies glucose deprivation effects on these genes expression possibly contributing to suppression of glioma cells proliferation [34].

Previously, we have shown that USP7 is regulated by IRE-1a signaling and hypoxia. Hypoxic regulation of USP7 was found to be independent from IRE-1a activity [6]. The precise mechanism of the exhibited by USP7 anti-proliferative effect is not clear. We hypothesized that anti-proliferative effect of USP7 is mediated through GSA7/ATG7. Indeed, cross-talk/ or final outcome of the activation of multiple stress signaling pathways, such as autophagy (ATG7)/ between ATG7 and UPR was implicated into regulation of cell proliferation, apoptosis and senescence [2, 9, 13, 20, 23, 24, 35].

Malignant gliomas are highly aggressive tumors with very poor prognosis and to date there is no efficient treatment available. The moderate efficacy of conventional clinical approaches therefore underlines the need for new therapeutic strategies. Glucose is important substrate for glycolysis, which is important to glioma development and a more agressive behaviour [36]. A better knowledge of tumor responses to glucose deprivation condition is required to elaborate therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms [37, 38].

The aim of this study was investigation the effect of glucose deprivation condition on the expression of a subset of ubiquitin specific peptidases and of ubiquitin activating enzyme E1-like protein/autophagy related 7 genes in glioma cells in relation to inhibition of signaling enzyme IRE1 with hopes of elucidating its mechanistic part in the development and progression of certain cancers and the contribution to unfolding protein response.

Materials and Methods

Cell Lines and Culture Conditions. In this study we used two sublines of U87 glioma cells, which are growing 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), streptomycin (0.1 mg/ml; Gibco) and penicillin (100 units/ml; Gibco) at 37 °C in a 5% CO_2 incubator. One subline was obtained

by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dominant-negative constructs (dnIRE1). This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of effects of glucose deprivation on the expression level of *USP1, USP4, USP10, USP22, USP25,* and *GSA7* 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 sensing and signaling enzyme of endoplasmic reticulum stress. The expression level of 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 glucose deprivation condition on the expression level of studied in cells with inhibited function of signaling enzyme IRE1. Clones were received by selection at 0.8 mg/ml geneticin (G418) and grown in the presence of this antibiotic at lower concentration (0.4 mg/ml). Glucose deprivation condition were created by changing the complete DMEM medium into culture plates on the DMEM medium without glucose, which we received from Gibco, Invitrogen, and supplemented with glutamine, 10% fetal bovine serum, streptomycin and penicillin. These 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 [39] 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 in 2 fold [40]. 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 [39]. 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.

Reverse transcription and quantitative PCR analysis. QuaniTect Reverse Transcription Kit (QIAGEN, Hilden, Germany) was used for cDNA synthesis as described previously [39]. The expression level of USP1, USP4, USP10, USP22, USP25, and GSA7 mRNA were measured in glioma cell line U87 and its subline (clone 1C5) by real-time quantitative polymerase chain reaction using "7500 HT Fast Real-Time PCR System" (Applied Biosystems) or "Mx 3000P QPCR" (Stratagene, USA) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, ABgene House, Epsom, Surrey, UK). Polymerase chain reaction was performed in triplicate.

The amplification of cDNA of the ubiquitin specific peptidases 1 (USP1; EC_number="3.4.19.12") was performed using forward primer (5′– CAGCATGATGCACAGGAAGT –3′) and reverse primer (5′– CCCATTTCCTTTTGGGAGTT–3′). These oligonucleotides correspond to sequences 1347–1366 and 1565–1546 of human USP1 cDNA (GenBank accession number NM_003368). The size of amplified fragment is 219 bp.

For amplification of the USP4 (EC_number="3.4.19.12") cDNA we used next primers: forward 5′– CTTATTGACAGCCGGTGGTT –3′ and reverse 5′– GTTTATTCCACGCCTCGGTA –3′. The nucleotide sequences of these primers correspond to sequences 185–204 and 389–370 of human USP4 cDNA (GenBank accession number NM_003363). The size of amplified fragment is 205 bp.

The amplification of cDNA of the ubiquitin specific peptidases 10 (USP10; EC_number="3.4.19.12") was performed using forward primer (5′– AGAGTGCATCACCTCCTGCT –3′) and reverse primer (5′– GATCCTCTGAAACCGGAACA –3′). These oligonucleotides correspond to sequences 1216–1235 and 1434–1415 of human USP10 cDNA (GenBank accession number NM_001272075). The size of amplified fragment is 219 bp.

For amplification of the USP14 (EC_number="3.4.19.12") cDNA we used next primers: forward 5′– CGTTCTGTGCCTGAACTCAA –3′ and reverse 5′– TTCACCTTTCTCGGCAAACT –3′. The nucleotide sequences of these primers correspond to sequences 586–605 and 789–770 of human USP14 cDNA (GenBank accession number NM_005151). The size of amplified fragment is 204 bp.

The amplification of cDNA of the USP22 (EC_number="3.4.19.12") was performed using forward primer (5′–

TGGAAATAATCGCCAAGGAG –3′) and reverse primer (5′– GAAGAAGTCCCG-CAGAAGTG –3′). These oligonucleotides correspond to sequences 575–594 and 816–797 of human USP22 cDNA (GenBank accession number NM_015276). The size of amplified fragment is 242 bp.

For amplification of the USP25 (EC_number="3.4.19.12") cDNA we used next primers: forward 5′– GGCACATAACGGAGG-AAGAA –3′ and reverse 5′– AGCTTGGCCTTC-GTGAACTA –3′. The nucleotide sequences of these primers correspond to sequences 1982–2001 and 2178–2159 of human USP25 cDNA (GenBank accession number NM_001283041). The size of amplified fragment is 197 bp.

The amplification of the ubiquitin activating enzyme E1-like protein/autophagy related 7 (GSA7/ATG7) cDNA was performed using forward primer (5′– TGAGCCTCCAACCTCTCTTG –3′) and reverse primer (5′– AGATCTCAGCAGCTTGGGTT –3′). These oligonucleotides correspond to sequences 1956–1975 and 2200–2181 of human USP10 cDNA (GenBank accession number NM_006395). The size of amplified fragment is 245 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 USP1, USP4, USP10, USP22, USP25, and GSA7 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 [41].

Results and Discussion

To determine if glucose deprivation regulates the genes of interest through the IRE1 branch of endoplasmic reticulum stress response, we investigated the effect of glucose deprivation condition on the expression of genes encoding *USP1, USP4, USP10, USP22, USP25,* and *GSA7* in two sublines of U87 glioma cells in relation to inhibition of IRE1 signaling enzyme, which is a major component of the unfolded protein response. As shown in Fig. 1, exposure of control glioma cells (transfected by empty vector) upon glucose deprivation condition leads to suppression of *USP1* mRNA expression (–27%). In glioma cells without functional activity of signaling enzyme IRE1 the expression of this gene is down-regulated (–22%) upon glucose deprivation. Thus, inhibition of IRE1 signaling enzyme function in U87 glioma cells by dnIRE1 does not change significantly the sensitivity of the expression of *USP1* gene to glucose deprivation condition and introduces sensitivity of this gene expression to glucose deprivation condition (Fig. 1).

Additionally, we found that expression of gene encoding for *USP4* is significantly up-regulated (+25%) upon glucose deprivation condition in control glioma cells (Fig. 2). In glioma cells containing dnIRE1, the effect glucose deprivation of on this mRNA expression was similar (+35%), indicating up-regulation of this gene expression by glucose deprivation condition in IRE1-independent manner (Fig. 2). As shown in Fig. 3, glucose deprivation condition significantly down-regulates (–19%) the expression level of *USP10* gene in control glioma cells, but inhibition of IRE1 signaling enzyme eliminates down-regulation of this gene expression upon glucose deprivation, indicating that down-regulation of this gene expression induced by glucose deprivation is dependent from IRE1 signaling.

At the same time, glucose deprivation condition does not change significantly *USP14* gene expression in both control glioma cells and cells without activity of signaling enzyme IRE1, indicating that this gene expression is resistant to glucose deprivation independently from IRE1 signaling (Fig. 4). We have also studied the expression of *USP22* gene. As shown in Fig. 5, exposure the control glioma cells upon glucose deprivation condition does not change significantly the expression of *USP22* mRNA in control glioma cells as compared to cells growing with glucose, indicating resistance of this gene expression

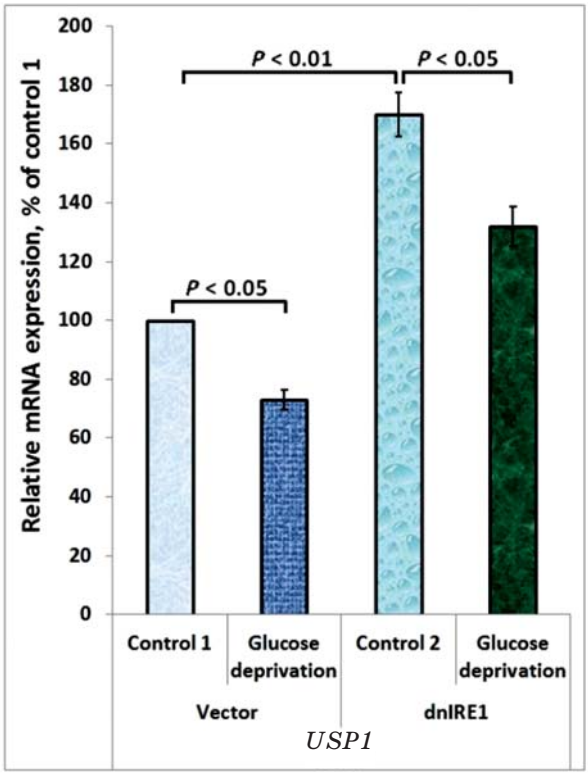

Fig. 1. **Effect of glucose deprivations on the expression level of *USP1* mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**
Thereafter: values of represented genes mRNA expressions were normalized to beta-actin mRNA and represe nted as percent of control 1 (100%); mean ± SEM; $n = 4$

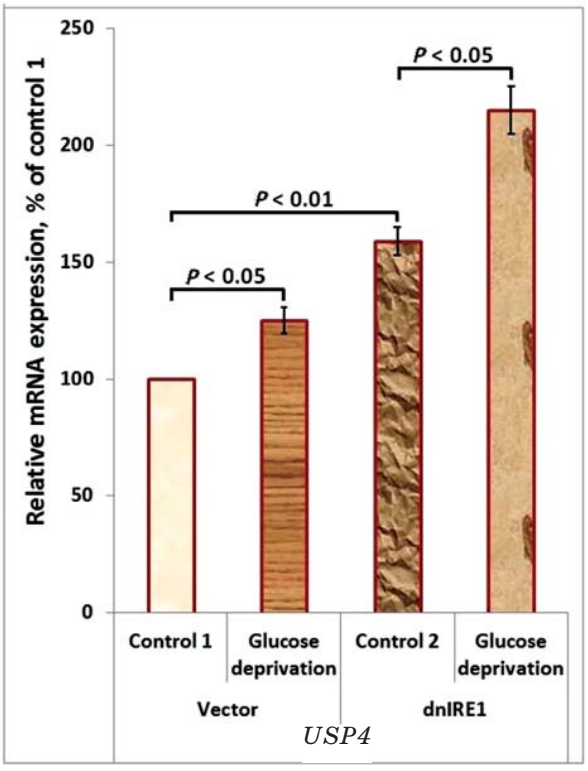

Fig. 2. **Effect of glucose deprivation on the expression level of *USP4* mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

to glucose deprivation. However, exposure of glioma cells containing dnIRE1 upon glucose deprivation condition leads to suppression of this mRNA expression (−19%), indicating regulation of this gene expression by glucose deprivation in IRE1-dependent manner (Fig. 5). As shown in Fig. 6, glucose deprivation condition significantly up-regulates the expression level of *USP25* mRNA in both control glioma cells and cells with knockdown of IRE1 signaling enzyme (+41% and +63%, correspondingly), but inhibition of IRE1 augments up-regulation of this gene expression upon glucose deprivation, indicating that up-regulation of this gene expression induced by glucose deprivation is dependent from IRE1 signaling.

Furthermore, we studied the effect of glucose deprivation condition on ubiquitin activating enzyme E1-like protein (GSA7/ATG7), which can mediate both the protein ubiquitination and cellular senescence, in both control glioma cells and cells with knockdown of IRE1 signaling. As shown in Fig. 7, ATG7 mRNA expression is resistant to glucose deprivation in control glioma cells, but inhibition of IRE1 signaling introduces small but statistically significant down-regulation of this gene expression by glucose deprivation condition (−14%), indicating that IRE1 participates in regulation of this gene expression by glucose deprivation. Furthermore, our results also demonstrate that the majority of the genes studied are up-regulated by inhibition of endoplasmic reticulum stress, mediated by IRE1 and that only *USP14* and *AGT7* mRNA are down-regulated (Fig. 1–7).

As shown in Fig. 8, inhibition of IRE-1a signaling does not significantly change the effect of glucose deprivation on the expression of *USP1*, *USP4*, and *USP14* genes, but modifies the sensitivity of *USP10*, *USP22*, *USP25*, and *ATG7* genes expression to glucose

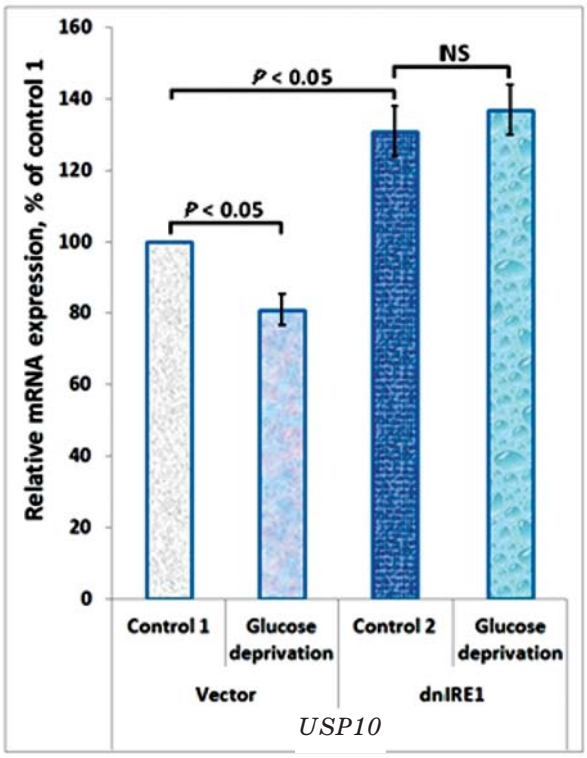

Fig. 3. **Effect of glucose deprivation on the expression level of *USP10* mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**
Thereafter: NS — no significant changes

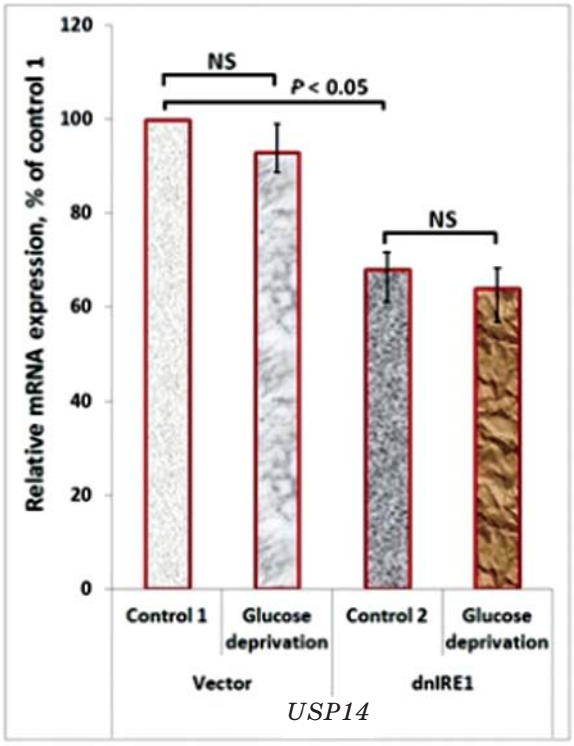

Fig. 4. **Effect of glucose deprivation on the expression level of *USP14* mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

deprivation in glioma cells by different ways: removes sensitivity of *USP10* gene to glucose deprivation, introduces sensitivity of *USP22* and *ATG7* genes and augments sensitivity of *USP25* gene to this treatment.

Thus, this study has demonstrated that glucose deprivation affects the expression of the majority of the genes encoding ubiquitin specific peptidases as well as ubiquitin activating enzyme E1-like protein in the IRE1-dependent manner and that these genes potentially contribute to regulation of cell proliferation, apoptosis, and metastasis. USP1 and USP4 have variable functions and increased level of *USP4* can be responsible for TNFa-induced apoptosis via deubiquitination of RIP1 (receptor-interacting serine-threonine kinase 1) [42]. Thus, the changes observed in the *USP4* gene correlate with slower cell proliferation in cells upon glucose deprivation and cells harboring dnIRE1. At the same time, there is also data that *USP4* overexpression may contribute to progression of certain tumors [9, 13].

We have also demonstrated that expression level of *USP10* is also up-regulated in glioma cells when IRE-1a function is inhibited. There is data that *USP10* protein suppresses the proliferation and growth of cancer cells through stabilizing p53 protein and up-regulation of its activity [15, 43], antagonizes c-MYC transcriptional activation through SIRT6 stabilization to suppress tumor formation [16] and that microRNA-191 promotes pancreatic cancer progression by targeting *USP10* [15]. Thus, our results that inhibition of IRE-1a via overexpression of dnIRE1 removes the sensitivity of the *USP10* gene expression to glucose deprivation are agree well with functional role of this deubiquitinating enzyme [15, 16, 43]. Moreover, these results fall in line with our previous data that indicate that the inhibition of IRE-1a up-regulates *TP53* gene expression [40]. We have shown that *USP14* gene expression is significantly down-regulated in glioma cells upon inhibition of IRE-1 signaling enzyme and, thus, down-regulation of this gene expression should contribute to suppression of glioma cell proliferation and tumor growth because *USP14* promotes tumor

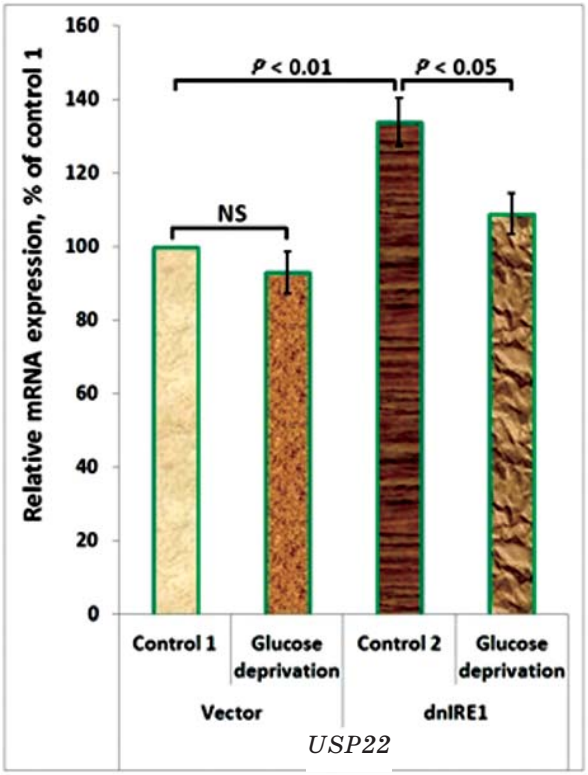

Fig. 5. **Effect of glucose deprivation on the expression level of *USP22* mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

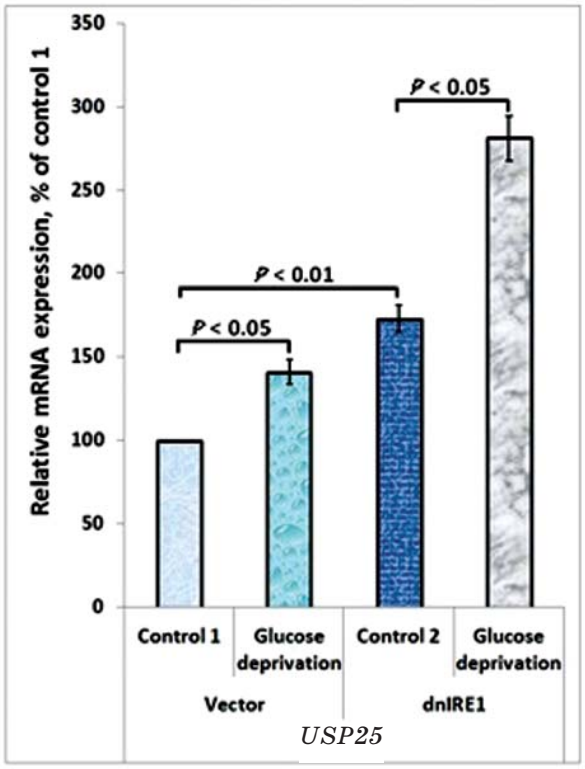

Fig. 6. **Effect of glucose deprivation on the expression level of *USP25* mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

progression through regulation of cellular proliferation and apoptosis of cancer cells, at least in hepatocellular carcinoma [17].

There is data that *USP22 i*nduces epithelial-mesenchymal transition, which play an important role in migration and invasion of the tumor cells [44]. However, it is known that this deubiquitinating enzyme is involved in tumor progression as regulator of the cell cycle and can remove ubiquitin from histones, thus regulating gene transcription [20, 21]. Moreover, p38 mitogen-activated protein kinase, which plays an important role in stress related transcription and cell cycle regulation, inhibits *USP22* transcription in HeLa cells [45]. Thus, this data agree with our results that IRE1 knockdown up-regulates *USP22* mRNA level.

So, in this study, we have shown that glucose deprivation as well as inhibition of IRE1 leads to up-regulation of *USP25* gene expression in glioma cells, which have decreased proliferation rate, but enhanced invasion and metastasis [6, 29]. Moreover, IRE1 knockdown increases sensitivity of this gene expression to glucose deprivation. Our results are consistent with recent data that shows [5] that miRNA-200c, which is involved in carcinogenesis and exerts tumor-suppressive effects for human non-small cell lung cancer, inhibits invasion and metastasis of these cells through the suppression of USP25 expression. At the same time, the expression of this gene is also necessary for ER-associated degradation of unfolded proteins: *USP25* counteracts ubiquitination of ERAD substrates by the ubiquitin ligase HRD1, rescuing them from degradation by the proteasome [23]. It is possible that up-regulation of *USP25* gene expression upon glucose deprivation as well as inhibition of IRE1 reflects suppressed ER stress signaling in glioma cells.

Our results, which demonstrate that inhibition of IRE1 down-regulates the expression of the GSA7/ATG7 mRNA, agree well with data that suppression of ATG7 in the intestinal epithelium decreases tumor growth [46]. Consequently, inhibition of IRE-1a signaling enzyme leads to up-

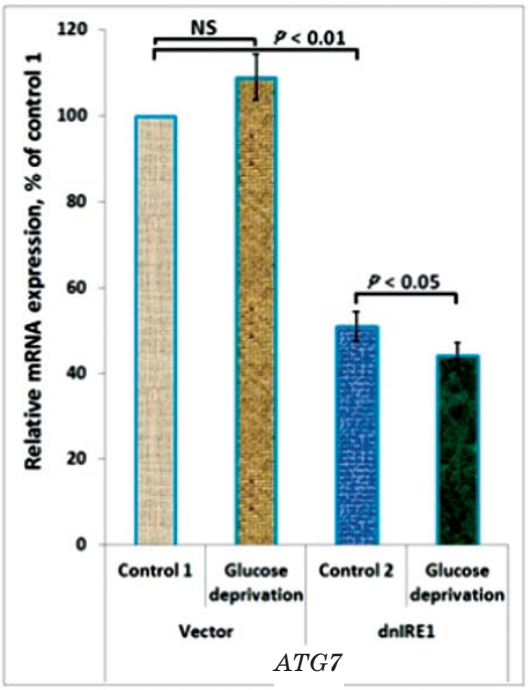

Fig. 7. **Effect of glucose deprivation on the expression level of ubiquitin activating enzyme E1-like protein/autophagy related 7 (*GSA7*/*ATG7*) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**

regulation of most enzymes responsible for deubiquitination, but down-regulation of the ubiquitin activating enzyme E1-like protein.

Biological significance of changes in diverse ubiquitin specific peptidases on cell proliferation and protein degradation is possibly related to *USP1-PHLPP1-Akt* signaling axis, TNFa-induced apoptosis via deubiquitination of RIP1 (*USP4*), regulation of p53 protein stability and activity, control of STAT3/MMP9 pathway, regulation of the cell cycle and stress related transcription [8, 15, 20, 28, 42–45].

In conclusion, our results demonstrate that the majority of the genes studied are both responsive to glucose deprivation in IRE1 dependent manner as well as to endoplasmic reticulum stress and potentially contribute to regulation of apoptosis and cell proliferation, but the mechanisms and functional significance of activation or suppression of their expression through IRE1 inhibition as well as glucose deprivation are different and warrant further investigation. Thus, the changes observed in the studied genes expression partially 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 [27, 39, 47, 48].

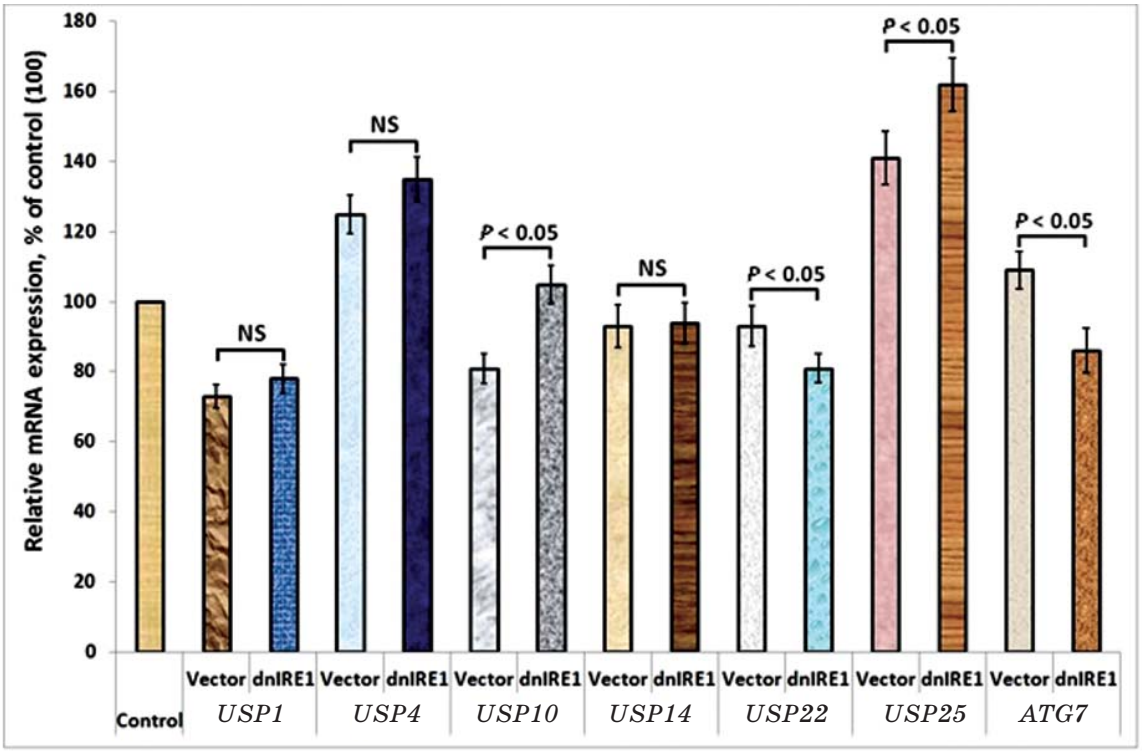

Fig. 8. **Comparative effect of glucose deprivation on the expression level of *ATG7* and a subset of USP genes in two types of glioma cells: control cells transfected by vector (Vector) and cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR**

REFERENCES

1. *Satija Y. K., Bhardwaj A., Das S.* A portrayal of E3 ubiquitin ligases and deubiquitylases in cancer. *Int. J. Cancer*. 2013, V. 133, P. 2759–2768.
2. *Mojsa B., Lassot I., Desagher S.* Mcl-1 ubiquitination: unique regulation of an essential survival protein. *Cells*. 2014, V. 3, P. 418–437.
3. *Zhang J., Zhang X., Xie F., Zhang Z., van Dam H., Zhang L., Zhou F.* The regulation of TGF-β/SMAD signaling by protein deubiquitination. *Protein Cell*. 2014, V. 5, P. 503–517.
4. *Kessler B. M., Edelmann M. J.* PTMs in conversation: activity and function of deubiquitinating enzymes regulated via post-translational modifications. *Cell. Biochem. Biophys*. 2011, V. 60, P. 21–38.
5. *Li J., Tan Q., Yan M., Liu L., Lin H., Zhao F., Bao G., Kong H., Ge C., Zhang F., Yu T., Li J., He X., Yao M.* miRNA-200c inhibits invasion and metastasis of human non-small cell lung cancer by directly targeting ubiquitin specific peptidase 25. *Mol. Cancer*. 2014, V. 13, P. 166.
6. *Danilovskyi S. V., Minchenko D. O., Karbovskyi L. L., Moliavko O. S., Kovalevska O. V., Minchenko O. H.* ERN1 knockdown modifies the hypoxic regulation of *TP53*, *MDM2*, *USP7* and *PERP* gene expressions in U87 glioma cells. *Ukr. Biochem. J*. 2014, 86 (4), 90–102.
7. *Kashiwaba S., Kanao R., Masuda Y., Kusumoto-Matsuo R., Hanaoka F., Masutani C.* USP7 is a suppressor of PCNA ubiquitination and oxidative-stress-induced mutagenesis in human cells. *Cell Rep*. 2015, V. 13, P. 2072–2080.
8. *Zhiqiang Z., Qinghui Y., Yongqiang Z., Jian Z., Xin Z., Haiying M., Yuepeng G.* USP1 regulates AKT phosphorylation by modulating the stability of PHLPP1 in lung cancer cells. *J. Cancer Res. Clin. Oncol*. 2012, V. 138, P. 1231–1238.
9. *Lee J. K., Chang N., Yoon Y., Yang H., Cho H., Kim E., Shin Y., Kang W., Oh Y. T., Mun G. I., Joo K. M., Nam D. H., Lee J.* USP1 targeting impedes GBM growth by inhibiting stem cell maintenance and radioresistance. *Neuro Oncol*. 2016, V. 18, P. 37–47.
10. *Villamil M. A., Liang Q., Chen J., Choi Y. S., Hou S., Lee K. H., Zhuang Z.* Serine phosphorylation is critical for the activation of ubiquitin-specific protease 1 and its interaction with WD40-repeat protein UAF1. *Biochemistry*. 2012, V. 51, P. 9112–9123.
11. *Garcia-Santisteban I., Zorroza K., Rodriguez J. A.* Two nuclear localization signals in USP1 mediate nuclear import of the USP1/UAF1 complex. *PLoS ONE*. 2012, V. 7, E38570.
12. *Li Z., Hao Q., Luo J., Xiong J., Zhang S., Wang T., Bai L., Wang W., Chen M., Wang W., Gu L., Lv K., Chen J.* USP4 inhibits p53 and NF-κB through deubiquitinating and stabilizing HDAC2. *Oncogene*. 2016, V. 35, P. 2902–2912.
13. *Yun S. I., Kim H. H., Yoon J. H., Park W. S., Hahn M. J., Kim H. C., Chung C. H., Kim K. K.* Ubiquitin specific protease 4 positively regulates the WNT/β-catenin signaling in colorectal cancer. *Mol. Oncol*. 2015, V. 9, P. 1834–1851.
14. *Zhang J., Zhang X., Xie F., Zhang Z., van Dam H., Zhang L., Zhou F.* The regulation of TGF-β/SMAD signaling by protein deubiquitination. *Protein Cell*. 2014, V. 5, P. 503–517.
15. *Liu H., Xu X. F., Zhao Y., Tang M. C., Zhou Y. Q., Lu J., Gao F. H.* MicroRNA-191 promotes pancreatic cancer progression by targeting USP10. *Tumour Bio*. 2014, V. 35, P. 12157–12163.
16. *Lin Z., Yang H., Tan C., Li J., Liu Z., Quan Q., Kong S., Ye J., Gao B., Fang D.* USP10 antagonizes c-Myc transcriptional activation through SIRT6 stabilization to suppress tumor formation. *Cell Rep*. 2013, V. 5, P. 1639–1649.
17. *Wang Y., Wang J., Zhong J., Deng Y., Xi Q., He S., Yang S., Jiang L., Huang M., Tang C., Liu R.* Ubiquitin-specific protease 14 (USP14) regulates cellular proliferation and apoptosis in epithelial ovarian cancer. *Med. Oncol*. 2015, V. 32, P. 379.
18. *Xu D., Shan B., Lee B. H., Zhu K., Zhang T., Sun H., Liu M., Shi L., Liang W., Qian L., Xiao J., Wang L., Pan L., Finley D., Yuan J.* Phosphorylation and activation of ubiquitin-specific protease-14 by Akt regulates the ubiquitin-proteasome system. *Elife*. 2015, V. 4, e10510.
19. *D'Arcy P., Wang X., Linder S.* Deubiquitinase inhibition as a cancer therapeutic strategy. *Pharmacol. Ther*. 2015, V. 147, P. 32–54.
20. *Tang B., Tang F., Li B., Yuan S., Xu Q., Tomlinson S., Jin J., Hu W., He S.* High USP22 expression indicates poor prognosis in hepatocellular carcinoma. *Oncotarget*. 2015, V. 6, P. 12654–12667.
21. *Liu Y. L., Zheng J., Tang L. J., Han W., Wang J. M., Liu D. W., Tian Q. B.* The deubiquitinating enzyme activity of USP22 is necessary for regulating HeLa cell growth. *Gene*. 2015, V. 572, P. 49–56.
22. *Ao N., Liu Y., Bian X., Feng H., Liu Y.* Ubiquitin-specific peptidase 22 inhibits colon cancer cell invasion by suppressing the signal transducer and activator of transcription 3/matrix metalloproteinase 9 pathway. *Mol. Med. Rep*. 2015, V. 12, P. 2107–2113.
23. *Blount J. R., Burr A. A., Denuc A., Marfany G., Todi S. V.* Ubiquitin-specific protease 25 functions in endoplasmic reticulum-associated degradation. *PLoS One*. 2012, V. 7, e36542.

24. *Kim S., Lee D., Lee J., Song H., Kim H. J., Kim K. T.* Vaccinia-related kinase 2 controls the stability of the eukaryotic chaperonin TRiC/CCT by inhibiting the deubiquitinating enzyme USP25. *Mol. Cell. Biol.* 2015, V. 35, P. 1754–1762.
25. *Galluzzi L., Bravo-San Pedro J. M., Kroemer G.* Autophagy mediates tumor suppression via cellular senescence. *Trend. Cell Biol.* 2016, V. 26, P. 1–3.
26. *Moenner M., Pluquet O., Bouchecareilh M., Chevet E.* Integrated endoplasmic reticulum stress responses in cancer. *Cancer Res.* 2007, V. 67, P. 10631–10634.
27. *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, a004424.
28. *Pluquet O., Dejeans N., Chevet E.* Watching the clock: endoplasmic reticulum-mediated control of circadian rhythms in cancer. *Ann. Med.* 2014, V. 46, P. 233–243.
29. *Auf G., Jabouille A., Guerit S., Pineau R., Delugin M., Bouchecareilh M., Magnin N., Favereaux A., Maitre M., Gaiser T., von Deimling A., Czabanka M., Vajkoczy P., Chevet E., Bikfalvi A., 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, V. 107, P. 15553–15558.
30. *Lenihan C. R., Taylor C. T.* The impact of hypoxia on cell death pathways. *Biochem. Soc. Trans.* 2013, V. 41, P. 657–663.
31. *Chesney J., Clark J., Klarer A. C., Imbert-Fernandez Y., Lane A. N., Telang S.* Fructose-2,6-bisphosphate synthesis by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) is required for the glycolytic response to hypoxia and tumor growth. *Oncotarget*. 2014, V. 5, P. 6670–6686.
32. *Hetz C., Chevet E., Harding H. P.* Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov*. 2013, V. 12, P. 703–719.
33. *Minchenko O. H., Tsymbal D. O., Moenner M., Minchenko D. O., Kovalevska O. V., Lypova N. M.* Inhibition of the endoribonuclease of ERN1 signaling enzyme affects the expression of proliferation-related genes in U87 glioma cells. *Endoplasm. Reticul. Stress Dis.* 2015, V. 2, P. 18–29.
34. *Mani 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, V. 307, P. 901–907.
35. *Minchenko O. H., Kryvdiuk I. V., Minchenko D. O., Riabovol O. O., Halkin O. V.* Inhibition of IRE1 signaling affects expression of a subset genes encoding for TNF-related factors and receptors and modifies their hypoxic regulation in U87 glioma cells. *Endoplasm. Reticul. Stress Dis.* 2016, V. 3, P. 1–15.
36. *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.
37. *Huber A. L., Lebeau J., Guillaumot P., P trilli V., Malek M., Chilloux J., Fauvet F., Payen L., Kfoury A., Renno T., Chevet E., Mani 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.
38. *Tsymbal D. O., Minchenko D. O., Riabovol O. O., Ratushna O. O., Minchenko O. H.* IRE1 knockdown modifies glucose and glutamine deprivation effects on the expression of proliferation related genes in U87 glioma cells. *Biotechnol. acta*. 2016, 9 (1), 26–37. doi: 10.15407/biotech8.06.009.
39. *Auf G., Jabouille A., Delugin M., Gu rit 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.
40. *Minchenko D. O., Danilovskyi S. V., Kryvdiuk I. V., Bakalets T. V., Lypova N. M., Karbovsky L. L., Minchenko O. H.* Inhibition of ERN1 modifies the hypoxic regulation of the expression of TP53-related genes in U87 glioma cells. *Endoplasm. Reticul. Stress Dis*. 2014, V. 1, P. 18–26.
41. *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, V. 99, P. 900–908.
42. *Hou X., Wang L., Zhang L., Pan X., Zhao W.* Ubiquitin-specific protease 4 promotes TNF-alpha-induced apoptosis by deubiquitination of RIP1 in head and neck squamous cell carcinoma. *FEBS Lett*. 2013, V. 587, P. 311–316.
43. *Sun X. X., Challagundla K. B., Dai M. S.* Positive regulation of p53 stability and activity by the deubiquitinating enzyme otubain 1. *EMBO J.* 2012, V. 31, P. 576–592.

44. *Hu J., Yang D., Zhang H., Liu W., Zhao Y., Lu H., Meng Q., Pang H., Chen X., Liu Y., Cai L.* USP22 promotes tumor progression and induces epithelial-mesenchymal transition in lung adenocarcinoma. *Lung Cancer*. 2015, V. 88, P. 239–245.
45. *Xiong J., Gong Z., Zhou X., Liu J., Jiang H. E., Wu P., Li W.* p38 mitogen-activated protein kinase inhibits USP22 transcription in HeLa cells. *Biomed Rep*. 2015, V. 3, P. 461–467.
46. *L vy J., Cacheux W., Bara M. A., L'Hermitte A., Lepage P., Fraudeau M., Trentesaux C., Lemarchand J., Durand A., Crain A. M., Marchiol C., Renault G., Dumont F., Letourneur F., Delacre M., Schmitt A., Terris B., Perret C., Chamaillard M., Couty J. P., Romagnolo B.* Intestinal inhibition of Atg7 prevents tumour initiation through a microbiome-influenced immune response and suppresses tumour growth. *Nat. Cell. Biol.* 2015, V. 17, P. 1062–1073.
47. *Backer M. V., Backer J. M., Chinnaiyan P.* Targeting the unfolded protein response in cancer therapy. *Meth. Enzymol*. 2011, V. 491, P. 37–56.
48. *Johnson G. G., White M. C., Grimaldi M.* Stressed to death: targeting endoplasmic reticulum stress response induced apoptosis in gliomas. *Curr. Pharm. Des*. 2011, V. 17, P. 284–292.

ЕКСПРЕСІЯ ГЕНІВ СПЕЦИФІЧНИХ ДО УБІКВІТИНУ ПЕПТИДАЗ У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87 ІЗ ПРИГНІЧЕНИМ IRE1 ЗА УМОВ ДЕФІЦИТУ ГЛЮКОЗИ

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Вивчено вплив дефіциту глюкози на експресію генів, які кодують специфічні до убіквітину пептидази (USP) та протеїн 7, що стосується автофагії (ATG7), у клітинах гліоми лінії U87 за умов пригнічення inositol requiring enzyme-1 (IRE1). Показано, що дефіцит глюкози знижує експресію генів *USP1* та *USP10* і підвищує *USP4* та *USP25* у контрольних (трансфікованих пустим вектором) клітинах гліоми. Водночас рівень експресії генів *USP14, USP22* та ATG7 у цих клітинах істотно не змінюється. Пригнічення функції сигнального ензиму IRE1 у клітинах гліоми лінії U87 модифікує ефект дефіциту глюкози на експресію більшості досліджених генів. Таким чином, дефіцит глюкози змінює рівень експресії більшості генів специфічних до убіквітину пептидаз залежно від функціональної активності ензиму IRE1, що контролює процеси проліферації та росту пухлин як центральний медіатор стресу ендоплазматичного ретикулума.

Ключові слова: експресія мРНК, гени USP, інгібування IRE1, дефіцит глюкози, клітини гліоми лінії U87.

ЭКСПРЕССИЯ ГЕНОВ СПЕЦИФИЧЕСКИХ К УБИКВИТИНУ ПЕПТИДАЗ В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87 С ИНГИБИРОВАННЫМ IRE1 ПРИ ДЕФИЦИТЕ ГЛЮКОЗЫ

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Изучено влияние дефицита глюкозы на экспрессию генов, кодирующих специфические к убиквитину пептидазы (USP) и протеин 7, что имеет отношение к аутофагии (ATG7), в клетках глиомы линии U87 при угнетении inositol requiring enzyme-1 (IRE1). Показано, что дефицит глюкозы снижает экспрессию генов *USP1* и *USP10* и повышает *USP4* и *USP25* в контрольных (трансфецированных пустым вектором) клетках глиомы. В то же время уровень экспрессии генов *USP14, USP22* и ATG7 в этих клетках существенно не изменяется. Угнетение функции сигнального энзима IRE1 в клетках глиомы линии U87 модифицирует эффект дефицита глюкозы на экспрессию большинства изученных генов. Таким образом, дефицит глюкозы изменяет уровень экспрессии большинства генов специфических к убиквитину пептидаз в зависимости от функциональной активности энзима IRE1, который контролирует процессы пролиферации и роста опухолей в качестве центрального медиатора стресса эндоплазматического ретикулума.

Ключевые слова: экспрессия мРНК, гены USP, ингибирование IRE1, дефицит глюкозы, клетки глиомы линии U87.