EXPERIMENTAL ARTICLES

GLUCOSE DEPRIVATION AFFECTS THE EXPRESSION OF LONP1 AND CATHEPSINS IN IRE1 KNOCKDOWN U87 GLIOMA CELLS

O. H. Minchenko[1]
O. V. Halkin[1]
O. O. Riabovol[1]
D. O. Minchenko[1, 2]
A. Y. Kuznetsova[1]
O. O. Ratushna[1]

[1]Palladin Institute of Biochemistry
 of the National Academy of Sciences of Ukraine, Kyiv

[2]Bohomolets National Medical University, Kyiv, Ukraine

E-mail: ominchenko@yahoo.com

To study the effect of glucose deprivation on the expression of genes encoding for LONP1/PRSS15 and cathepsins in U87 glioma cells in relation to inhibition of inositol requiring enzyme-1 (IRE1) was the aim of the research. It was shown that glucose deprivation up-regulated the expression of *CTSA, CTSB, CTSD, CTSK, CTSL, CTSO,* and *LONP1* genes and did not change the expression of *CTSC, CTSF,* and *CTSS* genes in control glioma cells (transfected by empty vector). Inhibition of IRE1 signaling enzyme function in U87 glioma cells modified effect of glucose deprivation on the expression of most studied genes: removed the effect of glucose deprivation on *CTSA* and *CTSO* genes, introduces on *CTSC* and *CTSS* genes, reduced — on *CTSK* gene, and enhanced — on *CTSL* gene. Therefore, glucose deprivation affect the expression level of most studied genes in relation to the functional activity of IRE1 enzyme, a central mediator of endoplasmic reticulum stress, which control cell proliferation and tumor growth.

Key words: mRNA expression, CTS, LONP1/PRSS15, IRE1 inhibition, glucose deprivation, U87 glioma cells.

The mitochondrial lon peptidase 1 (LONP1, also known as serine protease 15 (PRSS15), is a mitochondrial matrix protein that belongs to the Lon family of ATP-dependent proteases and mediates the selective degradation of misfolded, unassembled or oxidatively damaged polypeptides as well as certain short-lived regulatory proteins in the mitochondrial matrix, but not aggregated proteins [1, 2]. It may also have a chaperone function in the assembly of inner membrane protein complexes, and participate in the regulation of mitochondrial gene expression and maintenance of the integrity of the mitochondrial genome [3]. Lon peptidase 1 can be included in the growing class of proteins that are not responsible for oncogenic transformation, but that are essential for survival and proliferation of cancer cells, because it is a key enzyme controlling mitochondrial bioenergetics in cancer [3,

4]. Therefore, LONP1 is an essential protein for life and that it also performs a critical function in tumorigenesis by regulating the bioenergetics of cancer cells as a central regulator of mitochondrial activity in oncogenesis [2]. Recently was shown that inhibition of LONP1 by the anticancer molecule triterpenoid alters mitochondria and is associated to cell death in RKO human colon cancer cells [5]. LONP1 expression is induced by various stimuli, including hypoxia and reactive oxygen species, and provides protection against cell stress [6]. Furthermore, down-regulation of this enzyme is associated with with ageing, while up-regulation is observed in tumor cells, and is correlated with a more aggressive phenotype of cancer [6]. Consequently, the mitochondrial Lon peptidase 1 is at the crossroads of oxidative stress, ageing and cancer. Goo et al. [7] shown that mitochondrial proteins, LONP1 and

prohibitin, are overexpressed in HTRA2(-/-) mouse embryonic fibroblast cells and HTRA2 knock-down HEK293T cells, indicating that mitochondrial HTRA2 might be an upstream regulator of mitochondrial homeostasis.

Cathepsins are a group of lysosomal proteases that have a key role in cellular protein turnover as well as multiple roles in cancer and autophagy [8–10]. Cathepsin A (CTSA), also known as chaperone protective protein and protective protein for beta-galactosidase, is a lysosomal carboxypeptidase present at the cell surface, endoplasmic reticulum, nucleus and secreted outside the cell, which appears to be essential for both the activity of beta-galactosidase and neuraminidase, it associates with these enzymes and exerts a protective function necessary for their stability and activity and involved in tumor progression and metastasis by degrading the extracellular matrix [10, 11]. Cathepsin A plays a significant role in the processing of endogenous bioactive peptides and is also involved in inhibition of chaperon-mediated autophagy through proteolytic processing of LAMP-2A [8, 9]. Recently was shown that overexpression of CTSA associates with the cellular oxidative stress response [12].

CTSB is a lysosomal cysteine proteinase, which also present at the cell surface, nucleus and mitochondrion, implicated in tumor invasion and metastasis; toll-like receptor signaling pathway [13]. Recently was shown that inhibition of cathepsin B activity by clioquinol-ruthenium complex impairs tumor cell invasion [14]. Extracellular matrix remodeling by cell adhesion-related processes is critical for proliferation and tissue homeostasis and the actin-binding protein, filamin A, regulates the organization and remodeling of the pericellular collagen matrix through its effects on degradation pathways, particularly MMP-9 and cathepsin B, which is necessary for intracellular collagen digestion [15]. Huber et al. [16] shown that the urokinase plasminogen activator receptor strongly interacts with cysteine-rich angiogenic inducer 61 (CYR61) and the Y-box-binding protein 1(YB-1) in the triple-negative breast cancer and that both interactors significantly correlated with expression levels of cathepsin B, c-MET and the tumor grade. In addition, expression levels of CYR61 significantly correlated with cathepsin D [16]. Moreover, myeloid-derived suppressor cells are immune suppressive cells that are hallmarks of human cancer and apoptosis pathways play a key role of the in homeostasis of these cells through CTSB and CTSD [17]. Cathepsin B also participates in autophagy, which mediates tumor suppression via cellular senescence, particularly in TGF-β-induced autophagy [18−20].

Increased tissue activity of cathepsin C can be observed in many pathological conditions [11]. It is associated with an enhanced degradation of glycosaminoglycans, proteoglycans, and glycoproteins, and results in their decreased tissue content. Cathepsin C releases the glycosidases from complexes formed with cathepsin A, and reinstates their activity [11]. Furthermore, CTSB, CTSC, CTSD are increased in numerous tumors [21, 22]. Thus, human telomerase reverse transcriptase (hTERT) overexpression is responsible for the upregulation of the cysteine protease cathepsin D by regulating EGR-1 to activate invasiveness in cancer progression [22]. It is interesting to note that matrix-metalloproteinase-9 is cleaved and activated by cathepsin K [23]. Moreover, almost identical substrate specificities were determined for cysteine cathepsins K, L and S [24]. Cathepsin S also has relation to regulation of autophagy [25].

The unfolded protein response/endoplasmic reticulum stress is responsible for enhanced cancer cell proliferation and knockdown of IRE1, a major signaling pathway of endoplasmic reticulum stress, 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 [33]. 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 [28, 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].

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 critical substrate for glycolysis, which is important to glioma development and a more agressive behaviour [35]. 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 [36, 37].

The aim of this study was investigation the effect of glucose deprivation condition on the expression of LONP1/PRSS15 and a subset of cathepsins in glioma cells in 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 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 PRSS15 and a subset of cathepsins mRNA. 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 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 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 medium without glucose (from Gibco) and plates were exposed to this condition for 16 hrs.

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 [38] 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 [39]. 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 [38]. 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 [38]. The expression level of LONP1, CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, and CTSS 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 "RotorGene RG-3000" qPCR (Corbett Research, Germany) 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 mitochondrial lon peptidase 1 (LONP1; EC_number="3.4.21.-"), also known as hLON ATP-dependent protease and PRSS15 (protease, serine, 15), was performed using forward primer (5′− ATCTACCTGAGCGAC-ATGGG −3′) and reverse primer (5′− TTA-CGGTGGGTCTGCTTGAT −3′). These oligo-nucleotides correspond to sequences 1111−1130 and 1304−1285 of human LONP1 cDNA (GenBank accession number NM_004793). The size of amplified fragment is 194 bp.

For amplification of the cathepsin A (CTSA; EC_number="3.4.16.5"),

also known as chaperone protective protein and carboxypeptidase C, cDNA we used next primers: forward 5′– CAGCTGCTTCCACCTACCTC –3′ and reverse 5′– CTTCTGGTTGAGGGAATCCA –3′. The nucleotide sequences of these primers correspond to sequences 1432–1451 and 1682–1663 of human CTSA cDNA (GenBank accession number NM_000308). The size of amplified fragment is 251 bp.

The amplification of cDNA of the cathepsin B (CTSB; EC_number="3.4.22.1") was performed using forward primer (5′– CAAGCCACCCCAGAGAGTTA –3′) and reverse primer (5′– TAGAGGCCACCA-GAAACCAG –3′). These oligonucleotides correspond to sequences 360–379 and 680–661 of human CTSB cDNA (GenBank accession number NM_001908). The size of amplified fragment is 321 bp.

For amplification of the CTSC (EC_number="3.4.14.1") cDNA we used next primers: forward 5′– TCAGACCCCAATCCTAAGCC –3′ and reverse 5′– GCATGGAGAATCAGTGCCTG –3′. The nucleotide sequences of these primers correspond to sequences 949–968 and 1108–1089 of human CTSC cDNA (GenBank accession number NM_001814). The size of amplified fragment is 160 bp.

The amplification of cDNA for the CTSD (EC_number="3.4.23.5") was performed using forward primer (5′– CAAGTTCGATGGCATCCTGG –3′) and reverse primer (5′– CGGGTGACATTCAGGTAGGA –3′). These oligonucleotides correspond to sequences 712–731 and 930–911 of human CTSD cDNA (GenBank accession number NM_001909). The size of amplified fragment is 219 bp.

For amplification of the CTSF (EC_number="3.4.22.41") cDNA we used next primers: forward 5′– AGGAGCTCTTGGACTGTGAC –3′ and reverse 5′– TAGACCTTGGCCTTCTCTGC –3′. The nucleotide sequences of these primers correspond to sequences 1052–1071 and 1217–1198 of human CTSF cDNA (GenBank accession number NM_003793). The size of amplified fragment is 166 bp.

The amplification of the CTSK (EC_number="3.4.22.38") cDNA was performed using forward primer (5′– GCTCAAGGTTCTGCTGCTAC –3′) and reverse primer (5′– TCTTCACTGGTCATGTCCCC –3′). These oligonucleotides correspond to sequences 238–257 and 483–464 of human CTSK cDNA (GenBank accession number NM_000396). The size of amplified fragment is 246 bp.

For amplification of the CTSL (EC_number="3.4.22.15") cDNA we used next primers: forward 5′– ACAGCTTCACAATGGCCATG –3′ and reverse 5′– AAGCCCAACAAGAACCACAC –3′. The nucleotide sequences of these primers correspond to sequences 562–581 and 717–698 of human CTSL cDNA (GenBank accession number NM_001912). The size of amplified fragment is 210 bp.

The amplification of the CTSO (EC_number="3.4.22.42") cDNA was performed using forward primer (5′– ATTATGGCTGCAATGGAGGC –3′) and reverse primer (5′– GGGCCAAAGGTAAGAAGTGC –3′). These oligonucleotides correspond to sequences 549–568 and 768–749 of human CTSO cDNA (GenBank accession number NM_001334). The size of amplified fragment is 220 bp.

For amplification of the CTSS (EC_number="3.4.22.27") cDNA we used next primers: forward 5′– AACAAGGGCATCGACTCAGA –3′ and reverse 5′– AAGAAAGAAGGATGACGCGC –3′. The nucleotide sequences of these primers correspond to sequences 844–863 and 468–449 of human CTSS cDNA (GenBank accession number NM_004079). The size of amplified fragment is 197 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 LONP1, CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, and CTSS 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 [40].

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 LONP1/PRSS15, CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, and CTSS in U87 glioma cells in relation to inhibition 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 glucose deprivation condition leads to up-regulation of LONP1 mRNA expression (+30%) as compared to cells growing with glucose. In glioma cells without functional activity of signaling enzyme IRE1 the expression of this gene is also increased by glucose deprivation (+42%). Therefore, inhibition of IRE1 signaling enzyme function in U87 glioma cells by dnIRE1 enhances the sensitivity of *LONP1* gene expression to glucose deprivation.

Additionally, we found that expression of gene encoding for cathepsin A is also up-regulated (+49%) upon glucose deprivation condition in control glioma cells as compared to cells growing with glucose (Fig. 2). In glioma cells containing dnIRE1, the expression of CTSA mRNA was resistant to glucose deprivation, indicating up-regulation of this gene expression by glucose deprivation is IRE1-dependent. Consequently, inhibition of IRE1 signaling enzyme function in U87 glioma cells by dnIRE1 removes the sensitivity of *CTSA* gene expression to glucose deprivation. As shown in Fig. 3, glucose deprivation leads to small but statistically significant up-regulation (+16%) of the expression level of *CTSB* gene in control glioma cells as compared to cells growing with glucose, but inhibition of IRE1 signaling enzyme eliminates this effect of glucose deprivation, indicating that down-regulation of this gene expression induced by glucose deprivation is dependent from IRE1 signaling.

At the same time, the expression level of cathepsin C is resistant to glucose deprivation condition in control glioma cells, but inhibition
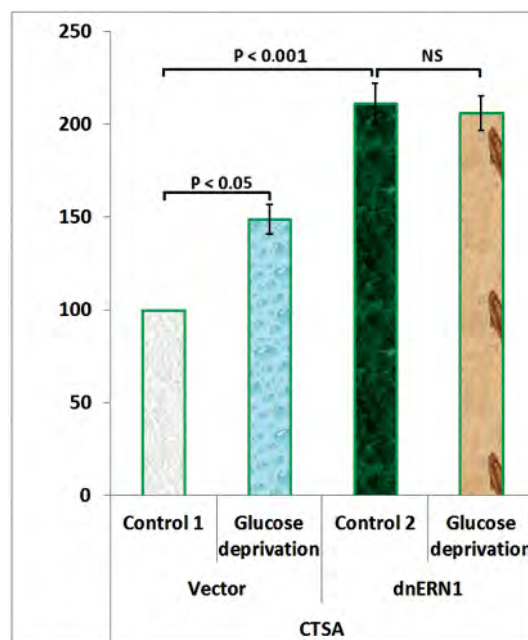

Fig. 1. **Effect of glucose deprivations on the expression level of mitochondrial lon peptidase 1 (LONP1), also known as PRSS15 (serine protease 15), mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR:**
values of LONP1/PRSS15 mRNA expressions were normalized to beta-actin mRNA and represented as percent of control 1 (100 %); hereafter mean ± SEM; *n* = 4

Fig. 2. **Effect of glucose deprivation on the expression level of cathepsin A (CTSA) mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR:**
hereafter: values of this mRNA expressions were normalized to beta-actin mRNA and represented as percent of control 1 (100 %); NS — no significant changes

of signaling enzyme IRE1 introduces the sensitivity of this gene expression to glucose deprivation (+23%), indicating that sensitivity of *CTSC* gene expression to glucose deprivation is dependent of IRE1 signaling (Fig. 4). We have also studied the expression of *CTSF* gene. As shown in Fig. 5, exposure the control glioma cells upon glucose deprivation condition does not change significantly the expression of CTSF mRNA in control glioma cells as compared to cells growing with glucose, indicating resistance of this gene expression to glucose deprivation. Furthermore, exposure of glioma cells containing dnIRE1 upon glucose deprivation condition also does not change the expression of this mRNA, indicating regulation of this gene expression by glucose deprivation is independent of IRE1 signaling.

As shown in Fig. 6, the expression level of CTSD mRNA significantly increases in both control glioma cells and cells with knockdown of IRE1 signaling enzyme (+60% and 55%, correspondingly) upon glucose deprivation condition, indicating that up-regulation of this gene expression by glucose deprivation is independent of IRE1 signaling enzyme function. Furthermore, we studied the effect of glucose deprivation on the expression level of cathepsin L in both control glioma cells and cells with knockdown of IRE1 signaling.

Investigation of cathepsin K gene expression shown that this gene is extremely sensitive to glucose deprivation in glioma cells with native IRE1 (+113%) and IRE1 inhibition reduces this glucose deprivation effect to +47% (Fig. 7). Thus, regulation of *CTSK* gene expression by glucose deprivation is complex and partly dependent of IRE1 signaling. As shown in Fig. 8, glucose deprivation leads to small but statistically significant up-regulation (+16%) of the expression level of CTSL mRNA in control glioma cells as compared to cells growing with glucose, but inhibition of IRE1 signaling enzyme function strongly enhances the effect of glucose deprivation on this gene expression (+74%), indicating that IRE1 participates in regulation of this gene expression by glucose deprivation.

Finally, we studied the expression level of genes encoding for cathepsin O and S (CTSO and CTSS) and found different regulation of these genes by glucose deprivation (Fig. 9 and 10). It was shown that exposure control glioma cells upon glucose deprivation condition leads to up-regulation of CTSO mRNA expression level (+24%), but did not change the level of CTSS mRNA expression as compared to cells growing with glucose. As shown in Fig. 9, glioma cells containing dnIRE1 are resistant to glucose deprivation. At the same
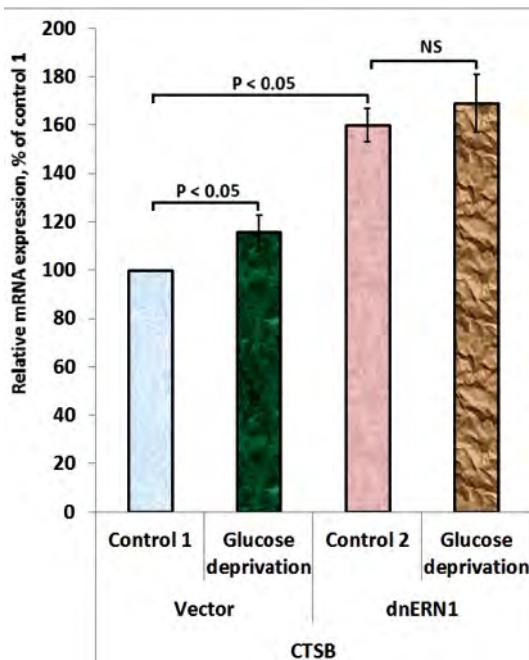

Fig. 3. **Effect of glucose deprivation on the expression level of CTSB mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**
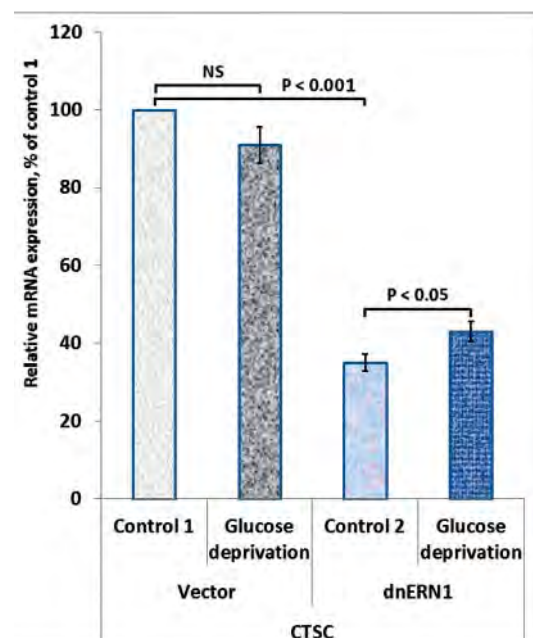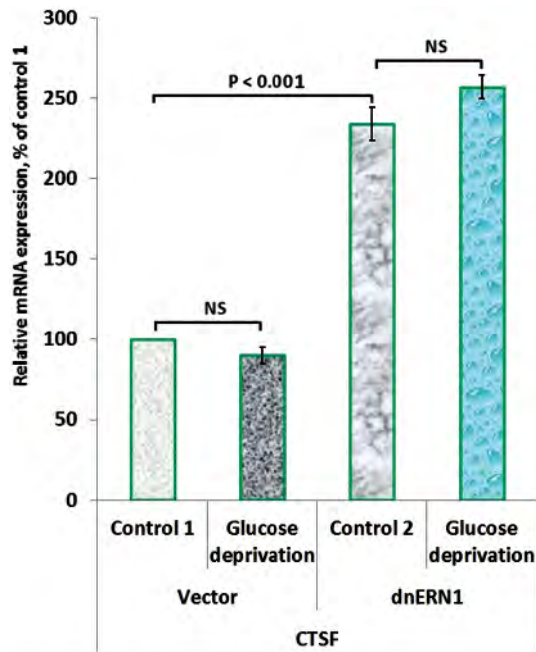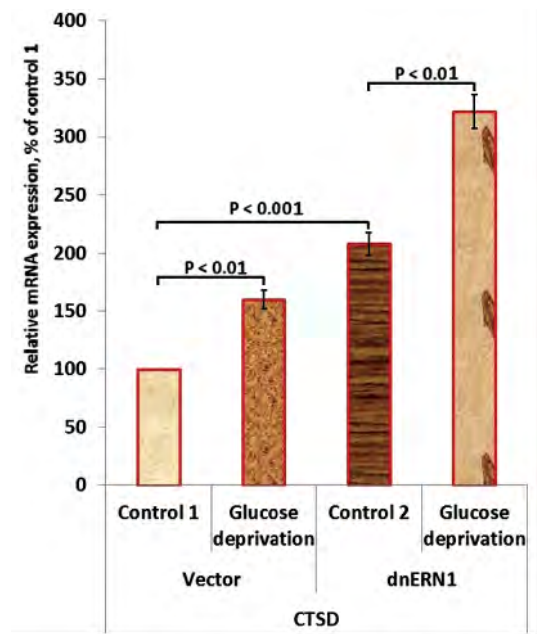

Fig. 4. **Effect of glucose deprivation on the expression level of CTSC mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR**
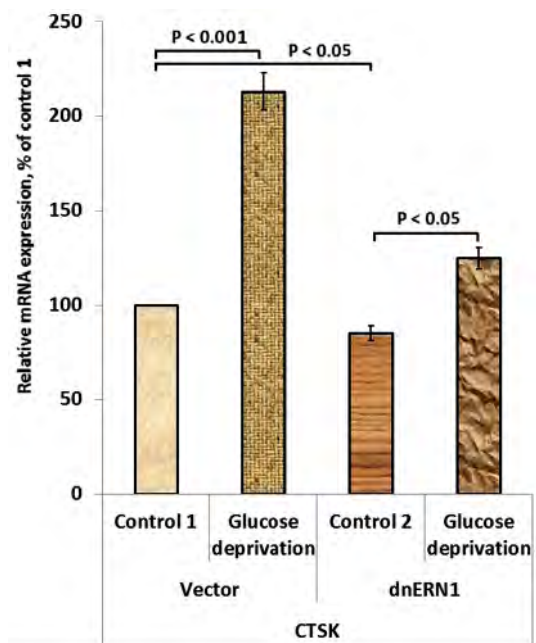
Fig. 5. Effect of glucose deprivation on the expression level of CTSF mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR

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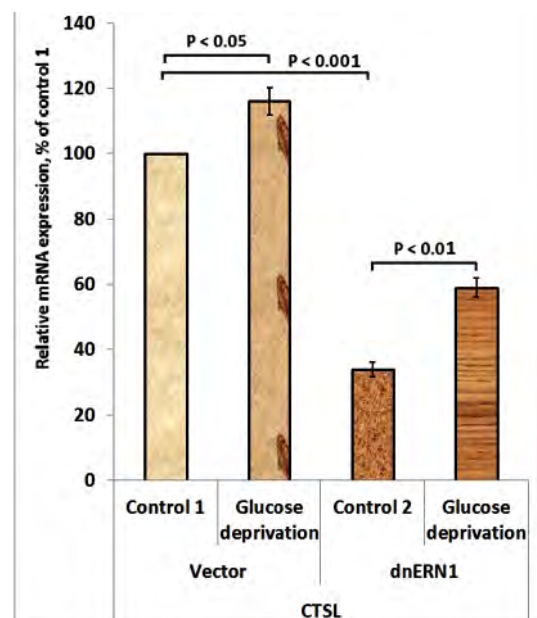

Fig. 7. Effect of glucose deprivation on the expression level of CTSK mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR
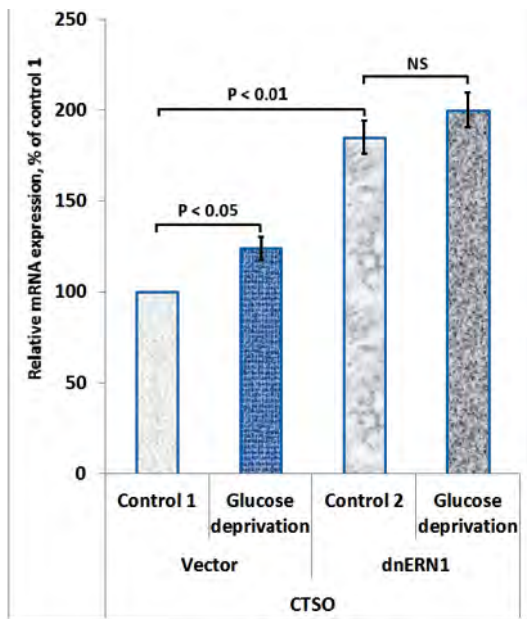

Fig. 8. Effect of glucose deprivation on the expression level of CTSL mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR

Fig. 9. Effect of glucose deprivation on the expression level of CTSO mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR
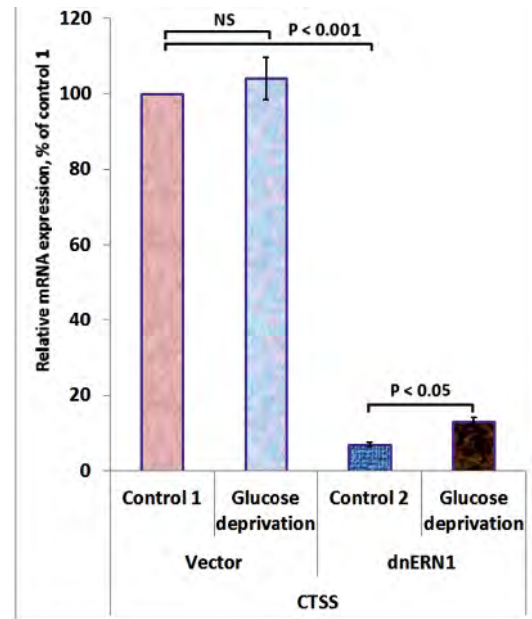

Fig. 10. Effect of glucose deprivation on the expression level of CTSS mRNA in control U87 glioma cells stable transfected with vector (Vector) and cells with inhibited function of signaling enzyme IRE1 (dnIRE1) measured by qPCR

time, inhibition of IRE1 signaling enzyme introduces the sensitivity of *CTSS* gene expression to glucose deprivation, indicating IRE1-dependent character of this gene expression regulation by glucose deprivation (Fig. 10). Consequently, inhibition of IRE1 signaling enzyme function in U87 glioma cells by dnIRE1 removes the sensitivity of *CTSO* gene expression to glucose deprivation, but introduces sensitivity of *CTSS* gene expression.

As shown in Fig. 11 and 12, inhibition of IRE-1α signaling does not significantly change the effect of glucose deprivation on the expression of *LONP1/PRSS15, CTSB,* and *CTSD* genes, but modifies the sensitivity of *CTSA, CTSC, CTSK, CTSL, CTSO,* and *CTSS* genes expression to glucose deprivation in glioma cells by different ways: removes sensitivity of *CTSA* and *CTSO* genes, introduces sensitivity of *CTSC* and *CTSS* genes, enhances *CTSL* gene and augments sensitivity of *CTSK* gene to this treatment.

Thus, this study has demonstrated that glucose deprivation affects the expression of the majority of the genes encoding cathepsins as well as LONP1/PRSS15 preferentially in the IRE1-dependent manner and that these genes

potentially contribute to regulation of cell proliferation, apoptosis, and metastasis.

LONP1 have variable functions and increased level of this gene transcript in control glioma cells upon glucose deprivation can be responsible for selective degradation of misfolded and certain short-lived regulatory proteins in the mitochondrial matrix, which should be induced by glucose deprivation [2]. At the same time, the IRE1 signaling enzyme knockdown in glioma cells does not significantly change the sensitivity of *LONP1* gene expression to glucose deprivation. It is possible that the regulation of this gene expression by glucose deprivation is not mediated by IRE1 and blockade of this signaling enzyme function does not affect the sensitivity of the expression of *LONP1* gene to glucose deprivation. Thus, the increased expression of the *LONP1* gene upon glucose deprivation is agreed well with functional role of this protease [3, 4]. It is possible that the regulation of this gene expression by glucose deprivation is mediated by other signaling pathways of endoplasmic reticulum stress like *ATF3*, *HOXC6*, and *FOXF1* genes [33]. Therefore, LONP1 protease is an essential enzyme and plays a critical function in

tumorigenesis by regulating the bioenergetics of cancer cells as a central regulator of mitochondrial activity in oncogenesis [2]. Furthermore, the expression of this mitochondrial protease is induced by various stimuli, including hypoxia and reactive oxygen species, and possibly provides protection against cell stress [6].

We have also shown that the expression of cathepsin A and B, which have multiple roles in cancer and autophagy [8, 9, 13, 19], is also increased in control glioma cells upon glucose deprivation, but the knockdown of IRE1 signaling enzyme in glioma cells removes the sensitivity of both these genes expression to glucose deprivation. It is possible that the regulation of *CTSA* and *CTSB* genes expression by glucose deprivation is mediated by IRE1 and blockade of this signaling enzyme function decreases the sensitivity of the expression of these genes to glucose deprivation. Thus, our results that inhibition of IRE1 via overexpression of dnIRE1 removes the sensitivity of the expression of *CTSA* and *CTSB* genes to glucose deprivation are agree well with functional role of these proteases and suppression of glioma cell proliferation [8, 9, 13, 27]. It is interesting to note that overexpression of chaperone protective protein CTSA associates with the cellular oxidative stress response [12] and involved in tumor progression and metastasis by degrading the extracellular matrix [10, 11]. Glucose deprivation is also induced the expression level of *CTSD, CTSK, CTSL,* and *CTSO* in control glioma cells, but the expression of *CTSC, CTSF,* and *CTSS* is resistant to glucose deprivation in these glioma cells. At the same time, inhibition of IRE1 had diverse effect on the sensitivity of these genes expression to glucose deprivation. It is possible that all these cathepsins participate in many pathological conditions and most of them are implicated in tumor invasion, metastasis, and autophagy [11, 22, 23, 25, 41, 42]. Moreover, almost identical substrate specificities were determined for cysteine cathepsins K, L and S, but almost all cathepsins have specific functions and consequently diverse changes upon glucose deprivation [24, 43].

In conclusion, our results demonstrate that the majority of the genes studied are both responsive to glucose deprivation in IRE1 dependent manner and potentially contribute to regulation of cell proliferation, metastasis, and apoptosis through various signaling pathways and stress related transcription, but the mechanisms and functional significance of activation 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, 38, 44−46].

REFERENCES

1. *Quirós P. M., Español Y., Acín-Pérez R., Rodríguez F., Bárcena C., Watanabe K., Calvo E., Loureiro M., Fernández-García M. S., Fueyo A., Vázquez J., Enríquez J. A., López-Otín C.* ATP-dependent Lon protease controls tumor bioenergetics by reprogramming mitochondrial activity. *Cell Rep.* 2014, 8 (2), 542−556.

2. *Bezawork-Geleta A., Brodie E. J., Dougan D. A., Truscott K. N.* LON is the master protease that protects against protein aggregation in human mitochondria through direct degradation of misfolded proteins. *Sci. Rep.* 2015, V. 5, P. 17397.

3. *Pinti M., Gibellini L., Nasi M., De Biasi S., Bortolotti C. A., Iannone A., Cossarizza A.* Emerging role of Lon protease as a master regulator of mitochondrial functions. *Biochim. Biophys. Acta.* 2016, 1857 (8), 1300−1306.

4. *Quirós P. M., Bárcena C., López-Otín C.* Lon protease: A key enzyme controlling mitochondrial bioenergetics in cancer. *Mol. Cell. Oncol.* 2014, 1 (4), e968505.

5. *Gibellini L., Pinti M., Bartolomeo R., De Biasi S., Cormio A., Musicco C., Carnevale G., Pecorini S., Nasi M., De Pol A., Cossarizza A.* Inhibition of Lon protease by triterpenoids alters mitochondria and is associated to cell death in human cancer cells. *Oncotarget.* 2015, 6 (28), 25466−25483.

6. *Pinti M., Gibellini L., Liu Y., Xu S., Lu B., Cossarizza A.* Mitochondrial Lon protease at the crossroads of oxidative stress, ageing and cancer. *Cell. Mol. Life Sci.* 2015, 72 (24), 4807−4824.

7. *Goo H. G., Rhim H., Kang S.* HtrA2/Omi influences the stability of LON protease 1 and prohibitin, proteins involved in mitochondrial homeostasis. *Exp. Cell Res.* 2014, 328 (2), 456−465.

8. *Timur Z. K., Akyildiz Demir S., Seyrantepe V.* Lysosomal cathepsin A plays a significant role in the processing of endogenous bioactive peptides. *Front. Mol. Biosci.* 2016, V. 3, P. 68.

9. *Kaminskyy V., Zhivotovsky B.* Proteases in autophagy. *Biochim. Biophys. Acta.* 2012, 1824 (1), 44–50.

10. *Haznedaroglu I. C., Malkan U. Y.* Local bone marrow renin-angiotensin system in the genesis of leukemia and other malignancies. *Eur. Rev. Med. Pharmacol. Sci.* 2016, 20 (19), 4089–4111.

11. *Minarowska A., Minarowski Ł., Karwowska A., Milewska A. J., Gacko M.* Role of cathepsin A and cathepsin C in the regulation of glycosidase activity. *Folia Histochem. Cytobiol.* 2012, 50 (1), 20–24.

12. *Petrera A., Kern U., Linz D., Gomez-Auli A., Hohl M., Gassenhuber J., Sadowski T., Schilling O.* Proteomic Profiling of Cardiomyocyte-Specific Cathepsin A Overexpression Links Cathepsin A to the Oxidative Stress Response. *J. Proteome Res.* 2016, 15 (9), 3188–3195.

13. *Aggarwal N., Sloane B. F.* Cathepsin B: multiple roles in cancer. *Proteomics Clin. Appl.* 2014, 8 (5–6), 427–437.

14. *Mitrović A., Kljun J., Sosič I., Gobec S., Turel I., Kos J.* Clioquinol-ruthenium complex impairs tumour cell invasion by inhibiting cathepsin B activity. *Dalton Trans.* 2016, 45 (42), 16913–16921.

15. *Mezawa M., Pinto V. I., Kazembe M. P., Lee W. S., McCulloch C. A.* Filamin A regulates the organization and remodeling of the pericellular collagen matrix. *FASEB J.* 2016, Oct. 30 (10), 3613–3627.

16. *Huber M. C., Falkenberg N., Hauck S. M., Priller M., Braselmann H., Feuchtinger A., Walch A., Schmitt M., Aubele M.* Cyr61 and YB-1 are novel interacting partners of uPAR and elevate the malignancy of triple-negative breast cancer. *Oncotarget.* 2016, 7 (28), 44062–44075.

17. *Liu F., Li X., Lu C., Bai A., Bielawski J., Bielawska A., Marshall B., Schoenlein P. V., Lebedyeva I. O., Liu K.* Ceramide activates lysosomal cathepsin B and cathepsin D to attenuate autophagy and induces ER stress to suppress myeloid-derived suppressor cells. *Oncotarget.* 2016, Nov. 17. doi: 10.18632/oncotarget.13438. [Epub ahead of print].

18. *Jiang Y., Woosley A. N., Sivalingam N., Natarajan S., Howe P. H.* Cathepsin-B-mediated cleavage of Disabled-2 regulates TGF-β-induced autophagy. *Nat. Cell Biol.* 2016, 18 (8), 851–863.

19. *Man S. M., Kanneganti T. D.* Regulation of lysosomal dynamics and autophagy by CTSB/cathepsin B. *Autophagy.* 2016, Oct 27, 1–2. [Epub ahead of print].

20. *Galluzzi L., Bravo-San Pedro J. M., Kroemer G.* Autophagy mediates tumor suppression via cellular senescence. *Trends Cell Biol.* 2016, V. 26, P. 1–3.

21. *Ruffell B., Affara N. I., Cottone L., Junankar S., Johansson M., DeNardo D. G., Korets L., Reinheckel T., Sloane B. F., Bogyo M., Coussens L. M.* Cathepsin C is a tissue-specific regulator of squamous carcinogenesis. *Genes Dev.* 2013, 27 (19), 2086–2098.

22. *Park Y. J., Kim E. K., Bae J. Y., Moon S., Kim J.* Human telomerase reverse transcriptase (hTERT) promotes cancer invasion by modulating cathepsin D via early growth response (EGR)-1. *Cancer Lett.* 2016, 370 (2), 222–231.

23. *Christensen J., Shastri V. P.* Matrix-metalloproteinase-9 is cleaved and activated by cathepsin K. *BMC Res. Notes.* 2015, V. 8, P. 322.

24. *Vizovisek M., Vidmar R., Van Quickelberghe E., Impens F., Andjelkovic U., Sobotic B., Stoka V., Gevaert K., Turk B., Fonovic M.* Fast profiling of protease specificity reveals similar substrate specificities for cathepsins K, L and S. *Proteomics.* 2015, 15 (14), 2479–2490.

25. *Huang C. C., Lee C. C., Lin H. H., Chen M. C., Lin C. C., Chang J. Y.* Autophagy-regulated ROS from xanthine oxidase acts as an early effector for triggering late mitochondria-dependent apoptosis in cathepsin S-targeted tumor cells. *PLoS ONE.* 2015, 10 (6), E0128045.

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. *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.

28. *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.

29. *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.

30. *Hetz C., Chevet E., Harding H. P.* Targeting the unfolded protein response in disease. *Nat. Rev. Drug Discov*. 2013, V. 12, P. 703–719.

31. *Lenihan C. R., Taylor C. T.* The impact of hypoxia on cell death pathways. *Biochem. Soc. Trans.* 2013, V. 41, P. 657–663.

32. *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.

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. *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, V. 307, C901-C907.

35. *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.

36. *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.

37. *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.

38. *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.

39. *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.

40. *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.

41. *Zhang W., Wang S., Wang Q., Yang Z., Pan Z., Li L.* Overexpression of cysteine cathepsin L is a marker of invasion and metastasis in ovarian cancer. *Oncol. Rep.* 2014, 31 (3), 1334–1342.

42. *Mori J., Tanikawa C., Funauchi Y., Lo P. H., Nakamura Y., Matsuda K.* Cystatin C as a p53-inducible apoptotic mediator that regulates cathepsin L activity. *Cancer Sci.* 2016, 107 (3), 298–306.

43. *Sudhan D. R., Rabaglino M. B., Wood C. E., Siemann D. W.* Cathepsin L in tumor angiogenesis and its therapeutic intervention by the small molecule inhibitor KGP94. *Clin. Exp. Metastasis.* 2016, 33 (5), 461–473.

44. *Wang X., Xiong L., Yu G., Li D., Peng T., Luo D., Xu J.* Cathepsin S silencing induces apoptosis of human hepatocellular carcinoma cells. *Am. J. Transl. Res.* 2015, 7 (1), 100–110.

45. *Backer M. V., Backer J. M., Chinnaiyan P.* Targeting the unfolded protein response in cancer therapy. *Methods Enzymol.* 2011, V. 491, P. 37–56.

46. *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.

ДЕФІЦИТ ГЛЮКОЗИ ЗМІНЮЄ ЕКСПРЕСІЮ LONP1 ТА КАТЕПСИНІВ У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87 З ПРИГНІЧЕНИМ IRE1

О. Г. Мінченко[1]
О. В. Галкін[1]
О. О. Рябовол[1]
Д. О. Мінченко[1,2]
А. Ю. Кузнєцова[1]
О. О. Ратушна[1]

[1]Інститут біохімії ім. О. В. Палладіна НАН України, Київ
[2]Національний медичний університет ім. О. О. Богомольця, Київ

E-mail: ominchenko@yahoo.com

Метою роботи було вивчення впливу дефіциту глюкози на експресію генів, що кодують LONP1/PRSS15 та катепсини у клітинах гліоми лінії U87 за умов пригнічення IRE1 — inositol requiring enzyme-1. Показано, що дефіцит глюкози посилював експресію генів CTSA, CTSB, CTSD, CTSK, CTSL, CTSO та LONP1, але не впливав на експресію генів CTSC, CTSF та CTSS у контрольних (трансфікованих порожнім вектором) клітинах гліоми. Пригнічення функції сигнального ензиму IRE1 у клітинах гліоми лінії U87 змінювало ефект дефіциту глюкози на експресію більшості досліджених генів: нівелювало ефект дефіциту глюкози на гени CTSA та CTSO, індукувало — на гени CTSC та CTSS, зменшувало — на ген CTSK і посилювало — на ген CTSL. Таким чином, дефіцит глюкози змінював рівень експресії більшості досліджених генів залежно від функціональної активності ензиму IRE1, центрального медіатора стресу ендоплазматичного ретикулума, що контролює процеси проліферації та росту пухлин.

Ключові слова: експресія мРНК, CTS, LONP1/PRSS15, пригнічення IRE1, дефіцит глюкози, клітини гліоми U87.

27