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ACUTE L-GLUTAMINE DEPRIVATION AFFECTS THE EXPRESSION OF *G6PD, GPI, TKT, TALDO1, PGLS* AND *RPIA* GENES IN U87 GLIOMA CELLS: EFFECT OF ERN1 KNOCKDOWN

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Pentose-phosphate cycle of glucose metabolism plays an important role in malignant tumor growth, including glioma. We have studied the expression level of mRNA of basic enzymes of this cycle (G6PD, TKT, TALDO1, PGLS and RPIA) as well as glucose-6-phosphate isomerase (GPI) in U87 glioma cells with knockdown of ERN1 (endoplasmic reticulum to nucleus signaling 1), the major sensor and signaling enzyme of endoplasmic reticulum stress, upon glutamine deprivation in the growing medium. It was shown that suppression of ERN1 signaling enzyme function leads to significant decrease of the level of TALDO1 and PGLS gene expressions in glioma cells, but the expression level of GPI gene is increased. At the same time, the expression level of other genes of pentose-phosphate cycle enzymes did not change significantly at this experimental condition. It was also shown that upon glutamine deprivation condition the expression level of G6PD and GPI genes is increased and decreased, correspondingly, but other pentose-phosphate cycle genes does not changed significantly in glioma cells. Moreover, the changes in the expression level of these genes upon glutamine deprivation is clearly dependent upon ERN1 signaling enzyme function. Thus, these results demonstrate that suppression of ERN1 signaling enzyme function in glioma cells affects the expression level of three studied genes only and that glutamine deprivation condition induces variable changes in the expression of most investigated genes but preferentially in glioma cells with ERN1 knockdown.

Key words: gene expression, ERN1 knockdown, G6PD, TKT, TALDO1, PGLS, RPIA, GPI, glutamine deprivation, U87 glioma cells

Introduction. Pentose phosphate pathway of glucose metabolism plays an important role in the regulation of various processes both in normal and pathological conditions, especially in glioma growth, and depends of endoplasmic reticulum stress, which is obligate component of cancer growth (Hetz et al., 2013; Moenner et al., 2007; Minchenko et al., 2013). Malignant gliomas are highly aggressive tumors and are characterized by marked angiogenesis and extensive tumor cell invasion into the normal brain parenchyma. Previously was shown that blockade of ERN1-mediated signaling pathway of endoplasmic reticulum stress leads to suppression of cell proliferation and tumor growth through changing in the expression level of genes, which responsible for control of glycolysis, cell cycle, apoptosis, angiogenesis and many other processes (Auf et al., 2010, 2013; Drogat et al., 2007; Minchenko et al., 2010, 2012a, 2012b, 2012c, 2014; Pluquet et al., 2013). Nutrient deprivation condition is also an important factor of cancer growth and participates in the induction of endoplasmic reticulum stress (Bi et al., 2005; Drogat et al., 2007).

The endoplasmic reticulum is a key organelle in the cellular response to ischemia, hypoxia, and some chemicals which activate a complex set of signaling pathways named the unfolded protein response. The endoplasmic reticulum stress is associated with accumulation of unfolded proteins in the endoplasmic reticulum and controls both neovascularization and proliferation processes (Moenner et al., 2007; Zhang and Kaufman, 2004). This adaptive unfolded protein response is activated upon the accumulation of misfolded proteins in the endoplasmic reticulum and is mediated by three endoplasmic reticulum-resident sensors named ERN1/IRE1 (Endoplasmic Reticulum to Nucleus signaling 1 / Inositol Requiring Enzyme-1), PERK (PRK-like ER kinase), and ATF6 (Activating Transcription Factor 6); however, ERN1 is the dominant sensor (Aragon et al., 209; Bi et al., 2005; Fels et al., 2006; Korennykh et al., 2009). Activation of the unfolded protein response tends to limit the de novo entry of proteins in to the endoplasmic reticulum and facilitate both the endoplasmic reticulum protein folding and its degradation to adapt cells for survival or, alternatively, to enter cell

death programs through endoplasmic reticulum-associated machineries (Aragon et al., 209; Moenner et al., 2007; Zhang and Kaufman, 2004). As such, it participates in the early cellular response to the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum, occurring under both physiological and pathological situations (Moenner et al., 2007; Zhang and Kaufman, 2004).

The signaling enzyme ERN1 is bifunctional and has two distinct catalytic domains: a serine/threonine kinase and an endoribonuclease. Both domains are contributed to ERN1 signaling (Moenner et al., 2007; Zhang and Kaufman, 2004). The ERN1-associated protein kinase autophosphorylates and dimerizes this enzyme in the endoplasmic reticulum membrane, leading to the activation of its endoribonuclease domain. It is possible that ERN1-associated protein kinase has some additional functions (Bakalets et al., 2013; Garmash et al., 2013). Endoribonuclease activity is responsible for degradation of a specific subset of mRNA and initiation of the pre-XBP1 (X-box binding protein 1) mRNA splicing (Acosta-Alvear et al., 2007; Hollien et al, 2009; Romero-Ramirez et al., 2004). Mature XBP1 mRNA splice variant (XBP1s) encodes a transcription factor that stimulates the expression more than five hundreds of unfolded protein response-specific genes (Acosta-Alvear et al., 2007). Moreover, XBP1s has several additional functions, especially in the regulation of glucose homeostasis. Thus, the p38 MAP kinase phosphorylates the spliced form of XBP1 and enhances its nuclear migration. Zhou et al. (2011) shown that XBP1s interacts with the FOXO1 (Forkhead box O1) transcription factor and directs it toward proteasome-mediated degradation. Moreover, the regulatory subunits of phosphatidyl inositol 3-kinase interact with XBP1 and also increase its nuclear translocation (Lee et al., 2011). At the same time, it was shown that an ERN1 kinase inhibitor activates the ERN1 endoribonuclease to confer cytoprotection against endoplasmic reticulum stress. It is possible that this activation of the ERN1 endoribonuclease is a result of its interaction with other sensor-signalling systems of endoplasmic reticulum stress.

Recently, there has been a renewed interest in the role of the pentose phosphate pathway in cancer (Du et al., 2013; Tsouko et al., 2014). This metabolic pathway is advantageous for rapidly growing cells because it provides nucleotide precursors and helps regenerate the reducing agent NADPH, which can contribute to reactive oxygen species (ROS) scavenging. Pentose phosphate pathway genes, such as *G6PD* (glucose-6-phosphate dehydrogenase), *TKT* (transketolase), *TALDO1* (transaldolase 1), *PGLS* (6-phosphoglucolactonase) та *RPIA* (ribose-5-phosphate isomerase), as well as *DPI* (glucose phosphate isomerase) gene play an important role in malignant tumor growth. These genes encode enzymes related to pentose formations, which are needed for nucleic acids synthesis and intensification of cell proliferation.

Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme of the pentose phosphate pathway and is involved in apoptosis as well as angiogenesis (Hu et al., 2013; Samland et al., 2009; Stanton et al, 2012; Zhang et al., 2014). Moreover, G6PD may regulate apoptosis and expression of cell cycle-related proteins through phosphorylation of transcription factors STAT3 and STAT5, thus mediating formation and growth of malignant cells (Hu et al., 2013). It is interesting to note that TAp73, a structural homologue of the pre-eminent tumor suppressor TP53, enhances the pentose phosphate pathway and supports cell proliferation through glucose-6-phosphate dehydrogenase, which has a critical role of in TAp73-mediated cell proliferation (Du et al., 2013; Jiang et al., 2013). Recent data indicates that the transcription factor NRF2 is responsible for regulating *G6PD* and *TKT* gene expressions and oncogenes can realize its effect via stabilization or degradation of the transcription factor (Shelton and Jaiswal, 2013).

Transaldolase is a key enzyme of the nonoxidative pentose phosphate pathway providing ribose-5-phosphate for nucleic acid synthesis and NADPH for lipid biosynthesis (Basu et al., 2011; Samland and Sprenger, 2009). This enzyme is involved in mitochondrial homoeostasis, Ca(2+) fluxing, oxidative stress, apoptosis, multiple sclerosis, and cancer (Berry, 2008; Qian et al., 2008; Samland et al., 2009).

Phosphoglucose isomerase (PGI) is a multifunctional enzyme that functions in glucose metabolism as a glycolytic enzyme catalyzing an interconversion between glucose and fructose inside the cell, while it acts as cytokine outside the cell, with properties that include autocrine motility factor (AMF)-regulating tumor cell motility. AMF/PGI mediates epithelial and mesenchymal phenotype conversions in breast cancer and its overexpression induces epithelial-to-mesenchymal transition with enhanced malignancy (Funasaka et al., 2009; Niinaka et al., 2010). Recent studies have revealed that silencing of AMF/PGI resulted in mesenchymal-to-epithelial transition of human lung fibrosarcoma cells and breast cancer cells with reduced malignancy. It was shown that overexpression of AMF/PGI significantly contributes to the aggressive phenotype of human colon cancer, but downregulation of its expression and subsequent abrogation of AMF/PGI secretion, which resulted in morphologic change with reduced growth, motility, and invasion (Niinaka et al., 2010; Tsutsumi et al., 2009).

Phosphoglucose isomerase also regulates endoplasmic reticulum stress and cell death through control of endoplasmic reticulum calcium release as well as promotes cell survival by the pAKT survival pathway (Fu et al., 2011). Its receptor, AMFR, is an E3 ubiquitin ligase implicated in endoplasmic reticulum-associated protein degradation (Fairbank et al., 2009). Moreover, AMF/PGI also protects against tunicamycin-induced endoplasmic reticulum stress and apoptosis (Fu et al., 2011). Furthermore, HER2 expression and AMF/PGI secretion were inversely related in breast carcinoma cells. Thus, AMF/PGI may contribute to HER2-mediated breast cancer progression (Kho et al., 2013).

The main goal of this study was investigation the role of the expression of pentose phosphate pathway genes (*G6PD, TKT, TALDO1, PGLS.* and *RPIA*) as well as glycolytic enzyme gene (*GPI*), those protein products participate in the regulation of cell proliferation, in glioma U87 cells with ERN1 knockdown and its regulation upon glutamine deprivation and to study the contribution of endoplasmic reticulum stress sensor ERN1 to fine tune their expression.

Materials and Methods. The U87 glioma cell line 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) and streptomycin (0.1 mg/ml; Gibco) at 37^{o}C in a 5% CO_2 incubator. In this work we used two sublines of this glioma cell line. 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 (dnERN1). This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of effects of glutamine deprivation condition on the expression level of *G6PD, GPI, TALDO1, TKT, PGLS,* and *RPIA* genes. Second subline was obtained by selection of stable transfected clones with overexpression of dnERN1 construct and has suppressed both protein kinase and endoribonuclease activities of ERN1 signaling enzyme. These cells were obtained from prof. M. Moenner (France) (Drogat et al., 2007; Auf et al., 2010). The expression level of *G6PD, GPI, TALDO1, TKT, PGLS,* and *RPIA* genes in these cells was compared with cells, transfected by vector (control 1). Moreover, glioma cells with blockade of ERN1 signaling enzyme function was also used as control 2 for investigation the effect of glutamine deprivation condition on the expression level of studied genes under blockade ERN1 function. Glutamine deprivation condition was created by changing the complete Dulbecco's modified Eagle's minimum essential medium on the medium without glutamine and culture plates were exposed to this condition for 16 hrs.

The suppression level of ERN1 both enzymatic activity in glioma cells that over express a dominant-negative construct of endoplasmic reticulum–nuclei-1 (dnERN1) was previously shown by analysis of the expression of XBP1 alternative splice variant (XBP1s), a key transcription factor in ERN1 signaling, and phosphorylated isoform ERN1 using cells treated by tunicamycin (0.01 mg/ml during 2 hours) (Minchenko et al., 2014).

Total RNA was extracted from glioma cells according to manufacturer protocols (Invitrogen, USA). RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water. For additional purification RNA samples were precipitated with 95 % ethanol and re-dissolved again in nuclease-free water. For synthesis of complementary DNA (cDNA) was used QuaniTect Reverse Transcription Kit (QIAGEN, Germany).

The expression levels of *G6PD, GPI, TALDO1, TKT, PGLS,* та *RPIA* mRNA were measured in glioma cell line U87 and its sublines (clone 1C5) by real-time quantitative polymerase chain reaction of cDNA using „Mx 3000P QPCR" (Stratagene, USA) and SYBRGreen Mix (AB gene, Great Britain). Polymerase chain reaction was performed in triplicate. For amplification of GPI (glucose-6-phosphate isomerase) cDNA we used forward (5'– cgcccaaccaactctattgt –3' and reverse (5'– ggtagaagcgtcgtgagagg –3') primers. The nucleotide sequences of these primers correspond to sequences 1554 – 1573 and 1766 – 1747 of human GPI cDNA (GenBank accession number NM_000175). The size of amplified fragment is 213 bp.

For amplification of G6PD (glucose-6-phosphate dehydrogenase) we were used forward (5'– gaggccgtgtacaccaagat –3' and reverse (5'– tacccaaggccgtacttgtc –3') primers**.** The nucleotide sequences of these primers correspond to sequences 1430 – 1439 and 1644 – 1625 of human G6PD cDNA (GenBank accession number NM_000402). The size of amplified fragment is 215 bp.

The amplification of TALDO1 (transaldolase 1) cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5'– ggctgtgacttcctcaccat –3' and reverse – 5'– ctcagggatgcgctactttc –3'. The nucleotide sequences of these primers correspond to sequences 795 – 814 and 1076 – 1057 of human TALDO1 cDNA (GenBank accession number NM_006755). The size of amplified fragment is 282 bp.

For amplification of TKT (transketolase) cDNA we used forward (5'– gacaaccttgtggccattct –3' and reverse (5'– tctgctcagccatgtttttg –3') primers. The

nucleotide sequences of these primers correspond to sequences 1530 – 1549 and 1834 – 1815 of human CCN2 cDNA (GenBank accession number NM_001901). The size of amplified fragment is 283 bp.

The amplification of PGLS (6-phosphogluconolactonase), also known as 6-phosphogluconolactone to 6-phosphogluconate (6PGL) cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5'– ctgctcactcttcccagacc –3' and reverse (5'– tccagttgccacaaagatga –3'). The nucleotide sequences of these primers correspond to sequences 515 – 534 and 665 – 646 cDNA of human PGLS (GenBank accession number NM_012088). The size of amplified fragment is 151 bp.

For amplification of RPIA (5-phosphate isomerase A) cDNA we used forward (5'– agtgctgggaattggaagtg –3' and reverse (5'– cgatcacgatgaagcgacta –3') primers. The nucleotide sequences of these primers correspond to sequences 335 – 354 and 627 – 608 of human RPIA cDNA (GenBank accession number NM_144563). The size of amplified fragment is 293 bp.

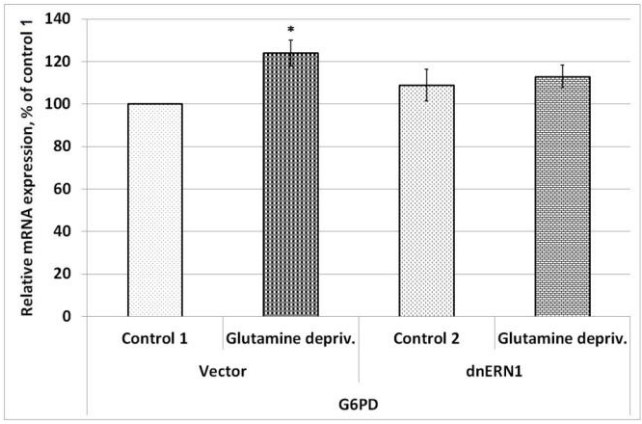
The amplification of beta-actin (ACTB) cDNA was performed using forward - 5'– ggacttcgagcaagagatgg –3' and reverse - 5'– agcactgtgttggcgtacag –3' primers. These primers nucleotide sequences correspond to 747 – 766 and 980 – 961 of human ACTB cDNA (GenBank accession number NM_001101). The size of amplified 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" (USA).

An analysis of quantitative PCR was performed using special computer program "Differential expression calculator" and statistical analysis using program OriginPro 7.5. The values of G6PD, GPI, TALDO1, TKT, PGLS and RPIA mRNA expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100 %). All values are expressed as M ± *m* from triplicate measurements performed in 4 independent experiments.

Results and Discussion. ***ERN1 modulates effect of glutamine deprivation condition on the expression of pentose phosphate genes in glioma cells.*** To test the effect of ERN1 on expression levels of glucose-6-phosphte dehydrogenase gene, we used the U87 glioma cells line, which constitutively expresses dominant-negative mutant of ERN1, the major component of endoplasmic reticulum stress signaling. Expression of this dnERN1 mutant was shown to have an inhibitory effect on ribonuclease and kinase activity of endogenous ERN1 (Minchenko et al., 2012c). Fig. 1 demonstrates that inhibition of ERN1 gene function in U87 glioma cells does not change significantly the expression level of *G6PD* gene, the rate-limiting enzyme of the pentose phosphate pathway.

To test whether ERN1 modulates expression of *G6PD* gene during acute L-glutamine deprivation condition, control glioma cells and cells harboring dnERN1 were cultured in the medium with L-glutamine (regular growing medium) and without L-glutamine (glutamine free medium) and expression level of *G6PD* gene was analyzed by qPCR. We found that expression levels of *G6PD* gene is significantly increased (+24 %) under acute L-glutamine deprivation condition in control glioma cells (stable transfected by vector), but did not change significantly in cells expressing dnERN1 (stably transfected with dnERN1) (Fig. 1). We therefore concluded that ERN1 down-regulation eliminates effect of acute L-glutamine deprivation condition on *G6PD* gene expression in glioma cells. It is possible that this effect of L-glutamine deprivation on the expression level of *G6PD* gene is realized through ERN1-mediated pathway of endoplasmic reticulum stress in U87 glioma cells. Moreover, nutrient deprivation condition is necessary component of tumor growth and induction of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the pentose phosphate pathway, can be responsible for cancer growth through the activation of nucleotide precursor synthesis and regeneration the reducing agent NADPH (Hu et al., 2013; Samland et al., 2009; Stanton et al, 2012; Zhang et al., 2014). At the same time, inhibition of ERN1 signaling enzyme function eliminate an induction of *G6PD* gene expression, which correlate with suppressed glioma cell proliferation (Auf et al., 2013).

As shown in Fig. 2, the level of *TKT* gene expression does also not change significantly in glioma cells expressing dnERN1 as compared to control glioma cells. No significant changes in the expression level of this gene were also found under L-glutamine deprivation condition in control glioma cells (stable transfected by vector). At the same time, acute L-glutamine glutamine deprivation condition leads to significant upregulation of *TKT* gene expression level in U87 glioma cells with ERN1 knockdown (Fig. 2). It is possible that ERN1 down-regulation induces effect of acute L-glutamine deprivation condition on *TKT* gene expression in U87 glioma cells and that this effect of L-glutamine deprivation is negatively realized through ERN1-mediated pathway of endoplasmic reticulum stress in these cells.



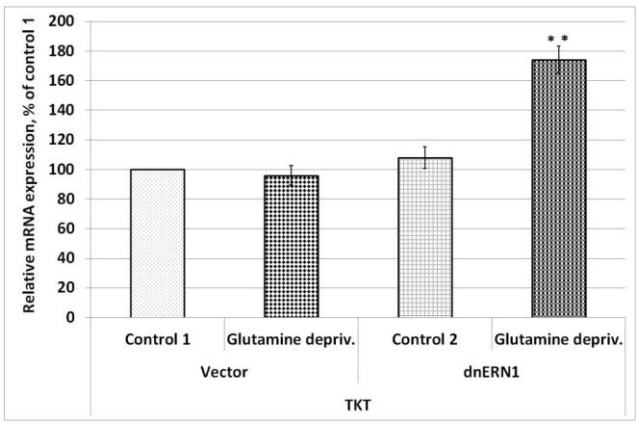


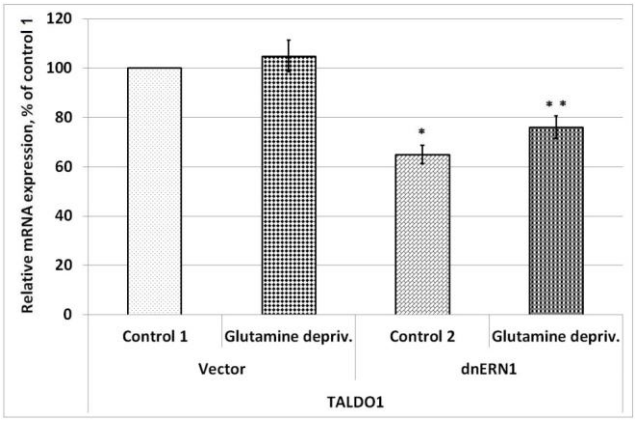
Fig. 1. Effect of glutamine deprivation on the expression level of G6PD (glucose-6-phosphate dehydrogenase) mRNA in U87 glioma cells stable transfected by dominant-negative construct of ERN1 in pcDNA3.1 vector (dnERN1) as compared to the cells transfected by vector pcDNA3.1 (Control), measured by quantitative polymerase chain reaction. The values of G6PD mRNA expression levels were normalized to the expression of beta-actin mRNA.

Fig. 2. Effect of glutamine deprivation on the expression level of TKT (transketolase) mRNA in U87 glioma cells stable transfected by dominant-negative construct of ERN1 in pcDNA3.1 vector (dnERN1) as compared to the cells transfected by vector pcDNA3.1 (Control), measured by quantitative polymerase chain reaction. The values of TKT mRNA expression level were normalized to the expression of beta-actin mRNA.

*Note: in Fig. 1 – 6 the changes in the expression of different mRNA in both types of glioma cells were compared to control 1 (Vector, 100 %); n = 4; * – P < 0,05 as compared to control 1, ** – P < 0,05 as compared with control 2 (dnERN1).*

Fig. 3 demonstrates that inhibition of ERN1 gene function in U87 glioma cells affects the expression of transaldolase gene, where the expression of this gene is decreased (-35 %) at this experimental condition as compared to control glioma cells, transfected by pcDNA3.1 vector. Analysis of mRNA expression of *TALDO1* gene has shown that acute L-glutamine glutamine deprivation condition does not affect this gene expression in control glioma cells in statistically significant manner, while cells harboring dnERN1 show slight but statistically significant increase (+17 %) in the expression of *TALDO1* gene during this experimental condition (Fig. 3). These results demonstrate that effect of acute L-glutamine deprivation on the expression level of *TALDO1* gene is also depended on endoplasmic reticulum stress mediated through ERN1signaling pathway in U87 glioma cells.

As shown in Fig. 4, the level of *PGLS* gene expression is slightly but statistically significant decreased (-22 %) in glioma cells expressing dnERN1 as compared to control glioma cells.



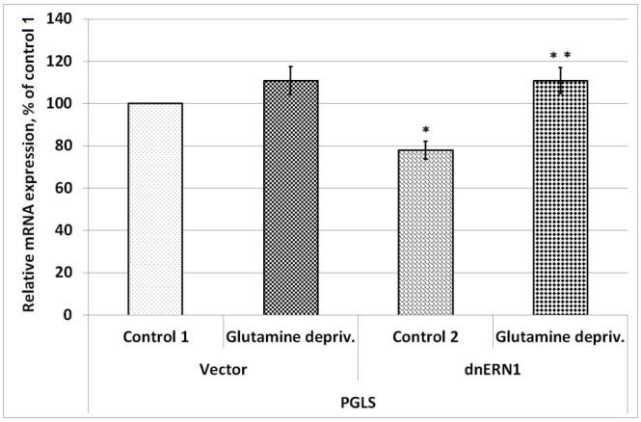
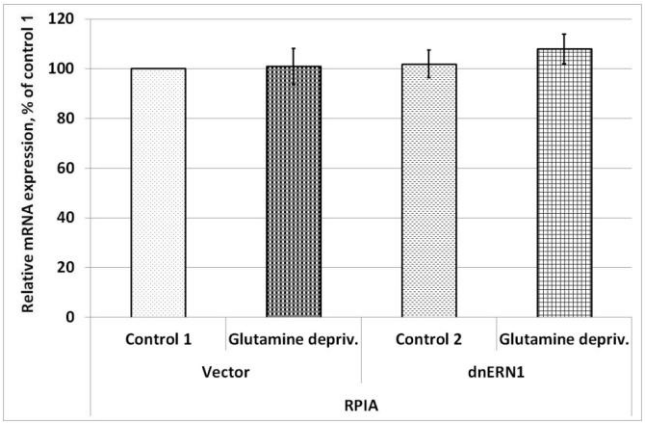


Fig. 3. Effect of glutamine deprivation on the expression level of TALDO1 (transaldolase) mRNA in U87 glioma cells stable transfected by dominant-negative construct of ERN1 in pcDNA3.1 vector (dnERN1) as compared to the cells transfected by vector pcDNA3.1 (Control), measured by quantitative polymerase chain reaction. The values of TALDO1 mRNA expression level were normalized to the expression of beta-actin mRNA.

Fig. 4. Effect of glutamine deprivation on the expression level of PGLS (6-phosphogluconolactonase) mRNA in U87 glioma cells stable transfected by dominant-negative construct of ERN1 in pcDNA3.1 vector (dnERN1) as compared to the cells transfected by vector pcDNA3.1 (Control) , measured by quantitative polymerase chain reaction. The values of PGLS mRNA expression level were normalized to the expression of beta-actin mRNA.



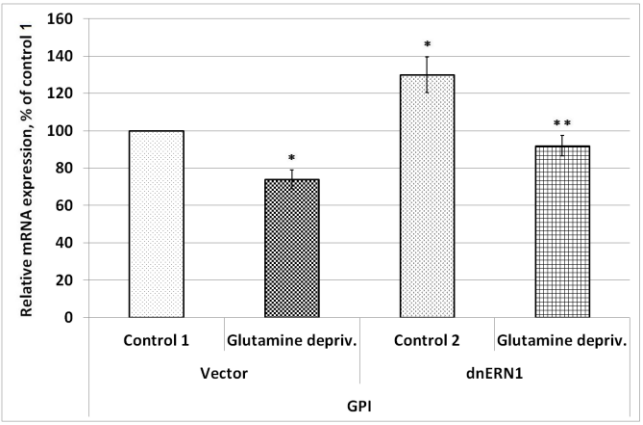


Fig. 5. Effect of glutamine deprivation on the expression level of RPIA (5-phosphate isomerase A) mRNA in U87 glioma cells stable transfected by dominant-negative construct of ERN1 in pcDNA3.1 vector (dnERN1) as compared to the cells transfected by vector pcDNA3.1 (Control), measured by quantitative polymerase chain reaction. The values of RPIA mRNA expression level were normalized to the expression of beta-actin mRNA.

Fig. 6. Effect of glutamine deprivation on the expression level of GPI (glucose-6-phosphate isomerase) mRNA in U87 glioma cells stable transfected by dominant-negative construct of ERN1 in pcDNA3.1 vector (dnERN1) as compared to the cells transfected by vector pcDNA3.1 (Control), measured by quantitative polymerase chain reaction. The values of GPI mRNA expression level were normalized to the expression of beta-actin mRNA.

At the same time, no significant changes in the expression level of this gene were also found under L-glutamine deprivation condition in control glioma cells (stable transfected by vector). However, acute L-glutamine glutamine deprivation condition leads to significant upregulation of *PGLS* gene expression level (+42 %) in U87 glioma cells with ERN1 knockdown (Fig. 4). It is possible that ERN1 knockdown induces effect of acute L-glutamine deprivation on *PGLS* gene expression in U87 glioma cells and that ERN1-mediated pathway of endoplasmic reticulum stress protects glioma cells of glutamine deprivation.

We also demonstrate that inhibition of ERN1 gene function in U87 glioma cells does not affect the expression level of 5-phosphate isomerase A gene as compared to control glioma cells (Fig. 5). Moreover, the level of *RPIA* gene expression was resistant to acute L-glutamine deprivation condition both in control and ERN1 knockdown glioma cells.

Thus, results of this study demonstrate that acute L-glutamine deprivation condition affects the expression of *G6PD* gene only in control glioma cells and *TKT*, *TALDO1*, and *PGLS* genes in ERN1 knockdown glioma cells and that inhibition of ERN1 signaling enzyme function modifies expression level of studied pentose phosphate pathway genes at this experimental condition. Moreover, inhibition of ERN1 signaling enzyme function leads to down-regulation of two pentose phosphate pathway genes (*TALDO1* and PGLS) and these changes can contribute to the suppression of glioma cell proliferation, which was shown previously (Auf et al., 2010, 2013; Minchenko et al., 2014). This data correlates to dysregulation of the expression of different genes of phosphoribosyl pyrophosphate synthetase in glioma cells with inhibition of ERN1 signaling enzyme function as well as to changes in these gene expressions under acute L-glutamine deprivation condition (Minchenko et al., 2013).

Expression of glycolytic enzyme gene glucose-6-phosphate isomerase in ERN1 knockdown glioma cells under glutamine deprivation condition. Fig. 6 demonstrates that inhibition of ERN1 signaling enzyme function in U87 glioma cells significantly increases (+30 %) the expression level of glucose-6-phosphate isomerase gene, a multifunctional enzyme that functions in glucose metabolism as a glycolytic enzyme catalyzing an interconversion between glucose and fructose inside the cell, while it acts as cytokine outside the cell, with properties that include autocrine motility factor regulating tumor cell motility.

To test whether ERN1 modulates expression of *GPI* gene during acute L-glutamine deprivation condition, control glioma cells and cells harboring dnERN1 were cultured in the medium with L-glutamine and without L-glutamine. We found that expression levels of *GPI* gene is decreased under acute L-glutamine deprivation condition in statistically significant manner both in control and ERN1 knockdown glioma cells (-26 and -30 %, correspondingly) (Fig. 6). We therefore concluded that ERN1 down-regulation does not change significantly the effect of acute L-glutamine deprivation condition on *GPI* gene expression in U87 glioma cells. It is possible that this effect of L-glutamine deprivation on the expression level of *G6PD* gene in these cells is not realized through

endoplasmic reticulum stress signaling pathway mediated by ERN.

Phosphoglucose isomerase as a multifunctional enzyme promotes cell migration in an autocrine manner in various tumor cells (Niinaka et al., 2010; Tsutsumi et al., 2009) can contribute to enhanced metastasis of glioma cells with ERN1 knockdown (Drogat et al., 2007; Auf et al., 2013). It is also possible that increased expression of *GPI* gene in glioma cells with blockade of ERN1 signaling enzyme function can modulate the endoplasmic reticulum stress as well as cell death through control of endoplasmic reticulum calcium release and protects cells against endoplasmic reticulum stress (Fu et al., 2011). Phosphoglucose isomerase can also participate in endoplasmic reticulum stress through its receptor, AMFR, which is an E3 ubiquitin ligase implicated in endoplasmic reticulum-associated protein degradation (Fairbank et al., 2009).

Thus, results of this investigation clearly demonstrate that inhibition of ERN1 signaling enzyme function affects the expression level of *GPI*, *TALDO1*, and *PGLS* genes and that acute L-glutamine deprivation condition changes the expression level of *G6PD* and *GPI* genes in control glioma cells and that ERN1 knockdown modifies the effect of glutamine deprivation on *G6PD*, *TKT*, *TALDO1*, and *PGLS* gene expressions. However, detailed molecular mechanisms of participation the pentose phosphate pathway genes as well as glycolytic enzyme gene *GPI* in suppression of tumor growth from ERN1 knockdown glioma cells warrants further study.

Conclusions.

1. It was shown that the expression level of *TALDO1* and *PGLS* genes is decreased in ERN1 knockdown U87 glioma cells, but GPI gene is increased.

2. Glutamine deprivation condition does not affect the expression level of *TALDO1*, *TKT*, *RPIA*, and *PGLS* genes, but increases *G6PD* and decreases *GPI* gene expressions in control glioma cells.

3. The changes in the expression level of *TALDO1*, *TKT*, *G6PD*, and *PGLS* genes upon glutamine deprivation is clearly dependent upon ERN1 signaling enzyme function.

4. Results of this study clearly demonstrated that suppression of ERN1 signaling enzyme function in glioma cells affects the expression level of *GPI*, *TALDO1*, and *PGLS* genes and that glutamine deprivation condition induces variable changes in the expression of most investigated genes but preferentially in glioma cells with ERN1 knockdown.

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ГОСТРИЙ ДЕФІЦИТ ГЛУТАМІНУ ЗМІНЮЄ ЕКСПРЕСІЮ ГЕНІВ ПЕНТОЗО-ФОСФАТНОГО ШЛЯХУ У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87: ЕФЕКТ ВИКЛЮЧЕННЯ ERN1

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Пентозо-фосфатний цикл метаболізму глюкози відіграє важливу роль у рості злоякісних пухлин. Нами проведено вивчення експресії мРНК основних ензимів цього циклу (G6PD, TKT, TALDO1, PGLS та RPIA) а також глюкозо-6-фосфатізомерази (GPI) у клітинах гліоми лінії U87 з пригніченою функцією ERN1 (сигналювання від ендоплазматичного ретикулуму до ядра-1), основного сенсорно-сигнального ензиму стресу ендоплазматичного ретикулуму, в залежності від рівня глутаміну у середовищі вирощування клітин. Встановлено, що за умов пригнічення функції сигнального ензиму ERN1 рівень експресії генів TALDO1 та PGLS у клітинах гліоми істотно знижувався, а гена GPI збільшувався. В той же час, рівень експресії генів інших ензимів пентозо-фосфатного циклу при цьому істотно не змінювався. Також показано, що рівень експресії генів G6PD та GPI підвищується і знижується, відповідно, у клітинах гліоми за умов дефіциту глутаміну, а генів інших ензимів пентозо-фосфатного циклу при цьому істотно не змінювався. Більше того, виявлені зміни в експресії досліджених генів за умов дефіциту глутаміну залежали від функції сигнального ензиму ERN1. Таким чином, результати даної роботи вказують на те, що у клітинах гліоми лінії U87 за умов пригнічення функції сигнального ензиму ERN1 змінюється рівень експресії лише трьох досліджених генів і що за умов дефіциту глутаміну у середовищі вирощування клітин гліоми по-різному змінюється рівень експресії більшості досліджених генів, але ці зміни виявлялися переважно у клітинах за умов виключення ERN1.

Ключові слова: експресія генів, блокада ERN1, G6PD, TKT, TALDO1, PGLS, RPIA, GPI, дефіцит глутаміну, клітини гліоми лінії U87.

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