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# GLUCOSE AND GLUTAMINE DEPRIVATIONS AFFECT THE EXPRESSION OF *MAP3K5, MAP4K3, CIB1, RIPK1,* AND *RIPK2* GENES IN U87 GLIOMA CELLS WITH BLOCKADE OF ERN1 SIGNALING ENZYME FUNCTION

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*Protein kinases play an important role in malignant tumor growth as key regulators of different metabolic processes. We studied the effect of glucose and glutamine deprivation conditions on the expression level of mitogen-activated protein kinase kinase kinase kinase 3 (MAP4K3), mitogen-activated protein kinase kinase kinase 5 (MAP3K5), receptor (TNFRSF)-interacting serine-threonine kinase 1 (RIPK1), and RIPK2 as well as protein kinase interacting protein CIB1 mRNA in U87 glioma cells. It was shown that the suppression of both enzymatic activities of sensor and signaling enzyme ERN1 (endoplasmic reticulum to nucleus signaling 1), the major component of endoplasmic reticulum stress signaling, decreases the expression level of genes encoding MAP4K3, RIPK2, and CIB1 in U87 glioma cells, but increases – RIPK1 gene expression. At the same time, no significant changes were observed in MAP3K5 gene expression in glioma cells with blockade of ERN1 signaling enzyme. Glutamine deprivation condition leads to increase the expression level of RIPK1 gene and to decrease – CIB1 gene in control glioma cells, but ERN1 knockdown modifies the effect of glutamine deprivation on the expression most of these genes. It was also shown that the expression level of MAP3K5, RIPK1, RIPK2, and CIB1 genes did not change significantly in control glioma cells at glucose deprivation condition, but in cells with ERN1 knockdown glucose deprivation enhances the expression of RIPK1 and RIPK2 genes. Thus, suppression of ERN1 enzyme function also changes the effect of glucose deprivation on the expression of most studied genes in glioma cells. Results of this investigation clearly demonstrated that the expression of MAP4K3, RIPK1, RIPK2, and CIB1genes in U87 glioma cells is dependent from blockade of ERN1-mediated endoplasmic reticulum stress and is mostly regulated by glutamine and glucose deprivation in dependence from ERN1 signaling enzyme function.*

*Key words: mRNA expression, ERN1 knockdown, MAP3K5, MAP4K3, RIPK1, RIPK2, CIB1, glutamine deprivation, glutamine deprivation, U87 glioma cells*

**Introduction.** Nutrient deprivation conditions as well as hypoxia are important factors of malignant tumor growth and as many other factors induce the endoplasmic reticulum stress and change the expression of many genes, which control different metabolic processes and proliferation (Denko et al., 2008; Johnson et al., 2008; Lenihan and Taylor, 2013; Moenner et al., 2007). The endoplasmic reticulum stress is associated with unfolded protein response and accumulation of unfolded proteins in the endoplasmic reticulum (Moenner et al., 2007; Wang and Kaufman, 2012). This adaptive response is activated upon the accumulation of misfolded proteins in the endoplasmic reticulum and is mediated by three endoplasmic reticulum-resident sensors named PERK (PRK-like ER kinase), ERN1 (Endoplasmic Reticulum to Nucleus signaling 1) also known as IRE1alpha (Inositol Requiring Enzyme-1alpha) and ATF6 (Activating Transcription Factor 6), however, endoplasmic reticulum to nuclei-1 is the dominant sensor (Bi et al., 2005; Fels et al., 2006; Zhang and Kaufman, 2006; Minchenko et al., 2013). Induction of endoplasmic reticulum stress is the early cellular response to the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum and tends to limit the de novo entry of proteins in to the endoplasmic reticulum and facilitate both the endoplasmic reticulum protein folding and degradation to adapt cells for survival, occurring under both physiological and pathological conditions (Hetz et al., 2013; Minchenko et al., 2014; Schröder, 2008).

The ERN1 has two distinct catalytic domains: for serine/threonine kinase and endoribonuclease, which

contribute to ERN1 signalling. The ERN1-associated protein kinase activity autophosphorylates and dimerizes this enzyme, leading to the activation of its endoribonuclease domain, which responsible for initiation of the pre-XBP1 (X-box binding protein 1) mRNA splicing and degradation of a specific subset of mRNA (Acosta-Alvear et al., 2007; Korennykh et al., 2009; Pluquet et al., 2013; Romero-Ramirez et al., 2004). Mature XBP1 mRNA splice variant encodes a transcription factor that stimulates the expression of hundreds of unfolded protein response-specific genes (Aragon et al., 2009; Hollien et al., 2009). Moreover, XBP1s has some additional functions, which are important for the regulation of glucose homeostasis (Lee et al., 2011; Park et al., 2010; Zhou et al., 2011). Thus, Zhou et al. (2011) shown that XBP1s interacts with the Forkhead box O1 (FOXO1) transcription factor and directs it toward proteasome-mediated degradation. Moreover, the p38 MAP kinase phosphorylates the alternative spliced form of XBP1 and enhances its nuclear translocation. Moreover, the regulatory subunits of phosphatidyl inositol 3-kinase interact with XBP1 and also increase its nuclear translocation (Park et al., 2010). At the same time, it was shown that a kinase inhibitor activates the ERN1 endoribonuclease to confer cytoprotection against ER 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.

Previously was shown that the complete blockade of ERN1 signal transduction pathway had anti-tumor effects both in glioma and lung cancer (Auf et al., 2010, 2013; Drogat et al., 2007). Thus, the endoplasmic reticulum stress response-signalling pathway is tightly linked to cell proliferation process and tumor growth. Malignant gliomas are highly aggressive tumors and are characterized by marked angiogenesis and extensive tumor cell invasion into the normal brain parenchyma (Bi et al., 2005). Moreover, nutrient deprivation condition is associated to glioma development and locally induce an adaptive response which confers to tumor cells an enhanced survival and a more agressive behaviour.

In malignant tumor growth an important role plays different protein kinases, including casein kinases, SNF1/AMP-activated protein kinases, mitogen-activated protein kinases MAP3K5 and MAP4K3, receptor (TNFRSF)-interacting serine-threonine kinases RIPK1 and RIPK2, the key regulators of different metabolic processes (Minchenko et al., 2012a, 2012b). There is data that stimulation of human aortic endothelial cells with TNF-alpha led to an increased expression of p73 protein and a reduction in the levels of p53 involving MAP3K5, which is an apoptosis signal-regulating kinase (Rastogi et al., 2012). Moreover, high glucose-induced cell apoptosis and activation of ASK1 in mesangial cells is prevented by knockdown of thioredoxin interacting protein (TXNIP) may be via reduction of oxidative stress (Shi et al., 2011). It is interested to note that apoptosis signal-regulating kinase 1 and cyclin D1 compose a positive feedback loop contributing to tumor growth in gastric cancer (Hayakawa et al., 2011). It was also shown that SERTAD1 (SERTA domain containing 1), also known as CDK4-binding protein P34SEI or SEI-1, inhibits ROS-induced cell death through by indirectly inducing ubiquitination of MAP3K5 (Hong et al., 2011). Protein kinase MAP4K3 activates key effectors in cell signalling, has relation to migration and invasion of cancer cells, and is required for leucine-induced mTORC1 activation (Schriever et al., 2013; Yan et al., 2010; Zhao et al., 2014). Moreover, this protein kinase orchestrates activation of BAX via the concerted posttranscriptional modulation of PUMA, BAD, and BIM (Lam et al., 2009).

Receptor-interacting serine-threonine kinase RIPK1 transduces cell-death signals the C-terminal domain of c-FLIPL specifically inhibits the interaction of the caspase 8 prodomain with the RIP1 death domain and, thereby, regulates caspase 8-dependent NF-kappaB activation (Matsuda et al., 2014). It is interested to note that TNFSF12 (tumor necrosis factor (ligand) superfamily, member 12), also known as TNF-related weak inducer of apoptosis (TWEAK), induces apoptosis through a death-signaling complex comprising receptor-interacting protein 1 (RIPK1) as well as Fas-associated death domain (FADD), and caspase-8 (Ikner and Ashkenazi, 2011). Recently was shown that shikonin mediated necroptosis in glioma cells is associated with the up-regulated expression of protein kinase RIPK1 and oxidative stress (Fu et al., 2013; Huang et al., 2013). It was shown that knockdown of receptor-interacting serine/threonine-protein kinase 2, also known as CARD-containing IL-1 beta ICE-kinase or receptor-interacting protein (RIP)-like interacting caspase-like apoptosis regulatory protein (CLARP) kinase, down-regulated nuclear factor kappa B (NF-κB)-dependent *PAI1* (plasminogen activator inhibitor type 1), also known as *SERPINE1* (serpin peptidase inhibitor, clade E, member 1), and *VIM* (vimentin) gene expressions (Wu et al., 2012). Protein kinase RIPK2 might play an important role in hepatic cell migration and inhibition of its tyrosine kinase activity limits NOD2-driven cytokine responses (Tigno-Aranjuez et al., 2010).

Protein kinase interacting protein CIB1 is a novel mediator of PKD2 (polycystic kidney disease 2)-

driven carcinogenesis, may play a role in regulation of apoptosis and angiogenesis and functions as a negative regulator of stress activated MAP kinase signaling pathways as well as POLO-like kinase 3 (Armacki et al., 2014; Leisner et al., 2013; Naik and Naik, 2011; Naik et al., 2011).

The main goal of this study was investigation the role of the blockade of both kinase and endoribonuclease activity of ERN1 signaling enzyme on the expression of *MAP3K5*, *MAP4K3*, *RIPK1*, *RIPK2*, and *CIB1* genes in glioma U87 cells and its regulation by nutrient (glucose or glutamine) deprivation condition.

**Materials and Methods.** The glioma cells U-87 MG (ATCC HTB-14) was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (DMEM; Gibco, Invitrogen, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco) and streptomycin (0.1 mg/ml; Gibco) at 37ºC in a 5% $CO_2$ incubator. In this study we used two sublines of this glioma cells. One subline was obtained by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dnERN1 (ERN1 dominant/negative constructs). This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of effects of nutrient (glutamine or glucose) deprivations on the expression level of different protein kinase and associated with kinase genes. Second subline was obtained by selection of stable transfected clones with overexpression of dnERN1 and has suppressed both protein kinase and endoribonuclease activities of ERN1 signaling enzyme (clone 1C5, which initially was obtained from prof. M. Moenner, France) (Auf et al., 2010; Drogat et al., 2007).

The expression level of mitogen-activated protein kinase kinase kinase 5 (MAP3K5), mitogen-activated protein kinase kinase kinase kinase 3 (MAP4K3), receptor (TNFRSF)-interacting serine-threonine kinase 1 (RIPK1), RIPK2, and calcium and integrin binding 1 (CIB1; calmyrin) mRNAs in cells with ERN1 knockdown was compared with cells, transfected by vector (control 1). The glioma cells with blockade of ERN1 was also used as control 2 for investigation the effect of glutamine and glucose deprivation conditions on the expression level of these genes upon ERN1 knockdown. Nutrient deprivation conditions were created by changing the complete Dulbecco's modified Eagle's minimum essential medium on the medium without glutamine or glucose and culture plates were exposed to these conditions for 16 hrs.

The suppression level of ERN1 both enzymatic activity in glioma cells that over express a dnERN1 was previously shown by analysis of ERN1 autophosphorylation and the expression of XBP1 alternative splice variant (XBP1s), a key transcription factor in ERN1 signaling, upon induction of endoplasmic reticulum stress by tunicamycin (0.01 mg/ml, 2 hours) (Minchenko et al., 2014).

Total RNA was extracted from glioma cells using Trizol reagent according to manufacturer protocols (Invitrogen, USA). The RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water. For additional purification RNA samples were re-precipitated with 95 % ethanol and re-dissolved again in nuclease-free water. QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis. Polymerase chain reaction was performed in triplicate.

The expression levels of MAP3K5, MAP4K3, RIPK1, RIPK2, and CIB1 mRNA were measured in glioma cell line U87 and its ERN1 knockdown subline (clone 1C5) by real-time quantitative polymerase chain reaction using „Mx 3000P QPCR" (Stratagene, USA) and SYBRGreen Mix (AB gene, Great Britain).

The amplification of MAP3K5 also known as apoptosis signal-regulating kinase 1 (ASK1) cDNA was performed using forward primer (5'– aaagaggcttgctggcataa –3') and reverse primer (5'– tctgcagacatggactctgg –3'). These oligonucleotides correspond to sequences 2839 – 2858 and 3090 – 3071 of human MAP3K5 cDNA (GenBank accession number NM_005923). The size of amplified fragment is 252 bp. For amplification of MAP4K3 also known as germinal center kinase-related protein kinase (GLK) cDNA we used forward (5'– gcatggagttttgtggaggt –3' and reverse (5'– cactgcccagagatcacaga –3') primers. The nucleotide sequences of these primers correspond to sequences 591 – 610 and 925 – 906 of human MAP4K3 cDNA (GenBank accession number NM_003618). The size of amplified fragment is 335 bp. The amplification of RIPK1 also known as cell death protein RIP cDNA for real time qRCR analysis was performed using two oligonucleotides primers: forward – 5'– tggaaaaggcgtgatacaca –3' and reverse – 5'– gacttctctgtgggctttgc –3'. The nucleotide sequences of these primers correspond to sequences 390 – 409 and 626 – 607 of human RIPK1 cDNA (GenBank accession number NM_003804). The size of amplified fragment is 237 bp. For amplification of RIPK2 also known as CARD-containing IL-1 beta ICE-kinase or receptor-interacting protein (RIP)-like interacting caspase-like apoptosis regulatory protein (CLARP) kinase cDNA we used forward (5'– ttccaattttgggaatttgc –3' and reverse (5'– atgcgccactttgataaacc –3') primers. The nucleotide sequences of these primers correspond to sequences 550 – 569 and 829 – 810 of

human RIPK2 cDNA (GenBank accession number NM_003821). The size of amplified fragment is 280 bp. The amplification of calcium and integrin binding 1 (CIB1; calmyrin) also known as SNK-interacting protein 2-28 (SIP2-28) cDNA was performed using forward - 5'– cattatgccttccgcatctt –3' and reverse - 5'– gctggcaaagtctggagaac –3' primers. These primers nucleotide sequences correspond to 487 – 506 and 717 – 698 of human CIB1 cDNA (GenBank accession number NM_006384). The size of amplified fragment is 231 bp. For amplification of beta-actin (ACTB) cDNA was used 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" (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 MAP3K5, MAP4K3, RIPK1, RIPK2, and CIB1 mRNA expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100 %). All values are expressed as mean ± SEM from triplicate measurements performed in four independent experiments.

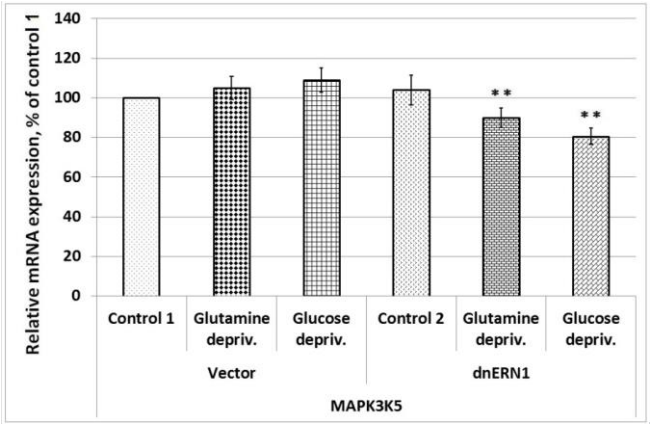
**Results and Discussion.** In this work we studied the effect of glucose and glutamine deprivation conditions on the expression level of two MAP kinases (mitogen-activated protein kinase kinase kinase kinase 3 and mitogen-activated protein kinase kinase kinase 5 and two RIP kinases (receptor (TNFRSF)-interacting serine-threonine kinase 1 and 2) as well as protein kinase interacting protein CIB1 mRNAs in U87 glioma cells with knockdown of ERN1, the major component of endoplasmic reticulum stress signaling. As shown in Fig. 1, the suppression of both enzymatic activities of sensor and signaling enzyme ERN1 did not change significantly the expression level of *MAP3K5* gene in U87 glioma cells. Moreover, glucose as well as glutamine deprivation condition also did not change significantly the expression level of mRNA for this protein kinase in control glioma cells, but in cells with blockade of ERN1 signaling enzyme function the expression level of *MAP3K5* gene is decreased upon both glucose and glutamine deprivation conditions: -27 % and -13 %, correspondingly (Fig, 1). Thus, blockade of ERN1 signaling enzyme function did not change the expression level of MAP3K5 mRNA, but modifies the effect of glucose as well as glutamine deprivation condition on the expression level of this gene in U87 glioma cells.

In this study we did not find any changes in mRNA expression level of protein kinase MAP3K5, which is an apoptosis signal-regulating kinase, in glioma cells with ERN1 knockdown; however, this kinase and cyclin D1 compose a positive feedback loop contributing to tumor growth in cancer (Hayakawa et al., 2011) and decreased expression of cyclin D1 in these cells (Minchenko et al., 2011) as well as up-regulated expression of MAP3K5 mRNA upon glucose and glutamine deprivation (Fig. 1) possibly contributes to suppression of cell proliferation after blockade of ERN1 (Auf et al., 2010).

Investigation of the expression of another member of mitogen-activated protein kinases, MAP4K3, demonstrates that knockdown of signaling enzyme ERN1 leads to significant (close to 2fold) decrease of its mRNA expression level in U87 glioma cells (Fig. 2). Moreover, the glutamine deprivation condition does not affect the expression level of *MAP4K3* gene in control glioma cells, but increases (+25 %) in cells with suppressed function of ERN1 signaling enzyme. At the same time, glucose deprivation condition up-regulates (+27 %) the expression level of MAP4K3 mRNA in control glioma cells, but blockade of ERN1 signaling enzyme function eliminates this effect of glucose deprivation (Fig. 2).

Thus, the regulation of MAP4K3 mRNA expression in glioma cells both by glucose and glutamine deprivation conditions is strongly depended upon ERN1 signaling enzyme function. Protein kinase MAP4K3 is multifunctional pro-proliferative kinase and decrease of its expression after knockdown of ERN1 signaling enzyme as well as elimination of this mRNA up-regulation upon glucose deprivation (Fig. 2) can contribute to anti-proliferative effect of ERN1 knockdown (Auf et al., 2010; Lam et al., 2009; Zhao et al., 2014).

As shown in Fig. 3, the *RIPK1* gene expression is up-regulated (almost to 2fold) in glioma cells with suppressed function of ERN1 signaling enzyme of endoplasmic reticulum stress. the expression level of this gene in glutamine deprivation condition is also increased (+25 %) in control glioma cells only; ERN1 knockdown eliminates this effect. At the same time, glucose deprivation condition does not affect the expression level of RIPK1 mRNA in control glioma cells, but up-regulated the level of this mRNA in glioma cells after blockade of ERN1 signaling enzyme function (Fig. 3). These results clearly demonstrated that knockdown of ERN1 modifies the dependence of *RIPK1* gene expression upon glucose and glutamine deprivation conditions.



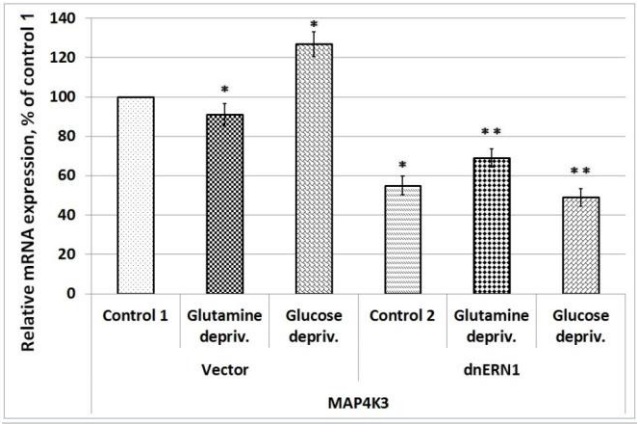




***Fig. 1. Effect of glutamine and glucose deprivation on the expression level of mitogen-activated protein kinase kinase kinase 5 (MAP3K5) mRNA also known as apoptosis signal-regulating kinase 1 (ASK1) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1) into vector pcDNA3.1, measured by quantitative real-time PCR. The level of this mRNA expression was normalized to the expression of beta-actin. The changes in the expression of MAP3K5 mRNA in the glutamine or glucose deprivation conditions in both types of glioma cells were compared with control 1 (100 %), but in cells, transfected with dnERN1, – with control 2; n = 4; * – P < 0,05 as compared to control 1, ** – P < 0,05 as compared with control 2.***

***Fig. 2. Effect of glutamine and glucose deprivation on the expression level of mitogen-activated protein kinase kinase kinase 4 (MAP4K3) mRNA also known as germinal center kinase-like kinase (GLK) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1) into vector pcDNA3.1, measured by quantitative real-time PCR. The level of this mRNA expression was normalized to the expression of beta-actin. The changes in the expression of MAP4K3 mRNA in the glutamine or glucose deprivation conditions in both types of glioma cells were compared with control 1 (100 %), but in cells, transfected with dnERN1, – with control 2; n = 4; * – P < 0,05 as compared to control 1, ** – P < 0,05 as compared with control 2.***

*Note: In fig. 1 – 5: Control 1 represents cells, transfected with vector pcDNA3.1 and Control 2 – cells, transfected with dnERN1.*

Ikner and Ashkenazi (2011) shown that TNF-related weak inducer of apoptosis (TWEAK or TNFSF12) induces apoptosis through a death-signaling complex comprising receptor-interacting protein 1 (RIPK1) as well as Fas-associated death domain (FADD), and caspase-8. This data correlates with our results that ERN1 knockdown strongly up-regulates the expression of *RIPK1* gene (Fig. 3) and suppressed proliferation rate of these cells (Auf et al., 2010).

We have also studied the expression level of *RIPK2* gene in glioma cells with ERN1 knockdown. As shown in Fig. 4, the expression level of RIPK2 mRNA is strongly down-regulated (more than 4fold) in glioma cells with suppressed function of signaling enzyme ERN1. It was also shown that the expression level of *RIPK2* gene did not change significantly in control glioma cells at both glucose and glutamine deprivation conditions, but in cells with ERN1 knockdown both glucose and glutamine deprivation enhances the expression of this gene (2.5fold and +22 %, correspondingly) (Fig. 4). Thus, the blockade of ERN1 enzyme function induces the up-regulation of *RIPK2* gene expression in glioma cells both in glucose and glutamine deprivation conditions. These results is argued with anti-proliferative effect of ERN1 knockdown because there is data that protein kinase RIPK2 plays an important role in hepatic cell migration and inhibition of its tyrosine kinase activity limits NOD2-driven cytokine responses and down-regulated NF-κB-dependent plasminogen activator inhibitor type 1 gene expression (Tigno-Aranjuez et al., 2010; Wu et al., 2012).

Moreover, the suppression of ERN1 enzyme function in U87 glioma cells leads to decreases (almost to 2fold) mRNA level of protein kinase interacting protein CIB1 (Fig. 5). At the same time, glucose deprivation condition does not affect the expression level of gene encoding CIB1 protein both in control and ERN1 knockdown U87 glioma cells, but glutamine deprivation causes slight, but statistically significant, decrease of this mRNA expression level in both types of studied glioma cells (Fig. 5). Thus, the suppression of ERN1 enzyme function does not change significantly the effect of glucose as well as glutamine deprivation on the expression of *CIB1* gene in glioma cells.

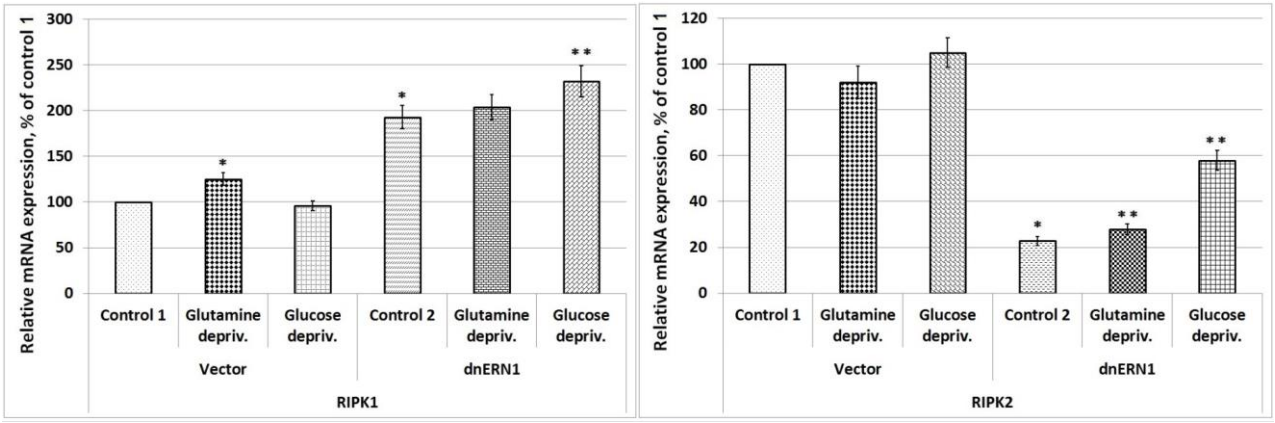



***Fig. 3. Effect of glutamine and glucose deprivation on the expression level of receptor (TNFRSF)-interacting serine-threonine kinase 1 (RIPK1) also known as cell death protein RIP mRNA in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1), measured by quantitative real-time PCR. The level of this mRNA expression was normalized to the expression of beta-actin. The changes in the expression of RIPK1 mRNA in the glutamine or glucose deprivation conditions in both types of glioma cells were compared with control 1 (100 %), but in cells, transfected with dnERN1, – with control 2; n = 4; * – P < 0,05 as compared to control 1, ** – P < 0,05 as compared with control 2.***

***Fig. 4. Effect of glutamine and glucose deprivation on the expression level of receptor-interacting serine-threonine kinase 2 (RIPK2) also known as receptor-interacting protein (RIP)-like interacting caspase-like apoptosis regulatory protein (CLARP) kinase mRNA in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1), measured by quantitative real-time PCR. The level of this mRNA expression was normalized to the expression of beta-actin. The changes in the expression of RIPK2 mRNA in the glutamine or glucose deprivation conditions in both types of glioma cells were compared with control 1 (100 %), but in cells, transfected with dnERN1, – with control 2; n = 4; * – P < 0,05 as compared to control 1, ** – P < 0,05 as compared with control 2.***

Down-regulation of the expression level of protein kinase interacting protein CIB1 in glioma cells ERN1 knockdown possibly also contributes to suppression of tumor growth from these cells (Auf et al., 2010) because this protein is a mediator of carcinogenesis and participates in regulation of apoptosis and angiogenesis (Armacki et al., 2014; Leisner et al., 2013).

Results of this investigation clearly demonstrated that the expression of *MAP4K3, RIPK1*, *RIPK2,* and *CIB1*genes in U87 glioma cells is dependent from blockade of ERN1-mediated endoplasmic reticulum stress and is mostly regulated by glutamine and glucose deprivation in dependence from ERN1 signaling enzyme function. Significant increase of the expression of *RIPK1* as well as decrease of *MAP4K3, RIPK2,* and *CIB1* genes in glioma cells with knockdown of signaling enzyme ERN1 correlates with suppressed proliferation rate of these cells and possibly contributes in this effect, because encoded be these genes proteins are multifunctional and play an important role in the regulation of proliferation and apoptosis (Armacki et al., 2014; Fu et al., 2013; Hayakawa et al., 2011; Lam et al., 2009; Leisner et al., 2013; Minchenko et al., 2014; Naik et al., 2011; Schriever et al., 2013).

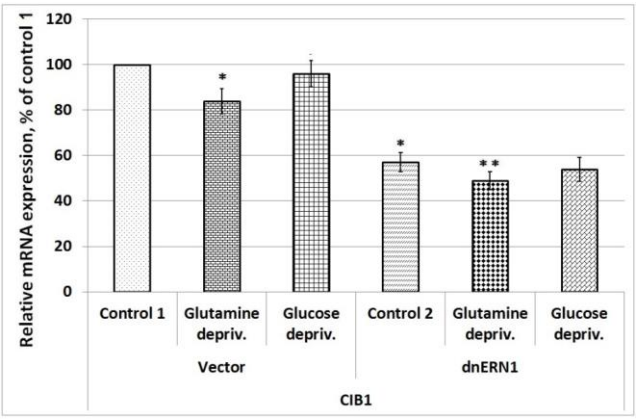



***Fig. 5. Effect of glutamine and glucose deprivation on the expression level of calcium and integrin binding 1 (CIB1) also known as SNK-interacting protein 2-28 (SIP2-28) mRNA in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dnERN1, measured by quantitative real-time PCR. The level of this mRNA expression was normalized to the expression of beta-actin. The changes in the expression of CIB1 mRNA in the glutamine or glucose deprivation conditions in both types of glioma cells were compared with control 1 (100 %), but in cells, transfected with dnERN1, – with control 2; n = 4; * – P < 0,05 as compared to control 1, ** – P < 0,05 as compared with control 2.***

**Conclusions.**

1. It was shown that suppression of both enzymatic functions of sensor and signaling enzyme ERN1 (endoplasmic reticulum to nucleus signaling 1), the major component of endoplasmic reticulum stress signaling, decreases the expression level of genes encoding MAP4K3, RIPK2, and CIB1 in U87glioma cells, but increases – *RIPK1* gene expression.

2. Glutamine deprivation condition leads to increase the expression level of *RIPK1* gene and to decrease – *CIB1* gene in control glioma cells, but ERN1 knockdown modifies the effect of glutamine deprivation on the expression most of these genes.

3. It was also shown that the expression level of *MAP3K5*, *RIPK1*, *RIPK2*, and *CIB1* genes did not change significantly in control glioma cells at glucose deprivation condition, but in cells with ERN1 knockdown glucose deprivation enhances the expression of *RIPK1* and *RIPK2* genes. Thus, suppression of ERN1 enzyme function also changes the effect of glucose deprivation on the expression of most studied genes in glioma cells.

4. Results of this investigation clearly demonstrated that the expression of *MAP4K3*, *RIPK1*, *RIPK2*, and *CIB1*genes in U87 glioma cells is dependent from endoplasmic reticulum stress mediated by ERN1 and that glutamine and glucose deprivation conditions mostly affect these gene expressions in dependence of ERN1 signaling enzyme function.

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## ДЕФІЦИТ ГЛЮКОЗИ ТА ГЛУТАМІНУ ЗМІНЮЄ ЕКСПРЕСІЮ ГЕНІВ *MAP3K5, MAP4K3, CIB1, RIPK1,* AND *RIPK2* У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87 З ВИКЛЮЧЕНОЮ ФУНКЦІЄЮ СИГНАЛЬНОГО ЕНЗИМУ ERN1

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*Протеїнкінази відіграють важливу роль у рості злоякісних пухлин як ключові регулятори різноманітних метаболічних процесів. Ми вивчали ефект дефіциту глюкози та глутаміну на рівень експресії мРНК протеїнкінази кінази кінази кінази 3, що активується міогеном, 3 (MAP4K3), протеїнкінази кінази кінази 3, що активується міогеном, 5 (MAP3K5), серин-треонінової кінази 1, що взаємодіє з рецептором TNFRSF (RIPK1), та RIPK2, а також протеїну CIB1, що взаємодіє з протеїнкіназою у клітинах гліоми лінії U87. Встановлено, що пригнічення обох ензиматичних активностей сенсорно-сигнального ензиму ERN1 (сигналювання від ендоплазматичного ретикулуму до ядра-1), основного сенсорно-сигнального ензиму стресу ендоплазматичного ретикулуму, знижує рівень експресії генів, що кодують MAP4K3, RIPK2 та CIB1, але збільшує експресію гена RIPK1. В той же час, не спостерігалось істотних змін в експресії гена MAP3K5 у клітинах гліоми за умов пригнічення сигнального ензиму ERN1. За умов дефіциту глутаміну спостерігалось збільшення рівня експресії гена RIPK1 і зниження – гена CIB1 у контрольних клітинах гліоми, але пригнічення ERN1 модифікувало ефект дефіциту глутаміну на експресію цих генів. Було також показано, що рівень експресії генів MAP3K5, RIPK1, RIPK2 та CIB1 істотно не змінювався у контрольних клітинах гліоми за умов дефіциту глюкози, в той час як у клітинах з пригніченим ERN1 дефіцит глюкози призводив до посилення експресії генів RIPK1 та RIPK2. Таким чином, пригнічення функції ензиму ERN1 також змінює ефект дефіциту глюкози на експресію більшості досліджених генів у клітинах гліоми. Результати цієї роботи чітко вказують на те, що експресія генів MAP4K3, RIPK1, RIPK2 та CIB1 у клітинах гліоми лінії U87 залежить від пригнічення опосередкованого ERN1 стресу ендоплазматичного ретикулуму і переважно змінюється за умов дефіциту глюкози і глутаміну в залежності від функції сигнального ензиму ERN1.*

*Ключові слова: експресія мРНК, блокада ERN1, MAP3K5, MAP4K3, RIPK1, RIPK2, CIB1, дефіцит глюкози, дефіцит глутаміну, клітини гліоми лінії U87.*

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