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ERN1 KNOCKDOWN MODIFIES THE EFFECT OF GLUTAMINE DEPRIVATION CONDITION ON THE EXPRESSION LEVEL OF *PLAU*, *PLAUR*, *SLURP1*, *PLAT*, *CCN2*, AND *ITGB1* GENES IN U87 GLIOMA CELLS

K. I. KUBAICHUK[1], D. O. MINCHENKO[1,2], I. V. KRYVDIUK[1], O. V. KOVALEVSKA[1], A. O. KULINICH[1,3], O. H. MINCHENKO[1,*]

[1]*Department of Molecular Biology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine;*
[2]*Department of Pediatrics, National Bogomolets Medical University, Kyiv, Ukraine;*
[3]*Department of Biochemistry, Dnipropetrovs'k State Medical Academy, Dnipropetrovs'k, Ukraine*
**e-mail: ominchenko@yahoo.com*

A set of complex intracellular signaling events known as the unfolded protein response is mediated predominantly by ERN1 (from endoplasmic reticulum to nuclei-1) signaling enzyme and is induced in cancer cells and is necessary for tumor growth. We have studied the expression of genes encoding factors, which control cell proliferation, in ERN1 knockdown glioma U87 cells in glutamine deprivation condition. It was shown that the blockade of ERN1 enzyme function leads to increase PLAT (tissue plasminogen activator), CCN2 (CCN family member 2), and ITGB1 (integrin beta-1) as well as to decrease of the expression level of PLAU (urokinase plasminogen activator), PLAUR (plasminogen activator, urokinase receptor), and SLURP1 (secreted LY6/PLAUR domain containing 1) mRNA expressions. Moreover, we have shown that glutamine deprivation condition does not affect the expression level of PLAUR, SLURP1, and ITGB1 mRNA, but increases CCN2 and decreases PLAU and PLAT mRNA in control glioma cells. At the same time, the expression level of PLAU mRNA is increased in ERN1 knockdown glioma cells under glutamine deprivation condition, but the expression level all other genes is decreased under this experimental condition. Furthermore, the blockade of ERN1 signaling enzyme function significantly enhances this effect of glutamine deprivation as compared to control glioma cells. Thus, results of this study clearly demonstrated that the expression level of all studied genes is affected by ERN1 knockdown and that effect of glutamine deprivation condition is mostly depended from ERN1 signaling enzyme function.

Key words: mRNA expression, PLAU, PLAUR, SLURP1, PLAT, CCN2, ITGB1, ERN1 knockdown, glioma cells, glutamine deprivation.

Introduction. Nutrient deprivation condition as well as endoplasmic reticulum stress is an important factor of malignant tumor growth (Johnson et al., 2008; Denko, 2008). The endoplasmic reticulum stress is associated with accumulation of unfolded proteins in the endoplasmic reticulum and controls the neovascularization and proliferation processes (Zhang and Kaufman , 2004; Moenner et al., 2007). 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 Nuclei-1 / Inositol Requiring Enzyme-1), PERK (PRK-like ER kinase), and ATF6 (Activating Transcription Factor 6); however, ERN1 is the dominant sensor (Bi et al., 2005; Aragón et al., 2009; Fels and Koumenis, 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 degradation to adapt cells for survival or, alternatively, to enter cell death programs through endoplasmic reticulum-associated machineries (Moenner et al., 2007; Romero-Ramirez et al., 2004; Acosta-Alvear et al., 2007; Hollien et al., 2009). 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 (Zhang and Kaufman, 2004; Moenner et al., 2007).

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 signalling. The ERN1-associated kinase autophosphorylates and dimerizes this enzyme in the endoplasmic reticulum membrane, leading to the activation of its endoribonucle-

ase domain. 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 (Moenner et al., 2007; Romero-Ramirez et al., 2004). Mature XBP1 mRNA splice variant encodes a transcription factor that stimulates the expression more than five hundreds of unfolded protein response-specific genes (Acosta-Alvear et al., 2007; Hollien et al., 2009). Moreover, XBP1s has several additional functions, especially in the regulation of glucose homeostasis (Lee et al., 2011; Zhou et al., 2013). Thus, the p38 MAP kinase phosphorylates the spliced form of XBP1 and enhances its nuclear migration. Zhou et al. (Zhou et al., 2013) 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 (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.

The endoplasmic reticulum stress response-signalling pathway is linked to the proliferation process and tumor growth because the complete blockade of ERN1 signal transduction pathway has anti-tumor effects in lung cancer and glioma cells (Moenner et al., 2007; Drogat et al., 2007; Auf et al., 2010). Malignant gliomas are highly aggressive tumors and are characterized by marked angiogenesis and extensive tumor cell invasion into the normal brain parenchyma (Moenner et al., 2007). Ischemia as well as hypoxia is associated to glioma development and locally induce an adaptive response which confers to tumor cells an enhanced survival and a more agressive behaviour (Moenner et al., 2007). A better knowledge of tumor responses to ischemia is required to elaborate therapeutical strategies of cell sensibilization and angiogenesis inhibition, based on the blockade of survival mechanisms.

Cell proliferation regulatory proteins PLAU, PLAUR, SLURP1, PLAT, CCN2, and ITGB1 play an important role in malignant tumor growth. Thus, PLAU (urokinase plasminogen activator) and its receptor PLAUR are responsible for tumor cell migration and proliferation (Hakelius et al., 2013; Grismayer et al., 2012). It is important to note that microRNA 137 can induce G1 cell cycle arrest in lung cancer cells as well as promotes transendothelial migration of cancer cells through beta1 integrin (Reymond et al., 2012). PLAUR mediates the proteolysis-independent signal transduction activation effects of PLAU and regulates single-chain PLAU-mediated angiogenesis through ITGB1 (beta1-integrin) and VEGFR2 (vascular endothelial growth factor receptor 2) (Larusch et al., 2013). It was also shown that activation of alpha(7)-nAChR by SLURP1 (secreted LY6/PLAUR domain containing 1) leads to up-regulation of NF-kB gene expression due to activation of the RAF1/MEK1/ERK1/2 cascade that proceeds via two complementary signaling pathways (Chernyavsky et al., 2010).

Protein CCN2 (CCN family member 2) also known as IGFBP8 (insulin-like growth factor binding protein 8) plays a role in cell proliferation, differentiation and, angiogenesis, cell adhesion in many cell types, and is related to platelet-derived growth factor (Yu et al., 2013). Loss of connective tissue growth factor activates miR-18b and promotes cell growth in nasopharyngeal carcinoma. Integrin beta1 (ITGB1) is involved in a variety of processes including cell proliferation, migration and invasion (Reymond et al., 2012; Larusch et al., 2013; Lee et al., 2013).

The main goal of this study was investigation the role of the expression of genes related to the control of cell proliferation (PLAU, PLAUR, SLURP1, PLAT, CCN2, and ITGB1) in glioma U87 cells with ERN1 signaling enzyme loss of function and its regulation upon glutamine deprivation condition.

Materials and Methods. The glioma cell line U87 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 *PLAU, PLAUR, SLURP1, PLAT, CCN2,* and *ITGB1* 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 this signaling enzyme. These cells were obtained from prof. M. Moenner (France) (Moenner et al., 2007; Drogat et al., 2007). The expression level of *PLAU, PLAUR, SLURP1, PLAT, CCN2,* and *ITGB1* genes in these cells was compared with cells, transfected by vector (control 1), but this subline was also used as control 2 for investigation the effect of glutamine deprivation condition on the expression level of these genes under blockade ERN1 function. Glutamine deprivation

condition was created by changing the complete DMEM 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., 2012).

Total RNA was extracted from glioma cells as described (Minchenko et al., 2012). RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water. For additional purification RNA samples were precipitated with 95 % ethanol and redissolved again in nuclease-free water.

QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis as described previously (Minchenko et al., 2012).

The expression levels of PLAU, PLAUR, SLURP1, PLAT, CCN2, and ITGB1 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 PLAU (urokinase plasminogen activator) cDNA we used forward (5'– tcaccaccaaaatgctgtgt –3' and reverse (5'– aggccattctcttccttggt –3') primers. The nucleotide sequences of these primers correspond to sequences 1210 – 1229 and 1432 – 1413 of human PLAU cDNA (GenBank accession number NM_002658). The size of amplified fragment is 223 bp.

The amplification of PLAUR (plasminogen activator, urokinase receptor) cDNA was performed using forward primer (5'– gccttaccgaggttgtgtgt–3') and reverse primer (5'– tgttgcagcatttcaggaag –3'). These oligonucleotides correspond to sequences 486 – 505 and 809 – 790 of human PLAUR cDNA (GenBank accession number NM_002659). The size of amplified fragment is 324 bp.

The amplification of SLURP1 (secreted LY6/PLAUR domain containing 1) cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5'– cctgatcttctgctgcttcc –3' and reverse – 5'– cctgatcttctgctgcttcc –3'. The nucleotide sequences of these primers correspond to sequences 293 – 312 and 476 – 457 of human SLURP1 cDNA (GenBank accession number NM_020427). The size of amplified fragment is 184 bp. 1189 339 1527

For amplification of CCN2 (CCN family member 2) cDNA we used forward (5'– actgtcccggagacaatgac –3' and reverse (5'– tgctcctaaagccacacctt –3') primers. The nucleotide sequences of these primers correspond to sequences 1189 – 1208 and 1527 – 1508 of human CCN2 cDNA (GenBank accession number NM_001901). The size of amplified fragment is 339 bp.

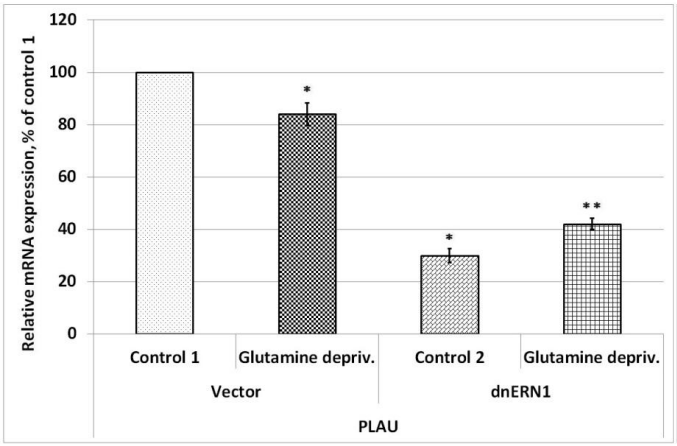
The amplification of PLAT (tissue plasminogen activator) cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5'– cagcaggccctgtacttctc –3' and reverse – 5'– ggctttgagtctcgatctgg –3'. The nucleotide sequences of these primers correspond to sequences 501 – 520 and 772 – 753 of human PLAT cDNA (GenBank accession number NM_000930). The size of amplified fragment is 272 bp.

For amplification of ITGB1 (integrin beta-1) cDNA we used forward (5'– cgaggtcatggttcatgttg –3' and reverse (5'– cagtgttgtgggatttgcac –3') primers. The nucleotide sequences of these primers correspond to sequences 2354 – 2373 and 2647 – 2628 of human ITGB1 cDNA (GenBank accession number NM_002211). The size of amplified fragment is 294 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" (USA).

An analysis of quantitative PCR was performed using special computer program "Differential expression calculator" and statistical analysis – in Excel program. The values of PLAU, PLAUR, SLURP1, PLAT, CCN2, and ITGB1 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 4 independent experiments.

Results and Discussion. As shown in fig. 1, the blockade of ERN1, the major component of endoplasmic reticulum stress signaling, leads to more than 3 fold decrease of the expression level of PLAU mRNA expression in U87 glioma cells. Moreover, exposure glioma cells under glutamine deprivation condition also decreased the expression level of PLAU mRNA in both types of U87 glioma cells, but ERN1 knockdown significantly modifies the effect of glutamine deprivation: the level of PLAU mRNA expression is significantly increased in these cells. Similar results were received with urokinase plasminogen activator receptor mRNA expression (Fig. 2). Thus, the expression level of PLAUR mRNA expression is decreased more than in two fold in ERN1 knockdown U87 glioma cells.



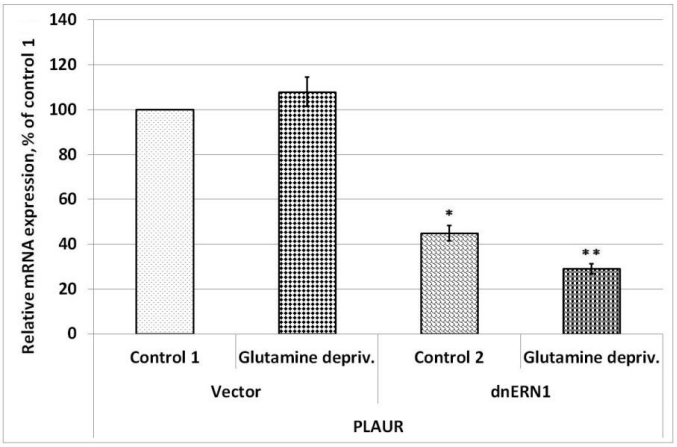


Fig. 1. Effect of glutamine deprivation condition on the expression level of PLAU (urokinase plasminogen activator) mRNA in U87 glioma cells, stable transfected with vector pcDNA3.1 (Vector) and cells, stable transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1), measured by quantitative real-time PCR. The level of PLAU mRNA expression was normalized to the expression of beta-actin.

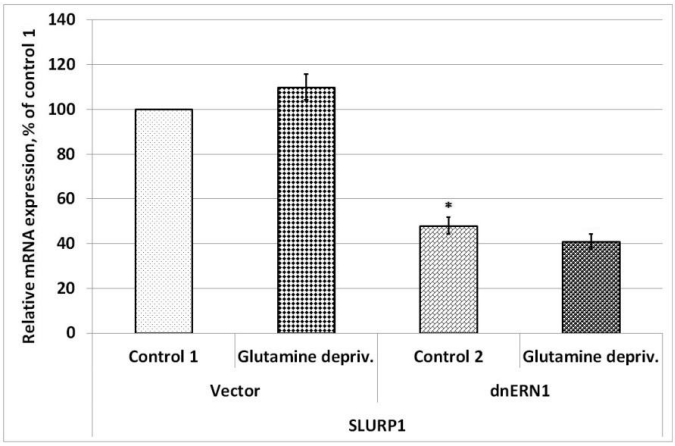
Fig. 2. Effect of glutamine deprivation condition on the expression level of PLAUR (plasminogen activator, urokinase receptor) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and ERN1 knockdown cells (dnERN1), measured by quantitative PCR. The level of PLAUR mRNA expression was normalized to the expression of beta-actin.

*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).*

However, glutamine deprivation condition also suppressed the PLAUR mRNA expression but only in ERN1 knockdown U87 glioma cells (Fig. 2).

Investigation of SLURP1 (secreted LY6/PLAUR domain containing 1) mRNA expression in U87 glioma cells is shown that the level of its mRNA is also decreased in ERN1 knockdown U87 glioma cells like PLAUR mRNA. As shown in fig. 3, the blockade of ERN1 leads to two fold decrease of the expression level of SLURP1 mRNA in glioma cells. However, an exposure glioma cell under glutamine deprivation condition does not affect the expression of SLURP1 mRNA in both control and ERN1 knockdown glioma cells.

The expression of three other genes (*PLAT*, *CCN2*, and *ITGB1*) is increased after blockade of ERN1 signaling enzyme function, being more significant for *CCN2* and *PLAT* (Fig. 4 – 6).



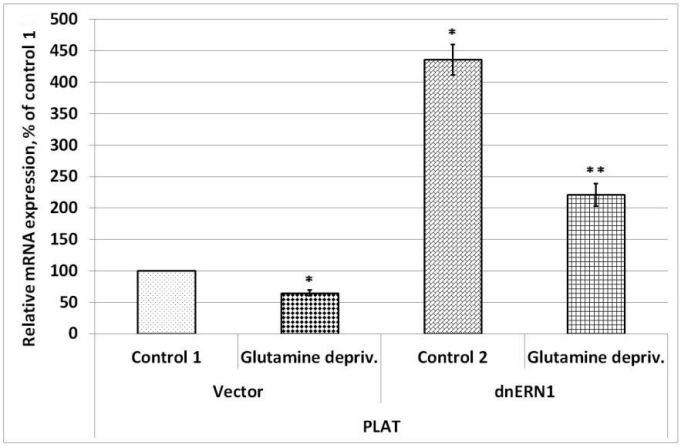


Fig. 3. Effect of glutamine deprivation condition on the expression level of SLURP1 (secreted LY6/PLAUR domain containing 1) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and ERN1 knockdown cells (dnERN1), measured by quantitative PCR. The level of SLURP1 mRNA expression was normalized to the expression of beta-actin.

Fig. 4. Effect of glutamine deprivation condition on the expression level of CCN2 (CCN family member 2) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and ERN1 knockdown cells (dnERN1), measured by quantitative PCR. The level of CCN2 mRNA expression was normalized to the expression of beta-actin.

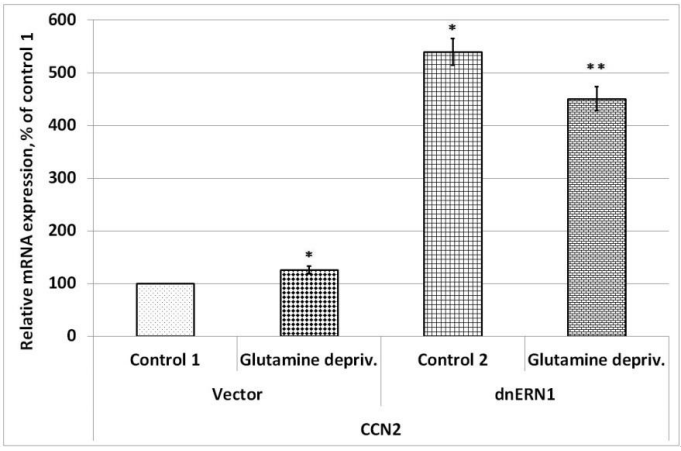


Fig. 5. Effect of glutamine deprivation condition on the expression level of PLAT (tissue plasminogen activator) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and ERN1 knockdown cells (dnERN1), measured by quantitative PCR. The level of PLAT mRNA expression was normalized to the expression of beta-actin.

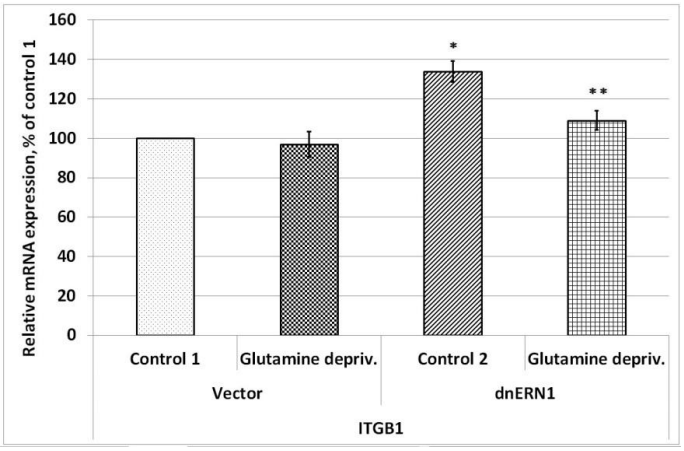


Fig. 6. Effect of glutamine deprivation condition on the expression level of ITGB1 (integrin beta-1) in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and ERN1 knockdown cells (dnERN1), measured by quantitative PCR. The level of PLAUR mRNA expression was normalized to the expression of beta-actin.

Thus, the expression levels of PLAT and CCN2 mRNA is increased in more than 4 and 5 fold, correspondingly, but ITGB1 mRNA – on 34 % only. We have also shown that glutamine deprivation condition does not affect the expression of ITGB1 mRNA in control glioma cells, increases CCN2 (+ 25 %) and decreases PLAT (- 35 %) mRNA (Fig. 4 – 6). Moreover, the blockade of ERN1 signaling enzyme function modifies the effect of glutamine deprivation condition on the expression of *PLAT*, *CCN2*, and *ITGB1* genes in U87 glioma cells. Thus, the expression level of PLAT, CCN2, and ITGB1 mRNA is decreased (-49 %, - 16 %, and – 18 %, correspondingly) (Fig. 4 – 6). Moreover, the knockdown of signaling enzyme ERN1 in U87 glioma cells leads to suppression of cell proliferation and tumor growth from these cells (Drogat et al., 2007; Auf et al., 2010) as well as create some morphological changes (Fig. 7).

Thus, results of this study clearly demonstrated that the expression levels of all tested genes encoded important regulatory factors controlling cell proliferation and malignant tumor growth is affected by ERN1 knockdown. Moreover, the decrease of PLAU and PLAUR mRNA expression levels in glioma cells after blockade of ERN1 signaling enzyme function is argued with suppression of tumor growth from these cells (Drogat et al., 2007; Auf et al., 2010), because both PLAU and PLAUR are up-regulated in the tumors as well as are responsible for cell proliferation and migration (Hakelius et al., 2013; Grismayer et al., 2012). Furthermore, the decrease of SLURP1 mRNA expression in U87 glioma cells after ERN1 knockdown can also participate in the suppression of tumor growth because there is data that SLURP1 protein can activate protein kinases and up-regulate the NF-kB gene expression (Chernyavsky et al., 2010).

Protein CCN2 is a multifunctional protein and plays a role in cell proliferation, differentiation, and angiogenesis. At the same time, loss of CCN2 activates miR-18b and promotes cell growth in nasopharyngeal carcinoma (Yu et al., 2013). Moreover, overexpression of MYC promoted vigorous tumor vascularization and growth without changes in VEGF level, but enhanced neovascularization correlated with down-regulation of anti-angiogenic thrombospondin-1 and CCN2 which binds vascular endothelial growth factor and inhibits VEGF-induced angiogenesis (Dews et al., 2006; Inoki et al., 2002).

There is data that induction of cell death is associated with increase of several gene expressions, including the PLAT, suggesting a mechanism by which the program of tissue atrophy coordinately removes extracellular matrix as cells die (Kenagy et al., 2011). It is possible, that overexpression of PLAT mRNA in ERN1 knockdown glioma cells is also associated with suppression of tumor growth from these cells (Drogat et al., 2007; Auf et al., 2010) by induction of cell death. Moreover, induction of PLAT by shark cartilage extract plays an essential role in its antiangiogenic activity (Simard et al., 2011).

Integrin beta1 (ITGB1) is also involved in a variety of processes including cell proliferation, migration and invasion as component of receptors for collagen, fibronectin, laminin, and thrombospondin (Reymond et al., 2012; Larusch et al., 2013; Lee et al., 2013). There are data that ITGB1 participate in PLAU-mediated angiogenesis through PLAUR (Larusch et al., 2013). It is possible that increase in ITGB1 expression in ERN1 knockdown glioma cells is connected with strong up-regulation of anti-angiogenic thrombospondin-1 (Kubaichuk et al., 2012).

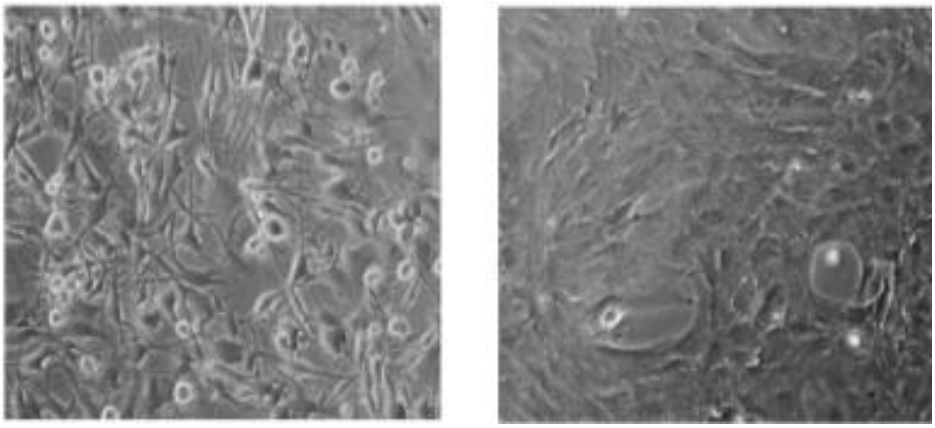


Fig. 7. Phase-contrast photomicrographs of control U87 glioma cells (left) ERN1 knockdown U87 glioma cells (right) are shown after 3 days in culture.

We have shown that glutamine deprivation condition affects the expression level most of studied genes and that ERN1 knockdown modifies the effect of glutamine deprivation on PLAT, PLAU, PLAUR, and ITGB1 mRNA expressions. Moreover, these changes usually decrease the effect of ERN1 knockdown on *PLAT*, *PLAU, CCN2,* and *ITGB1* gene expressions. However, detailed molecular mechanisms of participation the important growth regulatory factors, such as PLAU, PLAUR, SLURP1, PLAT, CCN2, and ITGB1, in suppression of tumor growth from ERN1 knockdown glioma cells warrants further study.

Conclusions.

1. It was shown that blockade of both enzymatic activities of signaling enzyme ERN1, the major component of endoplasmic reticulum stress signaling, decreases the expression level of genes encoding PLAU, PLAUR, and SLURP1 in U87 glioma cells.

2. The expression level of CCN2, PLAT, and ITGB1 mRNA is increased in ERN1 knockdown glioma cells, being more significant for CCN2 and PLAT.

3. Glutamine deprivation condition does not affect the expression level of PLAUR, SLURP1, and ITGB1 mRNA, but decreases PLAU and PLAT mRNA in control glioma cells.

4. The expression level of PLAU mRNA is increased, but the expression levels of all other genes are decreased in ERN1 knockdown glioma cells under glutamine deprivation condition. Furthermore, the blockade of ERN1 signaling enzyme function significantly enhances this effect of glutamine deprivation as compared to control glioma cells.

5. Results of this study clearly demonstrated that the expression levels of all studied genes is affected by ERN1 knockdown and that effect of glutamine deprivation condition is mostly depended from ERN1 signaling enzyme function.

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БЛОКАДА ФУНКЦІЇ ERN1 ЗМІНЮЄ ЕФЕКТ ДЕФІЦИТУ ГЛУТАМІНУ НА РІВЕНЬ ЕКСПРЕСІЇ ГЕНІВ *PLAU, PLAUR, SLURP1, PLAT, CCN2* ТА *ITGB1* У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87

К. І. Кубайчук, Д. О. Мінченко, І. В. Кривдюк, О. В. Ковалевська, А. О. Кулініч, О. Г. Мінченко

Реакція на не згорнуті протеїни являє собою комплекс складних внутрішньоклітинних подій, що опосередковується переважно сигнальним ензимом ERN1 (від ендоплазматичного ретикулуму до ядра) і виражено активується у клітинах злоякісних пухлин. Вона є необхідним фактором росту пухлин. Ми вивчали експресію генів, що кодують важливі для проліферації клітин фактори, у клітинах гліоми лінії U87 з пригніченою функцією ERN1 за умов дефіциту глутаміну. Встановлено, що блокада ERN1 призводить до збільшення рівня експресії мРНК PLAT (tissue plasminogen activator), CCN2 (CCN family member 2), та ITGB1 (integrin beta-1), а також до зниження рівня експресії мРНК PLAU (urokinase plasminogen activator), PLAUR (plasminogen activator, urokinase receptor), та SLURP1 (secreted LY6/PLAUR domain containing 1). Більше того, було показано, що рівень експресії мРНК PLAUR, SLURP1 та ITGB1 за умов дефіциту глутаміну істотно не змінюється у контрольних клітинах гліоми, але збільшується рівень експресії мРНК CCN2 та знижується рівень експресії мРНК PLAU і PLAT. В той же час, рівень експресії мРНК PLAU збільшується у клітинах гліоми з пригніченою функцією ензиму ERN1 за умов дефіциту глутаміну, але рівень експресії всіх інших генів за цих експериментальних умов знижується. Більше того, блокада функції сигнального ензиму ERN1 істотно посилює цей ефект дефіциту глутаміну у порівнянні з контрольними клітинами гліоми. Таким чином, отримані нами дані свідчать про те, що рівень експресії всіх досліджених генів змінюється за умов блокади ERN1 і що ефект дефіциту глутаміну у середовищі переважно залежить від функції сигнального ензиму ERN1.

Ключові слова: експресія мРНК, PLAU, PLAUR, SLURP1, PLAT, CCN2, ITGB1, виключення функції ERN1, клітини гліоми, дефіцит глутаміну.

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