UDC 577.112.7:61

EFFECT OF NUTRIENT DEPRIVATION ON THE EXPRESSION OF *PDGFC*, *PDGFRA*, AND *PDGFRB* GENES IN U87 GLIOMA CELLS DEPENDS ON ERN1 SIGNALING ENZYME FUNCTION

K. I. KUBAICHUK, D. O. MINCHENKO, O. O. RIABOVOL, O. V. HALKIN, O. H. MINCHENKO

Department of Molecular Biology, Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, 9 Leontovycha St., 01601, Kyiv, Ukraine;
Departments of Pediatrics, Bohomolets National Medical University, 13 Shevchenka Bvld., 01601, Kyiv, Ukraine;
e-mail: ominchenko@yahoo.com

Growth factors, which control angiogenesis, play an important role in malignant tumor progression. We studied the effect of glucose or glutamine deprivation conditions on the expression level of platelet derived growth factor C (PDGFC) and its receptors PDGFRA (platelet derived growth factor receptor A) and PDGFRB 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, upregulates the expression level of genes encoding PDGFC, PDGFRA, and PDGFRB in U87 glioma cells. Glutamine deprivation condition leads to increase the expression level of PDGFC gene and to decrease – PDGFRA and PDGFRB genes in control glioma cells, but ERN1 knockdown modifies the effect of glutamine deprivation on the expression of these genes. It was also shown that the expression level of PDGFC gene did not change significantly in control glioma cells at glucose deprivation condition, but in cells with ERN1 knockdown glucose deprivation decreases the expression of this gene. Results of this investigation clearly demonstrated that the expression of PDGFC, PDGFRA, and PDGFRB 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 of ERN1 signaling enzyme function.

Key words: mRNA expression, ERN1 knockdown, PDGFC, PDGFRA, PDGFRB, glutamine deprivation, glucose deprivation, U87 glioma cells

Introduction. 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 as well as hypoxia are associated to glioma development and more agressive behaviour as well as to cell surviving (Denko et al., 2008; Johnson et al., 2008; Lenihan and Taylor, 2013; Moenner et al., 2007).. The endoplasmic reticulum stress response-signalling pathways are tightly linked to angiogenesis and cell proliferation process as well as tumor growth because blockade of ERN1 signaling in glioma and lung cancer cells had anti-tumor effects (Auf et al., 2010, 2013; Drogat et al., 2007).

The unfolded protein response/endoplasmic reticulum stress is associated with accumulation of unfolded/misfolded proteins in the endoplasmic reticulum (Moenner et al., 2007; Wang and Kaufman, 2012). This adaptive response is activated upon the accumulation of unfolded proteins in the endoplasmic reticulum and is mediated by three endoplasmic reticulum-resident sensors named PERK (double stranded RNA activated protein Kinase (PRK)-like ER kinase), IRE1alpha (Inositol Requiring Enzyme-1alpha) also known as ERN1 (Endoplasmic Reticulum to Nucleus signaling 1) and ATF6 (Activating Transcription Factor 6); however, ERN1 is the dominant sensor and signaling enzyme (Bi et al., 2005; Fels et al., 2006; Manie et al., 2014; Minchenko et al., 2013; Zhang and Kaufman, 2006). 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 (Hetz et al., 2013; Minchenko et al., 2014; Schröder, 2008).

The ERN1 enzyme has two distinct catalytic domains: for kinase and endoribonuclease, both of 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; Maurel et al., 2014; 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). At the same time, it was shown that an inhibitor of ERN1 kinase activates the endoribonuclease of this enzyme to confer cytoprotection against endoplasmic reticulum stress. It is possible that activation of the ERN1 endoribonuclease after inhibition of kinase is a result of its interaction with other sensor-signalling systems of endoplasmic reticulum stress.

Growth factor PDGFC, also known as VEGFE (vascular endothelial growth factor E), is a member of the platelet-derived growth factor family and works as homodimer. It may play an essential role in the regulation of angiogenesis, cell proliferation, cell migration, survival, chemotaxis, and possibly responsible for invasiveness (Lee et al., 2013; Ruffini et al., 2013; Son et al., 2014; Wright et al., 2014). In the nucleus, PDGFC seems to have additional function. There is data that PDGFC mRNA expression is down-regulated in human papillary thyroid carcinomas containing infiltrated lymphocytes and inverse correlation exist between PDGFC expression and lymphocyte infiltration (Bruland et al., 2009). No other PDGF family member could be linked to lymphocyte specific gene expression in our collection of PTCs biopsies. Platelet-derived growth factor receptors (PDGFRs) are catalytic receptors that have intracellular tyrosine kinase activity and have roles in the regulation of many biological processes including angiogenesis, cell proliferation, apoptosis and differentiation, and contribute to the pathophysiology of some diseases, including cancer (Sciaccaluga et al., 2013; Sun et al., 2014). There are two isoforms of the PDGFR receptor (PDGFRA and PDGFRB, which can form homo- or heterodimers, which induce receptor dimerization and transphosphorylation at specific tyrosine residues and activates the intracellular kinase activity, initiating intracellular signaling through the MAPK, PI3-K and PKCG pathways (Cheng et al., 2013; Sciaccaluga et al., 2013; Sun et al., 2014).

The main goal of this study was investigation the role of the blockade of ERN1 signaling enzyme on the expression of *PDGFC*, *PDGFRA*, and *PDGFRB* genes in glioma U87 cells and its regulation by hypoxia and nutrient 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 37°C 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 (dominant/negative construct of ERN1). 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 (Auf et al., 2010; Drogat et al., 2007).

The effect of glutamine and glucose deprivation conditions on the expression level of PDGFC, PDGFRA, and PDGFRB mRNAs in glioma cells, transfected by vector, as well as effect of 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 PDGFC, PDGFRA, and PDGFRB mRNA were measured in glioma cell line U87 and its ERN1 knockdown subline by real-time quantitative polymerase chain reaction using „Mx

3000P QPCR" (Stratagene, USA) and SYBRGreen Mix (AB gene, Great Britain).

The amplification of PDGFC (platelet derived growth factor C) cDNA was performed using forward primer (5'– ctcctggttaaacgctgtgg –3') and reverse primer (5'– tatcctcctgtgctccctct –3'). These oligonucleotides correspond to sequences 1314 – 1333 and 1528 – 1509 of human PDGFC cDNA (GenBank accession number NM_016205). The size of amplified fragment is 215 bp. For amplification of PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) cDNA we used forward (5'– ggcacgctctttactccatg –3' and reverse (5'– aaggccgctgttgttttctt –3') primers. The nucleotide sequences of these primers correspond to sequences 262 – 281 and 571 – 552 of human PDGFRA cDNA (GenBank accession number NM_006206). The size of amplified fragment is 310 bp. The amplification of PDGFRB (platelet-derived growth factor receptor, beta polypeptide) cDNA for real time qRCR analysis was performed using two oligonucleotides primers: forward – 5'– cggagagcatcttcaacagc –3' and reverse – 5'– taaccttcgcccaacagtct –3'. The nucleotide sequences of these primers correspond to sequences 3084 – 3103 and 3366 – 3347 of human PDGFRB cDNA (GenBank accession number NM_002609). The size of amplified fragment is 283 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-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 PDGFC, PDGFRA, and PDGFRB 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 PDGFC and its receptors PDGFRA and PDGFRB 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 signaling enzyme ERN1 significantly upregulated (2.6 fold) the expression level of *PDGFC* gene in U87 glioma cells. Moreover, glutamine deprivation condition upregulates the expression level of mRNA for PDGFC (+35 %) in control glioma cells as compared to control 1, but in cells with blockade of ERN1 signaling enzyme function the expression level of this gene does not change significantly as compared to control 2 upon glutamine deprivation condition (Fig, 1).

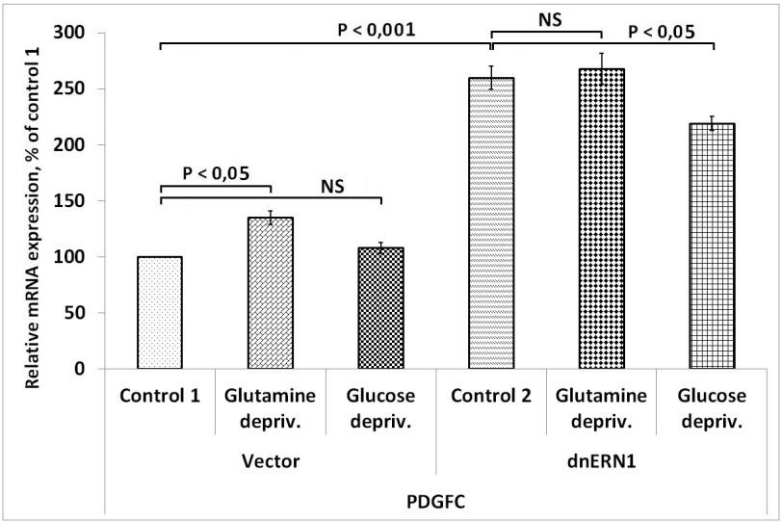

Fig. 1. Effect of glutamine and glucose deprivation on the expression level of PDGFC (platelet derived growth factor C) mRNA in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1) into vector pcDNA3.1, 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 PDGFC mRNA in the glutamine or glucose deprivation conditions in control glioma cells were compared to control 1 (100 %), but in cells, transfected with dnERN1, – to control 2; n = 4.
Note: in fig. 1 – 3: Control 1 represents cells, transfected with vector pcDNA3.1 and Control 2 – cells, transfected with dnERN1.

Thus, blockade of ERN1 signaling enzyme function eliminates the effect of glutamine deprivation condition on the expression level of *PDGFC* gene in U87 glioma cells. At the same time, glucose deprivation condition does not change significantly the expression level of *PDGFC* gene in control glioma cells, but slightly downregulates it expression (-16 %) in cells without ERN1 signaling enzyme function.

Investigation of the expression of *PDGFRA* gene demonstrates that knockdown of signaling enzyme ERN1 leads to significant (more than 2 fold) increase of its mRNA expression level in U87 glioma cells (Fig. 2). Moreover, the glutamine as well as glucose deprivation condition strongly downregulates the expression level of *PDGFRA* gene (2 fold) in control glioma cells; however, in cells with suppressed function of ERN1 signaling enzyme glutamine deprivation condition does not affects its expression (Fig. 2). At the same time, glucose deprivation condition also downregulates (+-64 %) the expression level of PDGFRA mRNA in glioma cells after blockade of ERN1 signaling enzyme function (Fig. 2). Thus, the regulation of PDGFRA mRNA expression in glioma cells both by glucose and glutamine deprivation conditions is strongly depended upon ERN1 signaling enzyme function.

As shown in Fig. 3, the *PDGFRB* gene expression is upregulated (more than in 3 fold) 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 strongly decreased (in 3 fold) in control glioma cells and ERN1 knockdown significantly decreases this effect (-29 % only). At the same time, glucose deprivation condition slightly decreases the expression level of PDGFRB mRNA both in control glioma cells (-17 % as compared to control 1) and in cells with blockade of ERN1 signaling enzyme function (-21 % as compared to control 2) (Fig. 3).

Upregulation of platelet-derived growth factor C in cells with blockade of ERN1 signaling enzyme function possibly not contributes to suppressed angiogenesis in tumors growing from these glioma cells, but it may responsible for enhanced invasiveness, because PDGFC plays an essential role in the regulation of cell migration and invasiveness, not only angiogenesis (Auf et al., 2010; Drogat et al., 2007; Lee et al., 2013; Ruffini et al., 2013; Son et al., 2014). Moreover, *PDGFC* gene expression is down-regulated in human papillary thyroid carcinomas (Bruland et al., 2009). These results agree with data Hose et al. (2009) concerning a complex network in the regulation of angiogenesis in malignant tumors. They demonstrate that purified myeloma cells from 300 untreated patients do not show a significantly higher median number of expressed pro-angiogenic (45) or anti-angiogenic (31) genes, but almost all of these myeloma cells samples aberrantly express at least one of the angiogenic factors. Furthermore, this complex angiogenic network is regulated by endoplasmic reticulum stress signaling at transcriptional and post-transcriptional levels that has been shown preferentially for VEGF-A (Pereira et al., 2010).

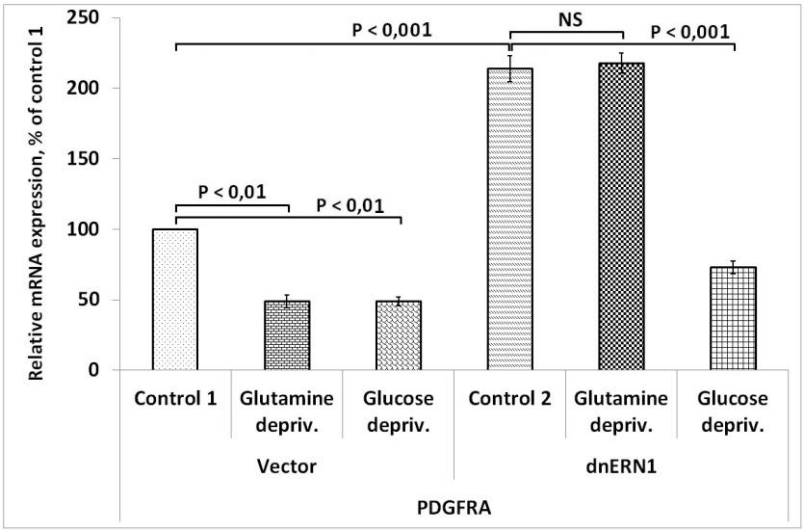

Fig. 2. Effect of glutamine and glucose deprivation on the expression level of PDGFRA (platelet derived growth factor receptor A) mRNA in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1) into vector 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 PDGFRA mRNA in the glutamine or glucose deprivation conditions in control glioma cells were compared to control 1 (100 %), but in cells, transfected with dnERN1, – to control 2; n = 4.

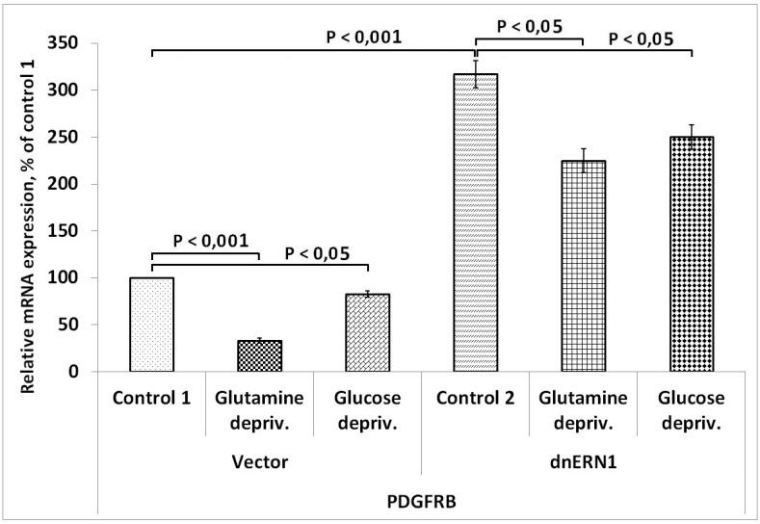

Fig. 3. Effect of glutamine and glucose deprivation on the expression level of PDGFRB (platelet derived growth factor receptor B) mRNA in U87 glioma cells, transfected with vector pcDNA3.1 (Vector) and cells, transfected with dominant/negative constructs of ERN1 signaling enzyme (dnERN1) into vector 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 PDGFRB mRNA in the glutamine or glucose deprivation conditions in control glioma cells were compared to control 1 (100 %), but in cells, transfected with dnERN1, – to control 2; n = 4.

We have also shown that both receptors of PDGF are upregulated in glioma cells with knockdown of ERN1 signaling enzyme. Thus, upregulation of PDGFC correlates to increased expression of *PDGFRA* and *PDGFRB* genes and possibly all these genes contributes to enhanced invasiveness of glioma cells without ERN1 function (Auf et al., 2010; Sciaccaluga et al., 2013; Sun et al., 2014). Moreover, glutamine deprivation suppresses the expression of *PDGFRA* and *PDGFRB* genes in glioma cells and this effect mostly depends on ERN1 signaling enzyme function. This data correlate with upregulation of both PDGF receptor genes in glioma cells with ERN1 knockdown and indicate possible participation of *PDGFRA* and *PDGFRB* genes in invasiveness of these glioma cells (Sciaccaluga et al., 2013).

Results of this investigation clearly demonstrated that the expression of *PDGFC*, *PDGFRA*, and *PDGFRB* 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 *PDGFC* as well as *PDGFRA* and *PDGFRB* genes in glioma cells with knockdown of signaling enzyme ERN1 correlates with suppressed proliferation rate of these cells and enhanced invasiveness and possibly contributes in these effects, because encoded be these genes proteins are multifunctional and play an important role in the regulation of angiogenesis, cell proliferation and migration (Lee et al., 2013; Ruffini et al., 2013; Sciaccaluga et al., 2013; Son et al., 2014; Sun et al., 2014).

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, upregulates the expression level of genes encoding PDGFC, PDGFRA, and PDGFRB in U87 glioma cells.
2. Glutamine deprivation condition leads to upregulation of the expression of *PDGFC* gene and to decrease – *PDGFRA* and *PDGFRB* genes in control glioma cells, but ERN1 knockdown eliminates the effect of glutamine deprivation on the expression of *PDGFC* and *PDGFRA* genes.
3. It was also shown that the expression level of *PDGFC* gene did not change significantly in control glioma cells at glucose deprivation condition, but decreased in cells with ERN1 knockdown. At the same time, the expression level of *PDGFRA* and *PDGFRB* genes is decreased in both types of glioma cells.
4. Results of this investigation clearly demonstrated that the expression of *PDGFC*, *PDGFRA*, and *PDGFRB* 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.

References:

1. Acosta-Alvear D., Zhou Y., Blais A., Tsikitis M., Lents N.H., Arias C., Lennon C.J., Kluger Y.,

Dynlacht D.D. XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks // Molecular Cell. – 2007. – 27. – P. 53 – 66.
2. Aragón T., van Anken E., Pincus D., Serafimova I.M., Korennykh A.V., Rubio C.A., Walter P. Messenger RNA targeting to endoplasmic reticulum stress signalling sites. // Nature. – 2009. – 457, N 7230. – P. 736 – 740.
3. Auf G., Jabouille A., Delugin M., Guérit S., Pineau R., North S., Platonova N., Maitre M., Favereaux A., Seno M., Bikfalvi A., Minchenko D., Minchenko O., Moenner M. High epiregulin expression in human U87 glioma cells relies on IRE1α and promotes autocrine growth through EGF receptor. // BMC Cancer. – 2013. – 13, N 1. – P. 597.
4. Auf G., Jabouille A., Guérit S., Pineau R., Delugin M., Bouchecareilh M., Favereaux A., Maitre M., Gaiser T., von Deimling A., Czabanka M., Vajkoczy P., Chevet E., Bikfalvi A., Moenner M. A shift from an angiogenic to invasive phenotype induced in malignant glioma by inhibition of the unfolded protein response sensor IRE1. // Proc. Natl. Acad. Sci. U.S.A. 2010. – 107, N 35. – P. 1555 – 15558.
5. Bi M., Naczki C., Koritzinsky M., Fels D., Blais J., Hu N., Harding H., Novoa I., Varia M., Raleigh J., Scheuner D., Kaufman R.J., Bell J., Ron D., Wouters B.G., Koumenis C. ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. // EMBO J. – 2005. – 24, N 19. – P. 3470 – 34815.
6. Bruland O., Fluge Ø., Akslen L.A., Eiken H.G., Lillehaug J.R., Varhaug J.E., Knappskog P.M. Inverse correlation between PDGFC expression and lymphocyte infiltration in human papillary thyroid carcinomas. // BMC Cancer. – 2009. – 9. – P. 425.
7. Cheng S., Li Y., Yang Y., Feng D., Yang L., Ma Q., Zheng S., Meng R., Wang S., Wang S., Jiang W.G., He J. Breast cancer-derived K172N, D301V mutations abolish Na+/H+ exchanger regulatory factor 1 inhibition of platelet-derived growth factor receptor signaling. // FEBS Lett. – 2013. – 587, N 20. – P. 3289 – 3295.
8. Denko N.C. Hypoxia, HIF1 and glucose metabolism in the solid tumour. // Nature Reviews Cancer. – 2008. – 8. – P. 705 – 713.
9. Drogat B., Auguste P., Nguyen D.T., Bouchecareilh M., Pineau R., Nalbantoglu J., Kaufman R.J., Chevet E., Bikfalvi A., Moenner M. IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. // Cancer Res. – 2007. – 67. – P. 6700 – 6707.
10. Fels D.R., Koumenis C. The PERK/eIF2a/ATF4 module of the UPR in hypoxia resistance and tumor growth. // Cancer Biology & Therapy. – 2006. – 5, N 7. – P. 723 – 728.
11. Hetz C., Chevet E., Harding H.P. Targeting the unfolded protein response in disease. // Nat. Rev. Drug Discov. – 2013. – 12, N 9. – P. 703 – 719.
12. Hollien J., Lin J.H., Li H., Stevens N., Walter P., Weissman J.S. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. // J. Cell. Biol. – 2009. – 186, N 3. – P. 323 – 331.
13. Hose D., Moreaux J., Meissner T., Seckinger A., Goldschmidt H., Benner A., Mahtouk K., Hillengass J., Rème T., De Vos J., Hundemer M., Condomines M., Bertsch U., Rossi J.F., Jauch A., Klein B., Möhler T. Induction of angiogenesis by normal and malignant plasma cells. // Blood. – 2009. – 114, N 1. – P. 128 – 143.
14. Johnson A. B., Denko N., Barton M. C. Hypoxia induces a novel signature of chromatin modifications and global repression of transcription. // Mutat. Res. – 2008. – 640. – P. 174 – 179.
15. Korennykh A.V., Egea P.F., Korostelev A.A., Finer-Moore J., Zhang C., Shokat K.M., Stroud R.M., Walter P. The unfolded protein response signals through high-order assembly of Ire1. // Nature. – 2009. – 457, N 7230. – P. 687 – 693.
16. Lee C., Zhang F., Tang Z., Liu Y., Li X. PDGF-C: a new performer in the neurovascular interplay. // Trends Mol. Med. – 2013. – 19, N 8. – P. 474 – 486.
17. Lee J., Sun C., Zhou Y., Lee J., Gokalp D., Herrema H., Park S.W., Davis R.J., Ozcan U. p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. // Nature Medicine. – 2011. – 17, N 10. – P. 1251 – 1260.
18. Lenihan C.R., Taylor C.T. The impact of hypoxia on cell death pathways. // Biochem. Soc. Trans. – 2013. – 41, N 2. – P. 657 – 663.
19. Manié SN, Lebeau J, Chevet E. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 3. Orchestrating the unfolded protein response in oncogenesis: an update. // Am. J. Physiol. Cell Physiol. – 2014. – 307, N 10. – P. C901 – 907.
20. Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. // Trends Biochem. Sci. – 2014. – 39, N 5. – P. 245 – 254.
21. Minchenko D.O., Hubenya O.V., Terletsky B.M., Moenner M., Minchenko O.H. Effect of hypoxia, glutamine and glucose deprivation on the expression of cyclin and cyclin-dependent kinase genes in glioma cell line U87 and its subline with suppressed activity of signaling enzyme endoplasmic reticulum-nuclei-1. Ukr. Biokhim. Zh. – 2011. – 83, N 1.– P. 18-29.
22. Minchenko O.H., Kharkova A.P., Bakalets T.V., Kryvdiuk I.V. Endoplasmic reticulum stress, its sensor and signaling systems and the role in the regulation of gene expressions in malignant tumor growth and hypoxia. // Ukr. Biochim. J. – 2013. – 85, N 5. – P. 5 – 16.
23. Minchenko O.H., Kubaichuk K.I., Minchenko D.O., Kovalevska O.V., Kulinich A.O., Lypova N.M. Molecular mechanisms of ERN1-mediated angiogenesis. // Int. J. Physiol. Pathophysiol. – 2014. – 5, N 1. – P. 1 – 22.
24. Moenner M., Pluquet O., Bouchecareilh M., Chevet E. Integrated endoplasmic reticulum stress responses in cancer. // Cancer Res. 2007. – 67, N 22. – P. 10631 – 10634.
25. Park S.W., Zhou Y., Lee J., Lu A., Sun C., Chung J., Ueki K., Ozcan U. The regulatory subunits of PI3K, p85alpha and p85beta, interact with XBP-1 and

increase its nuclear translocation. // Nature Medicine. – 2010. – 16, N 4. – P. 429 – 437.
26. Pereira E.R., Liao N., Neale G.A., Hendershot L.M. Transcriptional and post-transcriptional regulation of proangiogenic factors by the unfolded protein response. // PLoS One. – 2010. - 5, N 9. – P. e12521.
27. Pluquet O., Dejeans N., Bouchecareilh M., Lhomond S., Pineau R., Higa A., Delugin M., Combe C., Loriot S., Cubel G., Dugot-Senant N., Vital A., Loiseau H., Gosline S.J., Taouji S., Hallett M., Sarkaria J.N., Anderson K., Wu W., Rodriguez F.J., Rosenbaum J., Saltel F., Fernandez-Zapico M.E., Chevet E. Posttranscriptional regulation of PER1 underlies the oncogenic function of IREα. // Cancer Res. – 2013. – 73, N 15. – P. 4732 – 4743.
28. Romero-Ramirez L., Cao H., Nelson D., Hammond E., Lee A.H., Yoshida H., Mori K., Glimcher L.H., Denko N.C., Giaccia A.J., Le Q.-T., Koong A.C. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. // Cancer Res. – 2004. – 64, N 17. – P. 5943 – 5947.
29. Ruffini F., Tentori L., Dorio A.S., Arcelli D., D'Amati G., D'Atri S., Graziani G., Lacal P.M. Platelet-derived growth factor C and calpain-3 are modulators of human melanoma cell invasiveness. // Oncol. Rep. – 2013. – 30, N 6. – P. 2887 – 2896.
30. Schröder M. Endoplasmic reticulum stress responses. // Cell. Mol. Life Sci. – 2008. – 65, N6. – P. 862 – 894.
31. Sciaccaluga M., D'Alessandro G., Pagani F., Ferrara G., Lopez N., Warr T., Gorello P., Porzia A., Mainiero F., Santoro A., Esposito V., Cantore G., Castigli E., Limatola C. Functional cross talk between CXCR4 and PDGFR on glioblastoma cells is essential for migration. // PLoS ONE. – 2013. – 8, N 9. – P. E73426.
32. Son D., Na Y.R., Hwang E.S., Seok S.H. Platelet-derived growth factor-C (PDGF-C) induces anti-apoptotic effects on macrophages through Akt and Bad phosphorylation. // J. Biol. Chem. – 2014. – 289, N 9. – P. 6225 – 6235.
33. Sun Y, Zhang W, Chen D, Lv Y, Zheng J, Lilljebjorn H, Ran L, Bao Z, Soneson C, Sjogren HO, Salford LG, Ji J, French PJ, Fioretos T, Jiang T and Fan X. A glioma classification scheme based on coexpression modules of EGFR and PDGFRA. // Proc. Natl. Acad. Sci. U.S.A. – 2014. – 111, N 9. – P. 3538 – 3543.
34. Wang S., Kaufman R.J. The impact of the unfolded protein response on human disease. // J. Cell. Biol. – 2012. – 197, N 7. – P. 857 – 867.
35. Wright J.H., Johnson M.M., Shimizu-Albergine M., Bauer R.L., Hayes B.J., Surapisitchat J., Hudkins K.L., Riehle K.J., Johnson S.C., Yeh M.M., Bammler T.K., Beyer R.P., Gilbertson D.G., Alpers C.E., Fausto N., Campbell J.S. Paracrine activation of hepatic stellate cells in platelet-derived growth factor C transgenic mice: evidence for stromal induction of hepatocellular carcinoma. // Int. J. Cancer. – 2014. – 134, N 4. – P. 778 – 788.
36. Zhang K., Kaufman R.J. The unfolded protein response: a stress signaling pathway critical for health and disease. // Neurology. – 2006. – 66, N 2 (Suppl 1). – P. S102 – S109.
37. Zhou Y., Lee J., Reno C.M., Sun C., Park S.W., Chung J., Lee J., Fisher S.J., White M.F., Biddinger S.B., Ozcan U. Regulation of glucose homeostasis through a XBP-1-FoxO1 interaction. // Nature Medicine. – 2011. – 17, N 3. – P. 356 – 365.

ВПЛИВ ДЕФІЦИТУ ПОЖИВНИХ РЕЧОВИН НА ЕКСПРЕСІЮ ГЕНІВ *PDGFC*, *PDGFRA* ТА *PDGFRB* У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87 ЗАЛЕЖИТЬ ВІД ФУНКЦІЇ СИГНАЛЬНОГО ЕНЗИМУ ERN1

К.І. Кубайчук, Д. О. Мінченко, О. О. Рябовол, О.В. Галкін, О. Г. Мінченко

Фактори росту, що контролюють ангіогенез, відіграють важливу роль у рості злоякісних пухлин. Ми вивчали вплив дефіциту глюкози і глутаміну на рівень експресії мРНК PDGFC (platelet derived growth factor C) та його рецепторів PDGFRA (platelet derived growth factor receptor A) and PDGFRB у клітинах гліоми лінії U87. Встановлено, що пригнічення обох ензиматичних активностей сенсорно-сигнального ензиму ERN1 (endoplasmic reticulum to nucleus signaling 1), основного сигнального компоненту стресу ендоплазматичного ретикулуму, підвищує рівень експресії генів, що кодують PDGFC, PDGFRA та PDGFRB у клітинах гліоми лінії U87. За умов дефіциту глутаміну спостерігається збільшення рівня експресії гена PDGFC та зменшення – генів PDGFRA і PDGFRB у контрольних клітинах гліоми, але виключення ERN1 модифікує ефект дефіциту глутаміну на експресію цих генів. Було також показано, що за умов дефіциту глюкози рівень експресії гена PDGFC істотно не змінюється у контрольних клітинах гліоми, але у клітинах з виключеним ERN1 дефіцит глюкози зменшує експресію цього гена. Результати цього дослідження чітко продемонстрували, що рівень експресії генів PDGFC, PDGFRA та PDGFRB у клітинах гліоми лінії U87 залежить від блокади опосередкованого ERN1 стресу ендоплазматичного ретикулуму і переважно змінюється за умов дефіциту глутаміну та глюкози в залежності від функції сигнального ензиму ERN1.

Ключові слова: експресія мРНК, виключення ERN1, PDGFC, PDGFRA, PDGFRB, дефіцит глутаміну, дефіцит глюкози,клітини гліоми лінії U87

Одержано редколегією 12.04.2015 р.