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EFFECT OF HYPOXIA AND ISCHEMIA ON THE EXPRESSION OF PHOSPHOFRUCTOKINASE-1 AND LACTATE DEHYDROGENASE GENES IN GLIOMA U87 CELLS WITH ERN1 KNOCKDOWN

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Cancer cells preferentially utilize glycolysis in order to satisfy their increased energetic and biosynthetic requirements. We have studied effect of hypoxia and ischemia, which are important factors for tumor neovascularization and growth, on the expression of glycolytic enzymes genes phosphofructokinase-1 (PFKL, PFKM and PFKP) and lactate dehydrogenase (LDHA and LDHB) in the glioma cell line U87 with knockdown of endoplasmic reticulum–nuclei-1 (ERN1) sensing and signaling enzyme. It was shown that loss of the signaling enzyme ERN1 function leads to an increase in the expression levels of PFKL, PFKP and LDHA mRNA. However, expression level of PFKM mRNA decreases at this experimental condition. It was also shown that hypoxia strongly induces the expression of PFKL and PFKP mRNA in control glioma cells; however knockdown of ERN1 suppresses the effect of hypoxia on PFKP and especially PFKL mRNA expression. At the same time, hypoxia increases the expression levels of LDHA in both cell types and decreases PFKM expression in control cells only. Besides that, the expression levels of PFKL and PFKM mRNA increase in glucose and decrease in glutamine deprivation conditions but also in control glioma cells only. Expression levels of both LDHA and LDHB mRNA decrease in glutamine deprivation conditions in both cell types. Thus, the expression level of phosphofructokinase-1 and lactate dehydrogenase genes is mainly dependent on ERN1 signaling enzyme function in normal condition but there are gene specific changes in control glioma cells and cells with ERN1 loss of function at hypoxic and nutrient deprivation conditions. It is possible that different variants of phosphofructokinase-1 and lactate dehydrogenase proteins play different role in ERN1 signaling system associated with endoplasmic reticulum stress.

Key words: mRNA expression, PFKL, PFKM, PFKP, LDHA, LDHB, glioma cells, ERN1 knockdown, hypoxia, glucose and glutamine deprivation.

INTRODUCTION

A high rate of glycolytic flux, even in the presence of oxygen, is a central metabolic hallmark of most neoplastic tumors. The high glucose metabolism of cancer cells is caused by a combination of hypoxia-responsive transcription factors, activation of oncogenic proteins and the loss of tumor suppressor function. Over-expression of HIF-1 α or HIF-2 α and MYC, activation of ras and loss of TP53 and/or other tumor suppressor functions each have been found to stimulate glycolysis in part by activating a family of regulatory bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) and hexokinases [1, 2]. The PFKFB enzymes synthesize fructose-2,6-bisphosphate which allosterically activates 6-phosphofructo-1-kinase (PFK-1), a rate-limiting enzyme and essential control point in the glycolytic pathway [3–5]. Hypoxia responsive enzymes PFKFB3, PFKFB4 and PFKFB2 are overexpressed in different cancer tissues and activate PFK-1 via enhanced production of fructose-2,6-bisphosphate [6–9]. Overexpression of these enzymes as well as hexokinase 2 is an obligatory factor of tumor cell glycolysis and increased proliferation [10–12].

Three phosphofructokinase-1 isozymes exist in humans: muscle, liver and platelet, which are encoded by separate genes located at different chromosomes (21, 12

and 10) [13]. These isozymes function as subunits of the mammalian tetramer phosphofructokinase (EC 2.7.1.11), which catalyzes the phosphorylation of D-fructose-6-phosphate to D-fructose-1,6-bisphosphate, a key step in glycolysis. PFK1 from muscle is a homotetramer of M subunits, PF-1 from liver is a homotetramer of L-subunits, while PFK1 structure from other organs varies depending on tissue type. Recently, novel testis-specific and embryo-specific isoforms of the phosphofructokinase-1 muscle type gene were identified [13]. There is data that allosteric citrate binding sites on 6-phosphofructo-1-kinase M is important for enzymatic activity and substitution of residue at this citrate-binding site (D591V) resulted in the complete loss of activity [14]. Moreover, posttranslational modification of PFK1 enzyme is an important feature of cancer metabolism and might be the pivotal factor of deregulated glycolytic flux in tumors [15]. There is data that transcription factor KLF4 (Kruppel-like factor 4; zinc finger protein EZF) plays a role in maintenance of high glycolytic metabolism by transcriptional activation of the PFKP gene in breast cancer cells and can act both as activator and as repressor [16].

The protein encoded by lactate dehydrogenase genes (EC 1.1.1.27) catalyzes the conversion of L-lactate and NAD to pyruvate and NADH in the final step of anaerobic

glycolysis. High lactate dehydrogenase is associated with acute adult T-cell leukemia/lymphoma [17]. It was shown that lactate dehydrogenase expression in melanoma increases with disease progression and is associated with expression of Bcl-XL and Mcl-1, but not Bcl-2 proteins [18]. Moreover, upregulation of lactate dehydrogenase A by ErbB2 through heat shock factor 1 promotes breast cancer cell glycolysis and growth and knockdown of this enzyme in renal cancer cells results in significant reduction in tumor growth in a xenograft mouse model [19, 20].

Thus, the phosphofructokinase-1 isozyme family and lactate dehydrogenase proteins participate not only in the control of glucose metabolism via glycolysis, but also in the regulation of the cell proliferation and tumor growth.

The endoplasmic reticulum is a key organelle in the cellular response to ischemia, hypoxia, and some chemicals which activate a complex set of signaling pathways named the unfolded protein response. 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), IRE1/ERN1 (Inositol Requiring Enzyme-1/Endoplasmic Reticulum – Nuclei-1) and ATF6 (Activating Transcription Factor 6), however, endoplasmic reticulum – nuclei-1 is the dominant sensor [21–24]. 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 [21, 22, 25]. 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 conditions.

Two distinct catalytic domains of the bifunctional signaling enzyme endoplasmic reticulum – nuclei-1 were identified: a serine/threonine kinase and an endoribonuclease which contribute to ERN1 signalling. The ERN1-associated kinase activity autophosphorylates and dimerizes this enzyme, leading to the activation of its endoribonuclease domain, degradation of a specific subset of mRNA, and initiation of the pre-XBP1 (X-box binding protein 1) mRNA splicing [24–26]. Mature XBP1 mRNA splice variant encodes a transcription factor that has different C-terminus amino acid sequence and stimulates the expression of hundreds of unfolded protein response-specific genes [21, 25].

Moreover, the growing tumor requires the endoplasmic reticulum stress as well as hypoxia and ischemia for own neovascularization and growth and the complete blockade of ERN1 signal transduction pathway has anti-tumor effects [26, 27]. The endoplasmic reticulum stress response-signalling pathway is linked to the neovascularization process, tumor growth and differentiation as well as cell death processes [27, 28]. Thus, the blockade of the main unfolded protein response sensor ERN1 is important in studying the role of ERN1 signalling pathways in tumor progression, especially in malignant gliomas; it is important in the development of a

new understanding concerning molecular mechanisms of malignant tumors progression in relation to ischemia/hypoxia and it will help define the best targets for the design of specific inhibitors that could act as potent antitumor drugs. Gliomas are the most frequent primary brain neoplasms and represent a major challenge in cancer therapy as they are not easily accessible to current therapies.

The main goal of this work is to study the role of ERN1-signaling pathways in tumor progression by investigating the expression of phosphofructokinase-1 and lactate dehydrogenase genes in glioma U87 cells with ERN1 loss of function under normal, hypoxic and ischemic (glucose or glutamine deprivation) conditions.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. 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°C in a 5% CO₂ 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, which was used for creation of dnERN1. This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of the effect of hypoxia and glutamine or glucose deprivations on the expression level of PFK-1 and LDH genes. Second subline was obtained by selection of stable transfected clones with overexpression of ERN1 dominant/negative constructs (dnERN1) and has suppressed both protein kinase and endoribonuclease activities of this signaling enzyme [13]. The expression level of PFK-1 and LDH 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 hypoxia and glutamine or glucose deprivations on the expression level of PFK-1 and LDH genes under blockade ERN1 function.

Hypoxic conditions were created in special incubator with 3 % oxygen and 5 % carbon dioxide levels; culture plates were exposed to these conditions for 16 hrs. For glucose or glutamine deprivation the growing medium in culture plates was replaced with a medium without glucose or without glutamine and thus exposed for 16 hrs. The suppression level of ERN1 enzymatic activity in glioma cells that overexpress a dominant-negative construct of endoplasmic reticulum–nuclei-1 (dnERN1) was estimated 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).

RNA isolation. Total RNA was extracted from different tumor tissues and normal tissue counterparts as described [29]. RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water.

Reverse transcription and quantitative PCR analysis. The expression levels of PFKL, PFKM, PFKP,

LDHA and LDHB mRNA were measured in glioma cell line U87 and its subline with a deficiency of endoplasmic reticulum–nuclei-1 by quantitative polymerase chain reaction of complementary DNA (cDNA) using „Stratagene Mx 3000P cyclor” (USA) and SYBRGreen Mix (AB gene, Great Britain). QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis as described previously [29]. Polymerase chain reaction was performed in triplicate.

For amplification of phosphofruktokinaase-1 (ATP:D-fructose-6-phosphate-1-phosphotransferase; PFK1) liver type (PFKL) cDNA we used sequence 5'–GGAGCTTCGAGAACAACACTGG –3' as forward primer and 5'–CTGTGTGCCATGGGAGATG –3' as reverse primer. The nucleotide sequences of these primers correspond to sequences 1186 – 1205 and 1353 – 1334 of human PFKL cDNA (GenBank accession number NM_002626). The size of amplified fragment is 168 bp.

The amplification of the muscle isoform of phosphofruktokinaase-1 (PFKM) cDNA was performed using forward (5'–AGAGCGTTTCGATGATGCTT –3') and reverse (5'–CAGTGCCAATGGTCATATCG –3') primers. The nucleotide sequences of these primers correspond to sequences 397 – 416 and 720 – 701 of human PFKM cDNA (GenBank accession number NM_000289). The size of amplified fragment is 324 bp.

For amplification of the platelet isoform of phosphofruktokinaase-1 (PFKP) cDNA we used sequence 5'–GCTCCATTCTTGGGACAAAA –3' as forward primer and 5'–GATAGTGTTTCAGGGCGGTGT –3' as reverse primer. The nucleotide sequences of these primers correspond to sequences 1497 – 1516 and 1753 – 1734 of human PFKP cDNA (GenBank accession number NM_002627). The size of amplified fragment is 257 bp.

The amplification of lactate dehydrogenase-A (LDHA; cell proliferation-inducing gene 19 protein) cDNA was performed using forward primer (5'–ACGTCAGCAAGAGGGAGAAA –3') and reverse primer (5'–CGCTTCCAATAACACGGTTT –3'). These oligonucleotides correspond to sequences 566 – 585 and 756 – 737 of human LDHA cDNA (GenBank accession number NM_005566). The size of amplified fragment is 191 bp.

For amplification of lactate dehydrogenase-B (LDHB) cDNA we used sequence 5'–CCAACCCAGTGGACATTCTT –3' as forward primer and 5'–AAACACCTGCCACATTCACA –3' as reverse primer. The nucleotide sequences of these primers correspond to sequences 524 – 543 and 742 – 723 of human LDHB cDNA (GenBank accession number NM_002300). The size of amplified fragment is 219 bp.

The amplification of beta-actin cDNA was performed using forward - 5'–CGTACCACTGGCATCGTGAT –3' and reverse - 5'–GTGTTGGCGTACAGGTCTTT –3' primers. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The primers were received from “Sigma” (USA).

The amplification of XBP1 cDNA was performed using HotStarTaq Master Mix Kit (“QIAGEN”, Germany), “MasterCycler Personal” (“Eppendorf”, Germany) and primers: forward - 5'–

GGAGTTAAGACAGCGCTTGG –3' and reverse - 5'–TCACCCCTCCAGAACATCTC –3'. The nucleotide sequences of these primers correspond to sequences 441 – 460 and 608 – 589 of XBP1 mRNA (GenBank accession number NM_005080). The size of amplified fragment is 168 bp for non spliced variant and 142 bp for alternative splice variant (XBP1s). The phosphorylated isoform ERN1 was measured by Western analysis using IRE1p, phosphorylated (Ser724), US Biological.

An analysis of quantitative PCR was performed using special computer program “Differential expression calculator” and statistic analysis – in Excel program. The amplified DNA fragments were analyzed on a 2 % agarose gel and that visualized by 5x Sight DNA Stain (EUROMEDEA).

RESULTS AND DISCUSSION

In this study, we have used the human glioma cell line U87 and a genetically modified variant of these cells (deficient in the endoplasmic reticulum stress signaling enzyme ERN1) to investigate the expression of different genes that encode phosphofruktokinase-1 and LDH proteins as well as the involvement of the endoplasmic reticulum stress signaling system in the effect of hypoxia and glutamine or glucose deprivation on the expression of these genes. The level of suppression of the enzymatic activity of ERN1 was estimated by analysis of the expression of XBP1 and its splice variant, shorter isoform (XBP1s), a key transcription factor in ERN1 signaling, in U87 glioma cells that overexpress a dominant-negative construct of endoplasmic reticulum–nuclei-1 (dnERN1) compared with the control glioma cells transfected with a vector. As shown in fig. 1, inducer of endoplasmic reticulum stress, tunicamycin (10 mg/l), strongly induces alternative splicing of XBP1 only in control glioma cells, while having no effect on this process in transfected with dnERN1 cells. Moreover, the results presented in fig. 1 also shown that phosphorylated isoform of ERN1, measured by Western analysis using antibody against phosphorylated form of ERN1 (Ser724), also presents in control glioma cells only. This data clearly demonstrated the complete blockade the ERN1 enzyme function in glioma cells transfected with dnERN1.

We have also found that PFKL, PFKM, PFKP, LDHA and LDHB genes are expressed in the human glioma cell line U87 and the levels of its expression are mainly depend from ERN1 signaling enzyme function. As shown in fig. 2, the expression level of PFKL, PFKP and LDHA mRNA significantly increases in glioma cells, deficient in signaling enzyme ERN1 function, compared with the control value: +176, +72 and +31 %, respectively. However, PFKM mRNA expression levels are decreased (-43 %) in glioma cells with signalling enzyme ERN1 loss of function (fig. 2). At the same time, the suppression of ERN1 enzyme function does not change significantly the expression level of LDHB mRNA.

Exposure of cells to hypoxia for 16 hrs leads to an increase the expression level of PFKL (19 fold), PFKP (2.5 fold) and LDHA (+31 %) mRNA in control glioma cells, as compared to control 1, but to decrease the expression level of PFKL (-19 %; fig. 3 – 6). At the same

time, in glioma cells with suppressed function of the signaling enzyme ERN1 effect of hypoxia on the expression level of PFKL and PFKM was not observed, as compared to control 2 (fig. 3 and 4). However, the expression level of PFKP and LDHA mRNA increases under hypoxia in glioma cells with ERN1 loss of function: +44 and +23 %, respectively (fig. 5 and 6).

As shown in fig. 3 and 4, the expression level of PFKL and PFKM mRNA decreases in glucose deprivation condition in both tested glioma cell types: -38 and -25 % in control glioma cells and -25 and -31 % in ERN1 knockdown glioma cells, respectively. Exposure of cells to glucose deprivation condition leads also to a decrease of LDHA mRNA expression level in both tested glioma cell types: -29 and -32, respectively, but expression level of PFKP mRNA decreases in glioma cells with suppressed function of the signaling enzyme ERN1 only (fig. 5 and 6).

However, glutamine deprivation condition leads to an increase of PFKL and PFKM mRNA expression level (+20 and +44 %, respectively) in control glioma cells but does not change significantly the expression level of these mRNA in glioma cells with suppressed function of the signaling enzyme ERN1 (fig. 3 and 4). At the same time, the glutamine deprivation condition does not change significantly the expression level of PFKP and LDHA mRNA in control glioma cells, as compared to control 1, but decreases the expression level of these mRNA (-30 and -17 %, respectively) in ERN1 loss of function cells, as compared to control 2 (fig. 5 and 6).

Investigation of LDHB mRNA expression shown that in control glioma cells the level of this mRNA expression does not change significantly at all experimental conditions but decreased in cells with ERN1 loss of function under hypoxia and glutamine deprivation function, as compared to control 2 values: -19 and -20 %, respectively (fig. 7).

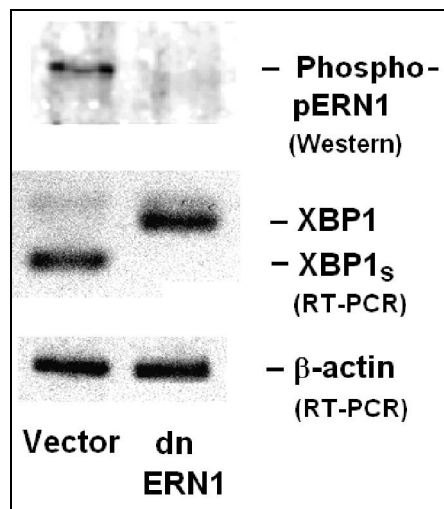


Fig. 1. Effect of tunicamycin (0.01 mg/ml – 2 hours) on the level of phosphoERK1 (Western analysis) and expression level of transcription factor XBP1 and its alternative splice variant (XBP1s) mRNA (qPCR analysis) in glioma cell line U87 stable transfected with vector (control) and its subline with blockade of signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1) stable transfected with dnERK1.

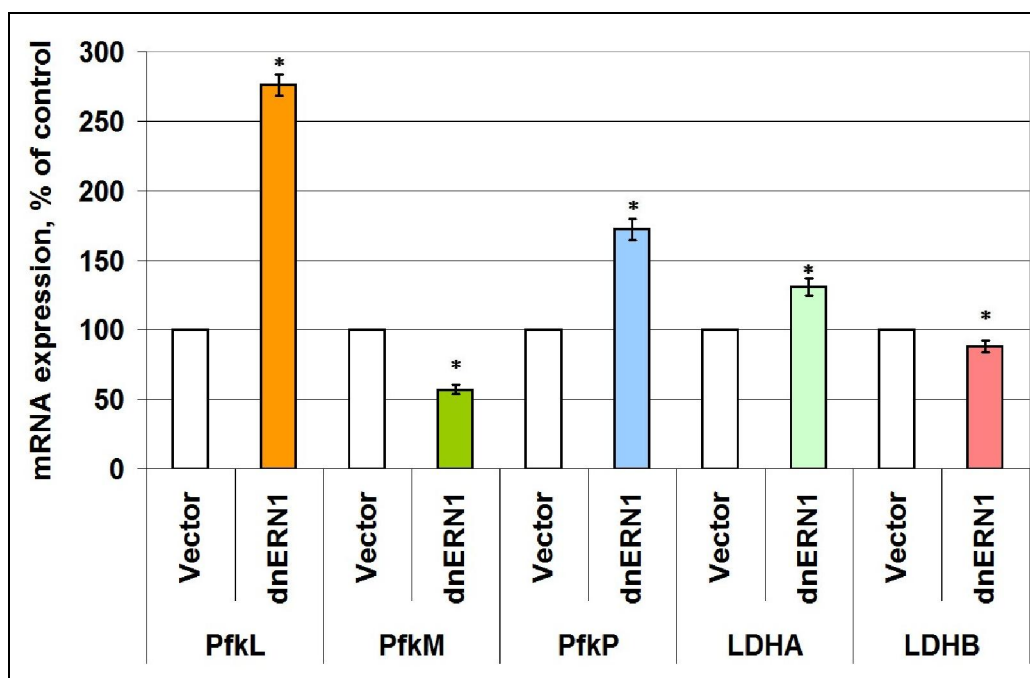


Fig. 2. Effect of blockade of signaling enzyme ERN1 on the expression levels of phosphofructokinase-1 type L (PFKL), type M (PFKM) and type P (PFKP) as well as lactate dehydrogenase A (LDHA) and B (LDHB) mRNA in glioma cell line U87 (Vector = control) and its subline with ERN1 deficiency (dnERK1) measured by quantitative polymerase chain reaction. Values of PFKL, PFKM, PFKP, LDHA and LDHB mRNA expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100 %); $n = 4$; * – $P < 0.05$ as compared to control.

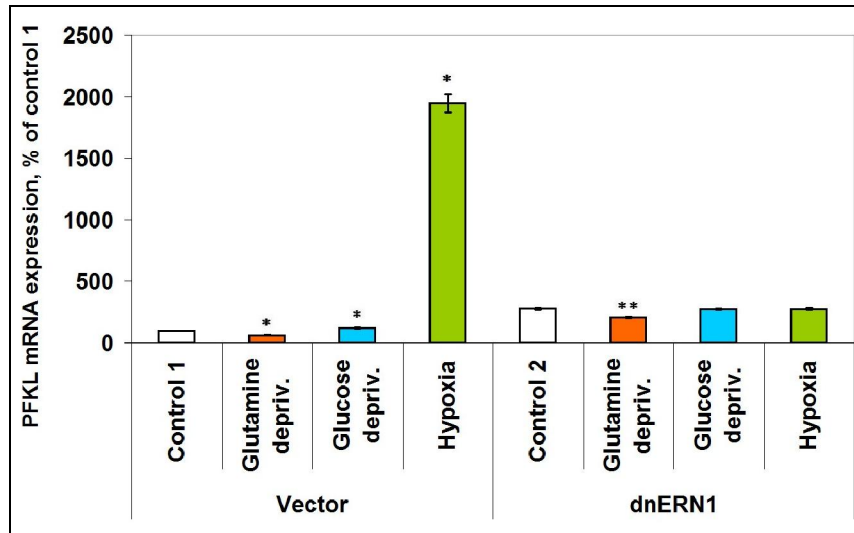


Fig. 3. Effect of hypoxia and glucose or glutamine deprivation on the expression of phosphofructokinase-1 type L (PFKL) mRNA in glioma cell line U87 (Control 1) and its subline with a deficiency of the signaling enzyme ERN1 (dnERN1 = Control 2) measured by quantitative polymerase chain reaction. Values of PFKL mRNA expressions were normalized to beta-actin mRNA expression and represent as percent for control 1 (100 %); $n = 4$; * – $P < 0.05$ as compared to control 1; ** – $P < 0.05$ as compared to control 2.

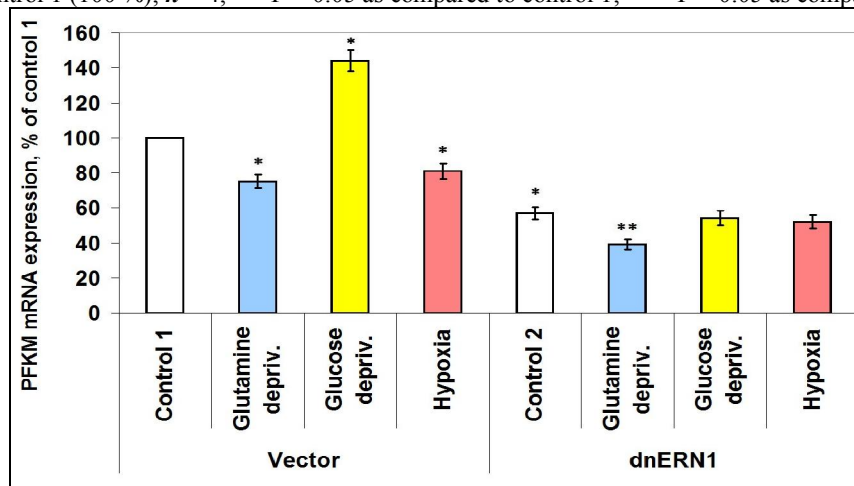


Fig. 4. Effect of hypoxia and glucose or glutamine deprivation on the expression of phosphofructokinase-1 type M (PFKM) mRNA in glioma cell line U87 and its subline with loss of the signaling enzyme ERN1 function (dnERN1) measured by quantitative polymerase chain reaction. Values of PFKM mRNA expressions were normalized to beta-actin mRNA expression and represent as percent for control 1 (100 %); $n = 4$; * – $P < 0.05$ as compared to control 1; ** – $P < 0.05$ as compared to control 2.

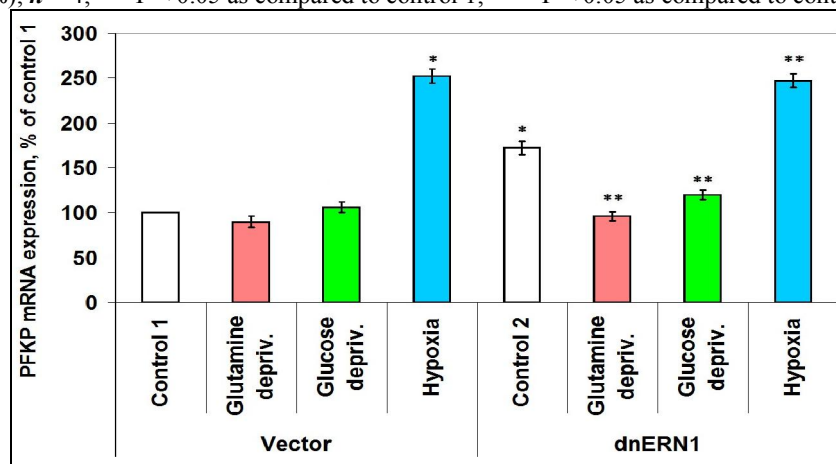


Fig. 5. Effect of hypoxia and glucose or glutamine deprivation on the expression of phosphofructokinase-1 type P (PFKP) mRNA in glioma cell line U87 and its subline with loss of the signaling enzyme ERN1 function (dnERN1) measured by quantitative polymerase chain reaction. Values of PFKP mRNA expressions were normalized to beta-actin mRNA expression and represent as percent for control 1 (100 %); $n = 4$; * – $P < 0.05$ as compared to control 1; ** – $P < 0.05$ as compared to control 2.

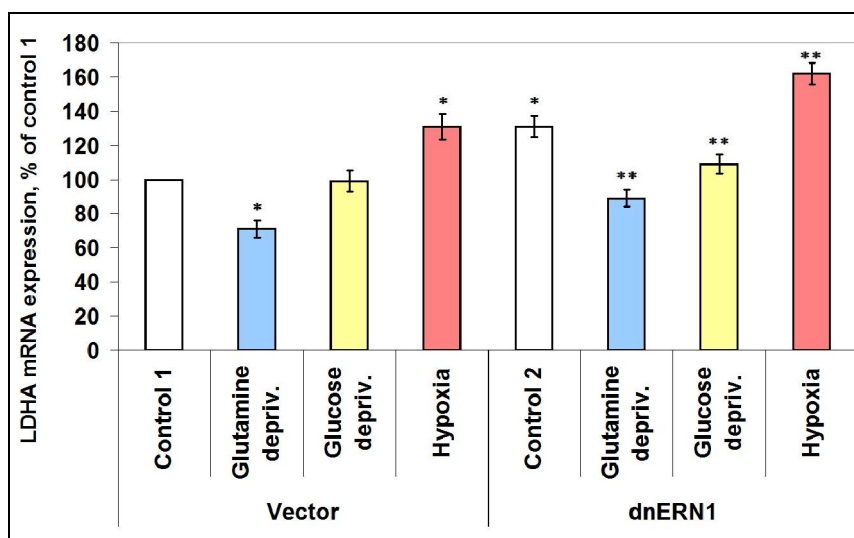


Fig. 6. Effect of hypoxia and glucose or glutamine deprivation on the expression of lactate dehydrogenase A (LDHA) mRNA in glioma cell line U87 and its subline with loss of the signaling enzyme ERN1 function (dnERN1) measured by quantitative polymerase chain reaction. Values of LDHA mRNA expressions were normalized to beta-actin mRNA expression and represent as percent for control 1 (100 %); $n = 4$; * – $P < 0.05$ as compared to control 1; ** – $P < 0.05$ as compared to control 2.

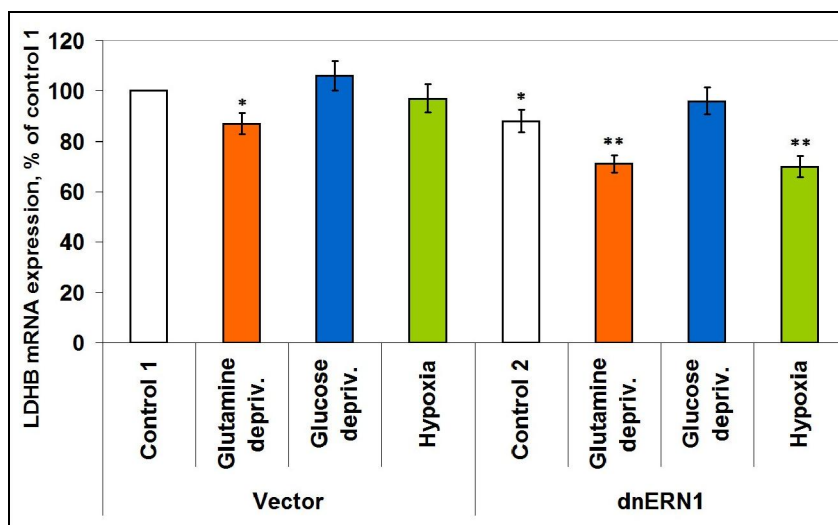


Fig. 7. Effect of hypoxia and glucose or glutamine deprivation on the expression of lactate dehydrogenase B (LDHB) mRNA in glioma cell line U87 and its subline with loss of the signaling enzyme ERN1 function (dnERN1) measured by quantitative polymerase chain reaction. Values of LDHB mRNA expressions were normalized to beta-actin mRNA expression and represent as percent for control 1 (100 %); $n = 4$; * – $P < 0.05$ as compared to control 1; ** – $P < 0.05$ as compared to control 2.

Bifunctional transmembrane signaling enzyme endoplasmic reticulum–nuclei-1 is a major proximal sensor of the unfolded protein response and participates in the early cellular response to the accumulation of misfolded proteins in the endoplasmic reticulum [27, 28]. It has been known that the endoplasmic reticulum stress sensing and signal transduction pathways are linked to the neovascularization process, tumor growth, and cellular death processes [27]. Moreover, the growing tumor requires endoplasmic reticulum stress, as well as ischemia and hypoxia both of which initiate endoplasmic reticulum stress. It is important for tumor neovascularization and growth as well as for inhibition of apoptotic processes [26, 28]. In this work, we studied the expression of several genes that encode phosphofructokinase-1 and lactate dehydrogenase proteins in glioma cells with ERN1 knockdown for the purpose of evaluating the dependence

of these genes upon the ERN1 signaling enzyme function but PFKM – decreases. Results of this investigation clearly demonstrated that the expression level of PFKL, PFKP and LDHA increases in glioma cells without ERN1 signaling enzyme function. This data is not completely correlate with anti-tumor effects of this ERN1 blockade, but correlates with enhanced invasiveness [26, 28]. It is well known that phosphofructokinase-1 isoenzymes and lactate dehydrogenase participate in glycolysis and proliferation and are the components of endoplasmic reticulum stress system, but its activity controls by different mechanisms, not only ERN1 signaling system [1, 2, 19, 27].

In this study we have studied effect of hypoxia on the expression of three phosphofructokinase-1 genes and two lactate dehydrogenase genes and shown that different phosphofructokinase-1 and lactate dehydrogenase genes

have significant difference in hypoxia responsibility. More strong induction of the expression was shown for PFKL and PFKP mRNA in control glioma cells compared with values in glioma cells without ERN1 signaling enzyme function. These results correlate with data concerning biological significance of different phosphofructokinase-1 and lactate dehydrogenase proteins in tumor growth [15, 16, 19, 30]. We have also shown that blockade of the activity of signaling enzyme endoplasmic reticulum–nuclei-1 leads to a complete reduction of hypoxic effect on the expression of PFKL and PFKM genes in glioma cells. This data demonstrated that effect of hypoxia on the expression of phosphofructokinase-1 genes mediates by ERN1 signaling system, at least partly and that blockade of the activity of ERN1 signaling enzyme by dnERN1 construct completely suppresses the formation of alternative splice variant of XBP1 and the main biological function of ERN1.

In this study we have also shown different sensitivity different phosphofructokinase-1 and lactate dehydrogenase genes to glutamine or glucose deprivation conditions both in control glioma cells and cells with ERN1 loss of function. Thus, the expression levels of PFKL and PFKM mRNA decrease in glucose deprivation condition in both used cell types, but expression levels of these mRNA significantly increase in glutamine deprivation condition only in control glioma cells. It is possible that increase of PFKL and PFKM mRNA expression in cells without activity of ERN1 enzyme function is sensitive to glutamine deprivation because its induction mediated by ERN1 signaling system.

Results of these investigations demonstrated that the expression of genes encoding the phosphofructokinase-1 and lactate dehydrogenase proteins in glioma cells is mainly regulated by hypoxia, glutamine or glucose deprivation and depends upon the activity of signaling enzyme endoplasmic reticulum–nuclei-1. However, PFKL, PFKP and LDHA are more sensitive to these experimental conditions as compared to PFKM and LDHB.

CONCLUSIONS

Thus, the major finding reported here is that the expression of PFKL, PFKM, PFKP and LDHA genes are dependent on the function of ERN1 signaling enzyme in normal condition but there are gene specific changes in control glioma cells and cells with ERN1 loss of function at hypoxic and nutrient deprivation conditions. It is possible that different variants of phosphofructokinase-1 and lactate dehydrogenase proteins play different role in ERN1 signaling system associated with endoplasmic reticulum stress. However, the detailed molecular mechanisms of regulation of phosphofructokinase-1 and lactate dehydrogenase genes by ERN1 signaling system under ischemic and nutrient deprivation conditions are complex and warrants further study.

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ВПЛИВ ГІПОКСІЇ ТА ШЕМІЇ НА ЕКСПРЕСІЮ ГЕНІВ ФОСФОФРУКТОКІНАЗИ-1 ТА ЛАКТАТДЕГІДРОГЕНАЗ У КЛІТИНАХ ГЛІОМИ U87 З ПРИГНІЧЕНОЮ ФУНКЦІЄЮ ERN1

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Клітини злоякісних пухлин переважно використовують гліколіз для задоволення своїх підвищених енергетичних та біосинтетичних потреб. Ми досліджували вплив гіпоксії та ішемії, що є важливими факторами неоваскуляризації та росту пухлин, на експресію генів таких ензимів гліколізу як фосфофруктокіназа-1 (PFKL, PFKM і PFKP) та лактатдегідрогеназа (LDHA і LDHB) у клітинах гліоми лінії U87 з пригніченою функцією сенсорно-сигнального ензиму ERN1 (від ендоплазматичного ретикулуму до ядра-1). Встановлено, що втрата функції сигнального ензиму ERN1 приводить до збільшення рівня експресії мРНК PFKL, PFKP та LDHA. Разом з тим, рівень експресії мРНК PFKM за цих експериментальних умов знижується. Було також показано, що гіпоксія виражено індукуює експресію мРНК PFKL та PFKP в контрольних клітинах гліоми, а виключення функції ERN1 пригнічує ефект гіпоксії на експресію мРНК PFKP та особливо PFKL. В той же час, гіпоксія збільшує рівні експресії LDHA в обох типах використаних нами клітин і знижує експресію PFKM, але лише в контрольних клітинах. Крім того, рівні експресії мРНК PFKL та PFKM збільшуються за умов дефіциту глюкози та зменшуються за умов дефіциту глютаміну, але також лише в контрольних клітинах. Показано, що рівні експресії мРНК як LDHA, так і LDHB, знижуються за умов дефіциту глютаміну в обох типах клітин. Таким чином, рівень експресії генів фосфофруктокінази-1 та лактатдегідрогенази залежать від функції сигнального ензиму ERN1 в нормальних умовах, але за умов гіпоксії та ішемії виявляються гено-специфічні зміни експресії в контрольних клітинах гліоми та в клітинах з втраченою функцією ензиму ERN1. Можливо, що різні варіанти протейнів фосфофруктокінази-1 та лактатдегідрогенази відіграють різну роль в системі сигналіну ERN1, асоційованого зі стресом ендоплазматичного ретикулуму.

Ключові слова: експресія мРНК, PFKL, PFKM, PFKP, LDHA, LDHB, клітини гліоми, виключення функції ERN1, гіпоксія, дефіцит глюкози та глютаміну.

ВЛИЯНИЕ ИПОКСИИ И ИШЕМИИ НА ЭКСПРЕССИЮ ГЕНОВ ФОСФОФРУКТОКИНАЗЫ-1 И ЛАКТАТДЕГИДРОГЕНАЗЫ В КЛЕТКАХ ГЛИОМЫ U87 С УГНЕТЕННОЙ ФУНКЦИЕЙ ERN1

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Клетки злокачественных опухолей преимущественно используют гликолиз для удовлетворения своих повышенных энергетических и биосинтетических потребностей. Мы исследовали влияние гипоксии и ишемии, которые есть важными факторами неоваскуляризации и роста опухолей, на экспрессию генов таких энзимов гликолиза как фосфофруктокиназа-1 (PFKL, PFKM и PFKP) и лактатдегидрогеназа (LDHA и LDHB) в клетках глиомы линии U87 с угнетенной функцией сенсорно-сигнального энзима ERN1 (от эндоплазматического ретикулума к ядру-1). Установлено, что потеря функции сигнального энзима ERN1 приводит к увеличению уровня экспрессии мРНК PFKL, PFKP и LDHA. Вместе с тем, уровень экспрессии мРНК PFKM при этих экспериментальных условиях снижается. Было же показано, что гипоксия выражено индуцирует экспрессию мРНК PFKL та PFKP в контрольных клетках глиомы, а выключение функции ERN1 угнетает эффект гипоксии на экспрессию мРНК PFKP и особенно PFKL. В то же время, гипоксия увеличивает уровни экспрессии LDHA в обоих типах использованных нами клеток и снижает экспрессию PFKM, но только в контрольных клетках. Кроме того, уровни экспрессии мРНК PFKL и PFKM увеличиваются в условиях дефицита глюкозы и уменьшаются в условиях дефицита глутамина, но также только в контрольных клетках. Показано, что уровни экспрессии мРНК как LDHA, так LDHB, снижаются в условиях дефицита глутамина в обоих типах клеток. Таким образом, уровни экспрессии генов фосфофруктокиназы-1 и лактатдегидрогеназы зависят от функции сигнального энзима ERN1 в нормальных условиях, но в условиях гипоксии и ишемии выявляются гено-специфические изменения экспрессии в контрольных клетках глиомы и в клетках с утерянной функцией энзима ERN1. Вполне возможно, что различные варианты протеинов фосфофруктокиназы-1 и лактатдегидрогеназы играют различную роль в системе сигналинга ERN1, ассоциированного со стрессом эндоплазматического ретикулума.

Ключевые слова: экспрессия мРНК, PFKL, PFKM, PFKP, LDHA, LDHB, клетки глиомы, выключение функции ERN1, гипоксия, дефицит глюкозы и глутамина.
