EXPRESSION OF 6-PHOSPHOFRUCTO-2-KINASE/FRUCTOSE-2,6-BISPHOSPHATASE-1 AND -2 AND ITS UNIQUE ALTERNATIVE SPLICE VARIANTS IN U87 GLIOMA CELLS WITH ERN1 LOSS OF FUNCTION

1Lypova N.M., 1,2Minchenko D.M., 1Kubaichuk K.I., 1Minchenko O.H.

1Palladin Institute of Biochemistry National Academy of Science of Ukraine, Kyiv, Ukraine;
2National O.O. Bogomolets Medical University, Kyiv 01601, Ukraine;
e-mail: ominchenko@yahoo.com

Was received 15.07.2011

The endoplasmic reticulum–nuclei-1 (ERN1) sensing and signaling enzyme mediates a set of complex intracellular signaling events known as the unfolded protein response. We have studied the effect of hypoxia and ischemic conditions (glucose or glutamine deprivation) on the expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-1 and -2 (PFKFB1 and PFKFB2) genes in glioma U87 cells and its subtype with suppressed function of ERN1 sensing and signaling enzyme. We have identified three unique alternative splice variants of PFKFB2 and PFKFB1 with insert or deletions in its N-terminal region which eliminate 6-phosphofructo-2-kinase activity. It was shown that blockade of ERN1 enzyme function, the key endoplasmic reticulum stress sensor, leads to an increase in the expression levels of PFKFB1 and PFKFB2 mRNAs as well as its alternative splice variants. Moreover, the expression level of PFKFB2 mRNA increases both in control glioma cells and decreases in genetically modified glioma cells treated by hypoxia. At the same time, expression level of PFKFB1 mRNA and its alternative splice variants does not change significantly at this experimental condition. Exposure cells under glutamine or glucose deprivation conditions leads to increase the expression level of PFKFB1 and PFKFB2 mRNA in control glioma cells only, but expression level of alternative splice variants of these mRNA also increases in control glioma cells and only in glutamate deprivation condition. It was shown also that blockade of ERN1 signaling enzyme function eliminate the increase of PFKFB1 and PFKFB2 mRNA as well as its alternative splice variants induced in glioma cells by glutamate and glucose deprivation conditions. Thus, results of this study clearly demonstrate that the expression level of PFKFB1 and PFKFB2 as well as its alternative splice variants without 6-phosphofructo-2-kinase activity increases in glioma cells with ERN1 signaling enzyme loss of function and that glutamine and glucose deprivation conditions also lead to increase the expression level of these PFKFB but in control glioma cells only. It is possible that increase of the expression level of PFKFB1 and PFKFB2 genes in glutamine and glucose deprivation conditions is mediated by ERN1 signaling system because it miss in glioma cells with ERN1 loss of function and is observed in control glioma cells only.

Ключові слова: mRNA expression, PFKFB1, PFKFB2, unique alternative splice variants, glioma cells, endoplasmic reticulum–nuclei-1 (ERN1), hypoxia, glucose and glutamine deprivation.

INTRODUCTION

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), ERN1 (Endoplasmic Reticulum – Nuclei-1) also known as IRE-1α (Inositol Requiring Enzyme-1α) and ATF6 (Activating Transcription Factor 6), however, ERN1 is the dominant sensor [1–6]. 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 [7, 8]. 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 ERN1 were identified: a serine/threonine kinase and an endoribonuclease which contribute to this enzyme signalling. The ERN1-associated kinase activity autophosphorylates and dimerizes this enzyme, leading to the activation of its endoribonuclease domain, initiation of the pre-XBP1 (X-box binding protein 1) mRNA splicing, and degradation of a specific subset of mRNA [9–11]. Mature XBP1 mRNA splice variant (XBP1s) encodes a transcription factor that has different C-terminal amino acid sequence and stimulates the expression of hundreds of unfolded protein response-specific genes [1, 9–12].

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 [4, 8, 13, 14]. The endoplasmic reticulum stress response-signalling pathway is linked to the neovascularization process, tumor growth and differentiation as well as cell death processes [12, 15, 16]. There is data that endoplasmic reticulum stress response-signaling pathway is involved in osteoblast differentiation induced by BMP2 as well as in induction of apoptosis by N-acetyl cysteine and penicillamine [17, 18]. 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 [19]; it is important in developing a new understanding concerning molecular mechanisms of malignant tumors progression in relation to ischemia/hypoxia and it can help define the best targets for the design of specific inhibitors that could act as potent antitumor drugs.

A high rate of glycolytic flux, even in the presence of oxygen, is a central metabolic hallmark of 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-1α or HIF-2α 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 [20–22]. Hexokinase 2 is a key mediator of aerobic glycolysis and promotes tumor growth in human glioblastoma multiforme, the most common malignant brain tumor [22]. The PFKFB enzymes synthesize fructose-2,6-bisphosphate which allosterically activates 6-phosphofructo-1-kinase, a rate-limiting enzyme and essential control point in the glycolytic pathway [23–25]. Different PFKFB are hypoxia responsive enzymes and overexpressed in different cancer tissues [26–29]. Overexpression of these enzymes is an obligatory factor of tumor cell glycolysis [20, 30, 31]. Recently, it was shown that nuclear targeting of 6-phosphofructo-2-kinase-3 increases proliferation via cyclin-dependent kinase [32]. Moreover, E3 ubiquitin ligase APC/C-CDH1 accounts for the Warburg effect by linking glycolysis to cell proliferation mainly via the glycolysis-promoting enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3, because this enzyme is degraded by the E3 ubiquitin ligase APC/C-CDH1, which also degrades cell-cycle proteins [33]. It was shown in two different cell types (neoplastic and non-neoplastic) that both proliferation and aerobic glycolysis are prevented by overexpression of CDH1 and enhanced by its silencing. Furthermore, activation of glycolysis, which is essential for cell proliferation, in the presence of active CDH1 does not result in proliferation [33]. There is data that ubiquitin ligase SKP1-CUL1-F (SCF)-beta-TrCP also sequentially regulates glycolysis during the cell cycle via PFKFB because this effect occurs only when PFKFB is present or is substituted by the downstream glycolytic enzyme 6-phosphofructo-1-kinase [34]. Besides that, the induction of de novo lipid synthesis from glucose in prostate cancer cells by androgen requires transcriptional up-regulation of PFKFB2 and phosphorylation of PFKFB2 generated by the PI3K/AKT signal pathway to supply the source for lipogenesis [35]. The increased glycolytic flux through the enhanced expression of PFKFB3 gene was observed after interaction of adenosine with macrophage Toll-4 receptor agonists [36]. Thus, the family of PFKFB proteins participates in the control of glucose metabolism via glycolysis as well as in the regulation of the cell cycle, proliferation, apoptosis and invasiveness.

Several alternative splice variants for PFKFB2, PFKFB3 and PFKFB4 were identified in normal and tumor cells which possibly have significance in cancer growth [37–42]. However, little is known about alternative splice variants for human PFKFB1 and PFKFB2 and its significance in tumor cell proliferation.

A better understanding of the impact of PFKFB gene networks regulation on glycolysis and cell cycle control as well as nutrient balance at the molecular, cellular and system levels promises to shed light on the emerging association between PFKFB, proliferation, endoplasmic reticulum stress response and cancer.

The main goal of this work is to study the role of ERN1-signaling pathways in tumor progression by investigating the expression of PFKFB1 and PFKFB2 and possible new alternative splice variants for these PFKFB in U87 glioma cells and 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 PFKFB1 and PFKFB2 mRNA. 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 PFKFB 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 PFKFB1 and PFKFB2 as well as its alternative splice variants 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, 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 [43]. RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water.

**Reverse transcription and quantitative PCR analysis.** The expression levels of PFKFB1, PFKFB2 and its alternative splice variants 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 cycler“ (USA) and SYBRGreen Mix (AB gene, Great Britain). QuantiTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis as described previously [43]. Polymerase chain reaction was performed in triplicate.

For amplification of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-1 cDNA we used sequence 5′– GTTTACCAGCTCGAGGCAAG –3′ as forward primer and 5′– AAAACCGCAACATGACCTTC –3′ as reverse primer. The nucleotide sequences of these primers correspond to sequences 102 – 121 and 203 – 207 bp. The amplified DNA fragments were analyzed on a 2% agarose gel and that visualized by 5x Sight DNA Stain calculator and statistical analysis – in Excel program.

For amplification of alternative splice variant of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-1 cDNA with 94 bases deletion was used forward primer sequence 5′– GGAGTTAACAACTAAAAGGCCAG –3′ and reverse – 5′– GTGTTGGCCGTACAGGTCTTT –3′ primers. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The primers were received from “Sigma“ (USA).

For amplification of alternative splice variant of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-1 cDNA with 109 bp insert was performed using forward (5′–GACATCCTGAGAGCTGCCATG –3′) and reverse (5′– GTAGGAGGCCCATGCTATAG –3′) primers. The amplification of alternative splice variant of PFKFB1 cDNA (GenBank accession number EU334168). The size of amplified fragment is 226 bp.

The amplification of alternative splice variant of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-2 cDNA with 94 bases deletion was used forward primer sequence 5′– GGAGTTAACAACTAAAAGGCCAG –3′ and reverse – 5′– GTGTTGGCCGTACAGGTCTTT –3′ primers. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The primers were received from “Sigma“ (USA).

**RESULTS AND DICUSSION**

To investigate the expression of PFKFB1 and PFKFB2 genes that encode different isoforms of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase as well as the involvement of endoplasmic reticulum stress signaling system in the effect of hypoxia and ischemia (glutamine and glucose deprivation conditions) on the expression of these genes we have used the human glioma cell line U87 and a variant of these cells with ERN1 signaling enzyme loss of function. The level of suppression of the enzymatic
activity of ERN1 was estimated by analysis of the expression of transcription factor XBP1 and its splice variant (XBP1s) in U87 glioma cells that overexpress a dominant-negative construct of ERN1 as compared to control glioma cells transfected with a vector. As shown in fig. 1, inductor of endoplasmic reticulum stress tunicamycin (0.01 mg/ml) strongly enhances the alternative splicing of XBP1, but in control glioma cells only, while having no effect on this process in transfected by dnERN1 subline glioma cells.

We have found that PFKFB1 and PFKFB2 mRNAs are expressed in the human glioma cell line U87 and the level of their expression is dependent on signaling enzyme ERN1 function. As shown in fig. 2, the level of PFKFB1 mRNA expression increases by 61% in glioma cells, deficient in signaling enzyme ERN1 function, as compared to control cells. PFKFB2 mRNA expression level also increases in glioma cells with signaling enzyme ERN1 loss of function by 78% as compared to control cells.

Exposure of cells to hypoxia for 16 hrs does not change significantly the expression level of PFKFB1 mRNA both in control glioma cells and genetically modified glioma cells (fig. 3). At the same time, hypoxia leads to an increase the expression level of PFKFB2 mRNA (+14%) in control glioma cells but decreases (-36%) in glioma cells with signaling enzyme ERN1 loss of function (fig. 4).

Moreover, exposure cells under glutamine or glucose deprivation conditions leads to increase the expression level of PFKFB1 and PFKFB2 mRNA but only in control glioma cells (fig. 3 and 4). It was shown also that blockade of ERN1 signaling enzyme function eliminate the increase of PFKFB1 and PFKFB2 mRNA induced by glutamine and glucose deprivation conditions.

Electrophoretic analysis of PFKFB1 amplified products reveals three bands one of which corresponds to known variant (244 bp) and two additional variants. It is known that this region of PFKFB1 gene contains two introns (GenBank accession number NM_002625). At the same time, in PFKFB2 amplified products was also identified additional band which has bigger size compared to known variant (215 bp). The PFKFB2 gene encoded this amplified region contains one intron (GenBank accession number NM_006212). We suggest that these additional bands in PFKFB1 and PFKFB2 amplified products represent alternative splice variants with deletion or insert. Full size cDNAs of PFKFB1 and PFKFB2 were synthesized and cloned in pCRRI-TOPO vector. Selected clones were analyzed by restriction analysis and sequenced. Consequently, we identified two unique alternative splice variants of PFKFB1 mRNA and one for PFKFB2 mRNA (fig. 5 and 6).

As shown in fig. 5, both identified alternative splice variants of PFKFB1 mRNA have deletion of exon 3 (94 bases) or its part (66 bases). Analysis of amino acid sequence of N-terminal part of PFKFB1 shown that 66 bases deletion leads to deletion of 22 amino acid residues without changing the reading frame but changes one amino acid residue (D instead V). This deletion eliminates two important catalytic domains of 6-phosphofructo-2-kinase. Thus, this alternative splice variant of PFKFB1 mRNA encoded isoenzymes without 6-phosphofructo-2-kinase activity. However, deletion of 94 bases changes reading frame and leads to create an anticipatory stop codon in seven amino acid residues (GSAPCQP) after deletion. Moreover, alternative splice variant of PFKFB1 mRNA with 94 bases deletion can be translated alternatively from ATG codon (position 753) before fructose-2,6-bisphosphatase part of PFKFB1 and encoded 244 amino acid residues polypeptide which correspond functional fructose-2,6-bisphosphatase.

Nucleotide sequence and predicted amino acid sequence of N-terminal part of alternative splice variant of PFKFB2 mRNA is shown in fig. 6. This alternative splice variant of PFKFB2 mRNA has 109 bases insert after exon 2 which also leads to create an anticipatory stop codon inside insert sequence, which eliminates N-terminal part of this enzyme. It is possible that this alternative splice variant of PFKFB2 mRNA encodes PFKFB isoenzymes with all catalytic domains as fructose-2,6-bisphosphatase and fructose-2,6-bisphosphatase without regulatory N-terminal region.

We have also studied the expression levels of these alternative splice variants of PFKFB1 and PFKFB2 in glioma cells with ERN1 signaling enzyme loss of function as well as effect of hypoxia and ischemia on its expression. As shown in Fig. 7 – 9, the expression level of all alternative splice variants of PFKFB1 and PFKFB2 mRNA increases in glioma cells, deficient in signaling enzyme ERN1 as compared to control 1. Exposure of cells to hypoxia for 16 hrs does not change significantly these alternative splice variants of PFKFB1 and PFKFB2 mRNA in control glioma cells and cells with ERN1 signaling enzyme loss of function except PFKFB2 splice variant, which expression decreases in glioma cells with suppressed function of ERN1 (-14%), as compared to control 2. However, under glutamine deprivation condition expression level of alternative splice variants of PFKFB1 and PFKFB2 mRNA increases in control glioma cells (+29, +17 and +18%, respectively, as compared to control 1), but it miss in glioma cells with ERN1 loss of function, as compared to control 2.

It has been known that the glycolysis and tumor growth processes are linked to the endoplasmic reticulum stress and its sensing and signal transduction pathways and ERN1 pathway, in particular, because the complete blockade of this signaling enzyme activity had anti-tumor effects [14, 16, 19]. Moreover, there is data that growing tumor requires ischemia and hypoxia which initiate the endoplasmic reticulum stress and its sensing and signal transduction pathways, for apoptosis inhibition [18, 19]. It is known that some PFKFB enzymes are components of the endoplasmic reticulum stress system; they participate in proliferation processes [21, 32, 33, 38].

In this study we have shown that blockade of ERN1, the key endoplasmic reticulum stress sensor, changes the expression levels of PFKFB1 and PFKFB2 isoenzymes,
which play an important role in the control of glycolysis and tumor growth [29, 35]. In this work we identified three unique alternative splice variants of PFKFB1 and PFKFB2 which can suppress glycolysis because these variants have only fructose-2,6-bisphosphatase activity. The PFKFB1 alternative splice variant with 66 bases deletion has not two important catalytic domains of 6-phosphofructo-2-kinase but it has native N-terminus for creation homodimers and can inactivate functionally active subunit of PFKFB1. This data agrees with idea that some PFKFB participate in endoplasmic reticulum stress signaling and are the important components of IRE-1 signaling [21, 32, 33]. We have found that the expression of PFKFB1 and PFKFB2 as well as its alternative splice variants increases in IRE-1 knockdown glioma cells and it is possible that this dysregulation is responsible, at least partly, for suppression of these cells proliferation [14] via inactivation of active subunit of PFKFB1 by splice variants and increased levels of PFKFB variants with fructose-2,6-bisphosphatase activity, because knockdown of PFKFB3 really suppress cell proliferation [33, 34]. The PFKFB2 alternative splice variant has all catalytic domains both 6-phosphofructo-2-kinase and fructose-2,6-bisphosphatase but it has not regulatory N-terminus for creation homodimers, necessary step for developing of 6-phosphofructo-2-kinase activity [25]. This alternative splice variant of PFKFB2 is similar to PFKFB4 splice variant from mice tissues and human melanoma cells [41, 42].

Thus, results of this study clearly demonstrate that the expression level of PFKFB1 and PFKFB2 as well as its alternative splice variants without 6-phosphofructo-2-kinase activity increases in glioma cells with ERN1 signaling enzyme loss of function and that glutamine and glucose deprivation conditions also lead to increase the expression level of these PFKFB but in control glioma cells only. It is possible that increase of the expression level of PFKFB1 and PFKFB2 genes in glutamine and glucose deprivation conditions is mediated by ERN1 signaling system because it miss in cells with ERN1 loss of function and is observed in control glioma cells only. However, the molecular mechanisms underlying these seemingly mutually exclusive behaviors have not been elucidated. This provides a rationale for the molecular analysis of expression signatures of different related genes in glioma cells which control the tumor growth and glycolysis for a comprehensive approach of these complex mechanisms.

The major finding reported here is that the expression of PFKFB genes is dependent on the function of ERN1 signaling enzyme – both in normal, hypoxic and glutamine or glucose deprivation conditions and possible participate in glioma cell proliferation and tumor growth via regulation of various signaling pathways. However, the detailed molecular mechanisms of regulation of genes encoding different PFKFB by ERN1 signaling system under ischemic and hypoxic stress conditions is complex and warrants further study.
Fig. 3. Effect of hypoxia and glutamine or glucose deprivation on the expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-1 (PFKFB1) mRNA in glioma cell line U87 (control 1) and its subline with blockade of signaling enzyme endoplasmic reticulum–nuclei-1 (dnERN1) activity (control 2) measured by quantitative polymerase chain reaction. Values of PFKFB1 mRNA expression 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 glutamine or glucose deprivation on the expression of PFKFB2 mRNA in glioma cell line U87 (control 1) and its subline with blockade of signaling enzyme endoplasmic reticulum–nuclei-1 (dnERN1) activity (control 2) measured by quantitative polymerase chain reaction. Values of PFKFB2 mRNA expression 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. Nucleotide sequence and predicted amino acid sequence of N-terminal part of PFKFB1 (NM_002625) and its alternative splice variants with deletion of 66 (-66) and 94 (-94) bases in glioma U87 cells. Deletion of 66 bases leads to deletion of 22 amino acid residues without changing of reading frame but changes one amino acid residue, labeled by red color. Deletion of 94 bases changes reading frame and leads to create an anticipatory stop codon in seven amino acid residues, labeled by red color, after deletion. PFKFB1 mRNA with 94 bases deletion can encoded 244 amino acid residues polypeptide which correspond functional fructose-2,6-bisphosphatase (N-terminal part of this polypeptide labeled by red color).
**Fig. 6.** Nucleotide sequence and predicted amino acid sequence of N-terminal part of basic variant of PFKFB2 (NM_006212) and its alternative splice variant (EU334168) with 109 bases insert in human glioma cells. 109 bases insert labeled by blue color leads to create an anticipatory stop codon in 24 amino acid residues, labeled by red color, inside this insertion. This alternative splice variant can be translated from methionine located in 206 bases after primary start of translation and can encoded 472 amino acid residues polypeptide with functional fructose-2,6-bisphosphatase (N-terminal part of this polypeptide labeled by red color).

**Fig. 7.** Effect of hypoxia and glutamine or glucose deprivation on the expression of PFKFB1 alternative splice variant (-66; part of exon 3; exon 3a) in glioma cell line U87 (control 1) and its subline with blockade of signaling enzyme endoplasmic reticulum–nuclei-1 (dnERN1) activity (control 2) measured by quantitative polymerase chain reaction. Values of the expression of this PFKFB1 alternative splice variant were normalized to beta-actin mRNA expression and represent as percent for control 1 (100 %); n = 5; * - P < 0.05 as compared to control 1; ** - P < 0.05 as compared to control 2.
Results of this investigation clearly demonstrate that the expression of PFKFB1 and PFKFB2 genes in glioma cells is regulated by hypoxia and glutamine or glucose deprivation and that this regulation depends on the functional activity of signaling enzyme ERN1. Thus, PFKFB1 and PFKFB2 isoenzymes participate in cell adaptive response to endoplasmic reticulum stress associated with hypoxia, glutamine or glucose deprivation.

CONCLUSIONS

References


ЕКСПРЕСІЯ 6-ФОСФОФРУКТО-2-КІНАЗИ/ФРУКТОЗО-2,6-БІСФОСФАТАЗИ-1-2 ТА ЇХ АЛЬТЕРНАТИВНИХ СПІЛЯС-ВАРІАНТІВ У КЛІТИНАХ ГЛІОМ У87 З ВТРАЧЕНОЮ ФУНКЦІЄЮ ERN1

Липова Н.М., Мінченко Д.А., Кубайчуц К.І., Мінченко О.Г.

Сенсорно-сигнальний ензим ERN1 (від ендоплазматичного ретикулуму до ядра) опосередковує комплекс складних внутрішньоклітинних сигнальних подій, відомих як реакція розротрання протеїнів. Ми досліджували вплив гіпокії та умов інші неіз (дефіцит глюкози або глютаміну) на експресію генів 6-фосфофруктук-2-кінази/фруктозо-2,6-бісфосфатаз-1 та -2 (PFKFB1 та PFKFB2) у клітинах глиом лінії U87 та їх сублінії з пригнічення функцією сенсорно-сигнального ензиму ERN1 і ідентифікували три унікальних альтернативних спіліяс-віаріантів PFKFB1 та PFKFB2 із вставкою або делецією в N-кінцевій ділянці, що приводить до втрати 6-фосфофрукто-2-кіназної активності. Встановлено, що блокада функції ензиму ERN1, ключового сенсора стресу ендоплазматичного ретикулуму приводить до збільшення рівня експресії мРНК PFKFB1 та PFKFB2, а також їх альтернативних спіліяс-віаріантів. Більше того, за умов гіпокії рівень експресії мРНК PFKFB2 збільшується як у контрольних клітинах глиом, так і в генетично модифікованих клітинах глиом. В той же час, рівень експресії мРНК PFKFB1 та її альтернативних спіліяс-віаріантів істотно не змінюється за цих експериментальних умов. Експресія клітин за умов дефіциту глюкози або глютаміну призводить до збільшення рівня експресії мРНК PFKFB1 та PFKFB2, а також їх альтернативних спіліяс-віаріантів. Більше того, за умов гіпокії рівень експресії мРНК PFKFB1 та її альтернативних спіліяс-віаріантів істотно не змінюється за цих експериментальних умов.

Ключові слова: експресія мРНК, PFKFB1, PFKFB2, унікальні альтернативні спіліяс-віаріанти, клітини глиом, від ендоплазматичного ретикулуму до ядра-1 (ERN1), гіпокія, дефіцит глюкози та глютаміну.
ЭКСПРЕССИЯ 6-ФОСФОФРУКТО-2-КІНАЗИ/ФРУКТОЗО-2,6-БІСФОСФАТАЗИ-1 И -2 И ИХ АЛЬТЕРНАТИВНЫХ СПЛАЙС-ВАРИАНТОВ В КЛЕТКАХ ГЛИОМЫ U87 С УТРАЧЕННОЙ ФУНКЦИЕЙ ERN1

Лыпова Н.М., Минченко Д.А., Кубайчук К.И., Минченко А.Г.

Сенсорно-сигнальный энзим ERN1 (от эндоплазматического ретикулума к ядру) является посредником комплексом сложных внутриклеточных сигнальных событий, известных как реакция развертывания протеинов. Мы исследовали влияние гипоксии и условий ишемии (дефицит глюкозы или глутамина) на экспрессию генов 6-фосфофрукто-2-кіназы/фруктозо-2,6-бісфосфатазы-1 та -2 (PFNFB1 и PFKFB2) в клетках глиомы линии U87 и их сублиниях с угнетенной функцией сенсорно-сигнального энзима ERN1. Идентифицировали три уникальных альтернативных сплайс-варианта PFKFB1 и PFKFB2 со вставкой или делециями в N-концевом участке, которые приводят к потере 6-фосфофрукто-2-кіназной активности. Установлено, что блокада функции энзима ERN1, ключевого сенсора стресса эндоплазматического ретикулума приводит к увеличению уровня экспрессии мРНК PFKFB1 и PFKFB2, а также их альтернативных сплайс-вариантов. Больше того, при гипоксии уровень экспрессии мРНК PFKFB1 и PFKFB2 увеличивается как в контрольных клетках глиомы, так и в генетически модифицированных клетках глиомы. В той же степени, уровень экспрессии мРНК PFKFB1 и ее альтернативных сплайс-вариантов существенно не изменяется при этих экспериментальных условиях. Экспозиция клеток в условиях дефицита глутамина и глукозы приводит к увеличению уровня экспрессии мРНК PFKFB1 и PFKFB2 только в контрольных клетках глиомы. В то же время, уровень экспрессии мРНК PFKFB1 и PFKFB2 в клетках с утраченной функцией ERN1 также увеличивается только в контрольных клетках глиомы, а альтернативных сплайс-вариантов этих мРНК также увеличивается только в контрольных клетках глиомы и только в условиях дефицита глутамина. Было также показано, что блокада функции сигнального энзима предотвращает увеличение экспрессии мРНК PFKFB1 и PFKFB2, а также их альтернативных сплайс-вариантов, что индуцируется в условиях дефицита глутамина в клетках глиомы. Таким образом, результаты этого исследования четко продемонстрировали, что уровень экспрессии мРНК PFKFB1 и PFKFB2, а также их альтернативных сплайс-вариантов с 6-фосфофрукто-2-кіназной активностью, увеличивается в клетках глиомы с утраченной функцией ERN1 и что в условиях дефицита глутамина и глукозы уровень экспрессии этих мРНК также увеличивается, но лишь в контрольных клетках глиомы. Возможно, что увеличение уровня экспрессии генов PFKFB1 и PFKFB2 в условиях дефицита глутамина и глукозы опосредуется сигнальной системой ERN1, поскольку оно отсутствует в клетках с утраченной функцией ERN1, а наблюдается лишь в контрольных клетках глиомы.

Ключевые слова: экспрессия мРНК, PFKFB1, PFKFB2, уникальные альтернативные сплайс-варианты, клетки глиомы, от эндоплазматического ретикулума к ядру-1 (ERN1), гипоксия, дефицит глукозы и глутамина.