ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ

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EFFECT OF GLUTAMINE OR 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 OF ENDOPLASMIC RETICULUM–NUCLEI-1

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Ischemia has been shown to induce a set of complex intracellular signaling events known as the unfolded protein response, which is mediated by endoplasmic reticulum–nuclei-1 sensing enzyme. We have studied the expression of several cyclin and cyclin-dependent kinase genes which participate in the control of cell cycle and proliferation under ischemic conditions (glucose or glutamine deprivation) in endoplasmic reticulum–nuclei-1-deficient glioma cells. It was shown that blockade of endoplasmic reticulum–nuclei signaling enzyme-1, the key endoplasmic reticulum stress sensor, leads to an increase of the expression levels of cyclin-dependent kinase-2 and cyclin A2, D3, E2 and G2 genes but suppresses cyclin D1. Moreover, the expression level of cyclin-dependent kinase-2 as well as cyclin A2, D3 and E2 mRNAs is significantly decreased under glucose or glutamine deprivation conditions both in control and endoplasmic reticulum–nuclei-1-deficient glioma cells. However, cyclin-dependent kinase-4 and -5 mRNA expressions is increased, but in glucose deprivation conditions only. Results of this study have shown that the expression of most tested genes of encoded cyclins and cyclin-dependent kinases is dependent on endoplasmic reticulum–nuclei-1 signaling enzyme function both in normal and glutamine and glucose deprivation conditions and possibly participates in cell adaptive response to endoplasmic reticulum stress associated with ischemia.

Key words: mRNA expression, cyclin A2, D1, D3, E2 and G2, cyclin-dependent kinase-2, -4 and -5, glioma cells, endoplasmic reticulum–nuclei-1, glucose and glutamine deprivation.

Ischemia and hypoxia have been shown to induce a set of complex intracellular signaling events known as the unfolded protein response, which is mediated by endoplasmic reticulum–nuclei-1 signaling enzyme (also named as inositol requiring enzyme-1α), to adapt cells for survival or, alternatively, to enter cell death programs through endoplasmic reticulum-associated machineries [1–5]. As such, it participates in the early cellular response to the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum, occurring under both physiological and pathological situations.

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 endoplasmic reticulum–nuclei-1 signalling. The endoplasmic reticulum–nuclei-1-associated kinase activity autophosphorylates and dimerizes this enzyme, leading to the activation of its endoribonuclease domain, to the degradation of a specific subset of mRNA and to the initiation of the pre-XBP1 (X-box binding protein 1) mRNA splicing [6–9]. Mature XBP1 mRNA splice variant encodes a transcription factor that stimulates the expression of hundreds of unfolded protein response-specific genes [3, 10–13]. Greenman et al. [14] recently showed that single mutations in endoplasmic reticulum–nuclei-1 gene were detected in different human cancers and encoded by this gene enzyme was proposed as a major contributor to

tumor (including glioblastoma) progression among protein kinases. The endoplasmic reticulum–nuclei-1 signal transduction pathway is linked to the neovascularization process, tumor growth and cell death processes because the complete blockade of this sensing enzyme activity had anti-tumor effects [15–19].

It is known that many cyclins, cyclin-dependent kinases and their inhibitors, retinoblastoma proteins, and E2F transcription factors are components of endoplasmic reticulum stress and they participate in the control of cell cycle and proliferation [20–23]. Cyclin D1 (CCND1) forms a complex with, and functions as a regulatory subunit of cyclin-dependent kinases-4 or -6, whose activity is required for cell cycle G1/S transition. It interacts with tumor suppressor retinoblastoma protein and the expression of this gene is regulated positively by retinoblastoma protein. Mutation, amplification and overexpression of Cyclin D1 are observed frequently in a variety of tumors and may contribute to tumorigenesis. There is data that cyclin D1 blocks the anti-proliferative function of RUNX3 by interfering with RUNX3-p300 interaction and reduces G1 cell cycle arrest despite constitutive expression of cyclin E2 in ovarian cancer cells [24, 25]. Cyclin D3 (CCND3) is the primary driver of the cell cycle, in cooperation with CCND1 that integrates extracellular mitogenic signaling [26]. Cyclin D3 has been shown to specifically interact with cyclin-dependent kinase-2 and CIP/KIP family of CDK inhibitors, and plays a role in cell cycle G1/S transition. A significantly increased expression level of CCND3 gene as well as repression of CDK inhibitors were observed in tumor-derived cells [27, 28]. It was shown that the repression of p14ARF and INK4 genes is mainly a result of epigenetic promoter methylation [29]. Moreover, ectopic expression of PFKFB3 increased the expression of several key cell cycle proteins, including cyclin-dependent kinase-1, cell division cycle 25C and cyclin D3 and decreased the expression of the cell cycle inhibitor p27 [30].

It is known that cyclin E2, which is increased in tumor derived cells, binds and activates kinase cell division cycle-2 or cyclin-dependent kinases-2, and thus promotes both cell cycle G1/S and G2/M transitions [31]. However, gastric cancer progression correlates with expression levels of cyclin G2, but not cyclin E: cyclin G2 appears to be a negative cell-cycle regulator in gastric cancer, and its expression seems to be inversely related to gastric cancer progression [32–34]. There is data that down-regulation of transcription factor E2F1, cyclins E1 and E2 by siRNA shows reduction in the phosphorylation levels of the retinoblastoma protein pRB and a decrease in the amount of cyclin A2, which expression is also controlled by the transcription factor E2F [35]. There is data that cyclin A2 and human epidermal growth factor 2 are associated with proliferation and high recurrence, particularly when combined [36, 37].

The cyclin-dependent kinase 2 is associated with and regulated by the regulatory subunits of the complex including cyclin A or E, cyclin dependent kinase inhibitor p21 (CDKN1A) and p27 (CDKN1B) [27]. It is a catalytic subunit of the cyclin-dependent kinase complex that is important for cell cycle. The activity of cyclin-dependent kinase 4 is controlled by the regulatory subunits D-type cyclins and cyclin-dependent kinase inhibitor p16 (INK4a). This kinase was shown to be responsible for the phosphorylation of carboxy-terminus of retinoblastoma gene product. The cyclin-dependent kinase 5 is one of the kinases studied in neuronal cell system. Recently, the involvement of cyclin-dependent kinase 5 in phosphorylation p53 has been shown in certain cancer types: phosphorylation of overexpressed p53 following inhibition of protein phosphatase 2A. Cyclin-dependent kinase 5 has several very important functions: negative regulation of cell cycle, protein export from nucleus, protein ubiquitination, it induces cell cycle arrest/apoptosis and inhibits tumor progression [38, 39].

In this work we have studied the effect of glucose and glutamine deprivation on the expression of different cyclin (A2, D1, D3, E2 and G2) and cyclin-dependent kinase-2, -4 and -5 genes in glioma cell line U87 and modified glioma cells without endoplasmic reticulum–nuclei signaling enzyme-1 kinase and endoribonuclease activities for evaluation of cyclin and cyclin-dependent kinase genes responsibility from this signaling enzyme-1 function.

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 (Gibco, Invitrogen, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio,Inc., USA), penicillin (100 units/ml; Gibco) and streptomycin (100 μg/ml; Gibco) at 37 °C in a 5% CO_2 incubator. In this work we used two sublines of this glioma cell line. One subline has suppressed both protein kinase and endoribonuclease activities of sensor endoplasmic reticulum–nuclei signaling enzyme-1 which was obtained by selection of stable transfected clones with overexpression of endoplasmic reticulum–nuclei-1 dominant/negative constructs (dnERN1) [15]. The second subline was obtained by selection

of stable transfected clones with overexpression of vector, which was used for creation of dnERN1. This subline was used as control 1. Cells were synchronized and grown to 80% confluence before the experiment was started.

For glucose or glutamine deprivation the growing medium in culture plates was replaced on the Gibco medium without glucose or glutamine and exposed for 16 hours.

RNA isolation. Total RNA was extracted from different tumor tissues and normal tissue counterparts using Trizol reagent according to manufacturer protocol (Invitrogen, USA) [40]. RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water.

Reverse transcription and quantitative PCR analysis. The expression of cyclin A2, D1, D3, E2 and G2, as well as CDK4 and CDK5 mRNA was measured in glioma cell line U87 and its subline with endoplasmic reticulum–nuclei-1-deficiency by quantitative polymerase chain reaction of complementary DNA (cDNA) using «Stratagene Mx 3000P cycler» (USA) and SYBRGreen Mix (AB gene, Great Britain). QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis as described previously [40]. Polymerase chain reaction was performed in triplicate.

For amplification of cyclin D1 (CCND1) cDNA we used forward (5′-GAGGAAGAGGAGGAGGAGGA-3′ and reverse (5′-GAGATGGAAGGGGGAAAGAG-3′) primers. The nucleotide sequences of these primers correspond to sequences 1029-1048 and 1264-1245 of human CCND1 cDNA (GenBank accession number NM_053056).

The amplification of cyclin D3 (CCND3) cDNA was performed using forward primer (5′-GTGGCCACTAAGCAGAGGAG-3′) and reverse primer (5′-AGCTTGACTAGCCACCGAAA-3′). These oligonucleotides correspond to sequences 1074-1093 and 1261-1242 of human CCND3 cDNA (GenBank accession number NM_001136017).

The amplification of cyclin A2 (CCNA2) cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5′-TTATTGCTGGAGCTGCCTTT-3′ and reverse – 5′- CTCTGGTGGGTTGAGGAGAG-3′. The nucleotide sequences of these primers correspond to sequences 1366-1385 and 1589-1570 of human CCNA2 cDNA (GenBank accession number NM_001237).

Two other primers were used for real time RCR analysis of E2 (CCNE2) cDNA expression: forward – 5′-TCAGAAAAGGGGGACAGTTG-3′ and reverse – 5′-GAATTGGCTAGGGCAATCAA-3′. The nucleotide sequences of these primers correspond to sequences 1236-1255 and 1438-1419 of human CCNE2 cDNA (GenBank accession number NM_057749).

For amplification of cyclin G2 (CCNG2) cDNA we used forward (5′-AGCCATCAAATGGGGTAGTG-3′ and reverse (5′-CCCTGCTAAGGAAGCACTTG-3′) primers. The nucleotide sequences of these primers correspond to sequences 1507-1526 and 1739-1720 of human CCNG2 cDNA (GenBank accession number NM_004354).

For real time RCR analysis of cyclin-dependent kinase 2 cDNA expression we used the next primers: forward – 5′-CATTCCTCTTCCCCTCATCA-3′ and reverse – 5′-CAGGGACTCCAAAAGCTCTG-3′. The nucleotide sequences of these primers correspond to sequences 531-550 and 703-684 of human CDK2 cDNA (GenBank accession number NM_001798).

The amplification of cyclin-dependent kinase 4 cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5′-GAAACTCTGAAGCCGACCAG-3′ and reverse – 5′-AGGCAGAGATTCGCTTGTGT-3′. The nucleotide sequences of these primers correspond to sequences 874-893 and 1086-1067 of human CDK4 cDNA (GenBank accession number NM_000075).

Two other primers were used for real time RCR analysis of cyclin-dependent kinase 5 cDNA expression: forward – 5′-GTCCATCGACATGTGGTCAG-3′ and reverse – 5′-CTCCCTGTGGCATTGAGTTT-3′. The nucleotide sequences of these primers correspond to sequences 660-679 and 896-877 of human CDK5 cDNA (GenBank accession number NM_004935).

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

Quantitative PCR was performed on “Stratagene Mx 3000P cycler”, using SYBR Green Mix. 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 separated on a 2% agarose gel and that visualized by 5x Sight DNA Stain (EUROMEDEA).

Results

In this study, we have used human glioma cell line U87 and its genetically modified variant (deficient in signaling enzyme of endoplasmic re-

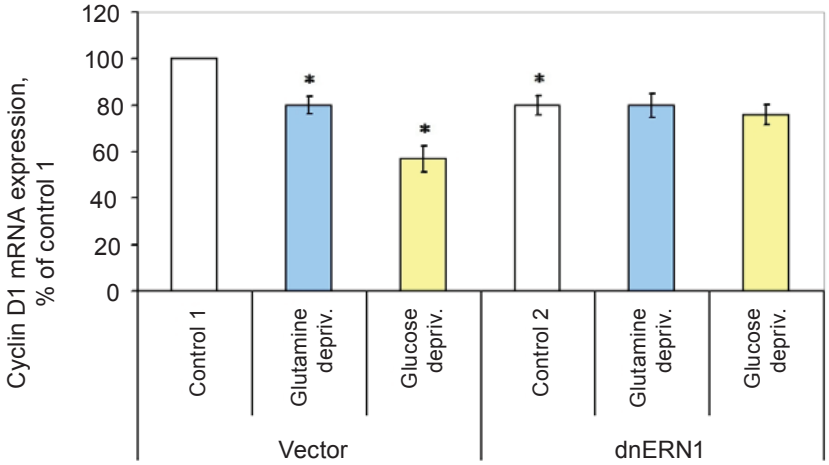

*Fig. 1. Expression of cyclin D1 mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of cyclin D1 mRNA expressions were normalized to β-actin mRNA expression and represented as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1*

ticulum–nuclei-1) to investigate the involvement of endoplasmic reticulum stress system in the effect of glutamine and glucose deprivation on the expression of different genes of cyclins and cyclin-dependent kinases. For this aim, the cells were incubated at 37 °C before harvesting in regular DMEM medium (control) and in the medium without glucose or glutamine for 16 hours. Total RNA was extracted from cells, converted into complementary DNA and readily quantified by real time polymerase chain reaction.

We have found that cyclin D1, D3, A2, G2 and E2 mRNA are expressed in the human glioma cell line U87 and the level of its expression is dependent on endoplasmic reticulum – nuclei signaling enzyme-1 function as well as on glutamine or glucose deprivation. As shown in Fig. 1, the level of cyclin D1 mRNA expression is decreased by 20% in glioma cells, deficient in signaling enzyme endoplasmic reticulum–nuclei-1, as compared to control cells. The exposure of cells during 16 hours in glutamine or glucose deprivation conditions leads to a decrease of cyclin D1 mRNA expression level, but only in control cells (-20 and -41%, correspondingly). No significant changes were found in genetically modified cells with suppressed function of signaling enzyme of endoplasmic reticulum–nuclei-1 after the exposure under glutamine or glucose deprivation conditions.

At the same time, the level of cyclin D3 mRNA expression is significantly increased (+74%) in glioma cells with suppressed activity of signaling enzyme of endoplasmic reticulum–nuclei-1 as compared to control glioma cells (Fig. 2). Investigation of the effect of glucose or glutamine deprivation conditions on the expression of cyclin D3 mRNA level is shown that both in control and endoplasmic reticulum–nuclei-1 signaling enzyme-deficient glioma cells there was a significant decrease of the expression level of cyclin D3 mRNA; in the control cells, however, it was slightly less.

As shown in Fig. 3, the level of cyclin A2 mRNA expression is also increased (+39%) in glioma cells with suppressed activity of signaling enzyme of endoplasmic reticulum–nuclei-1 as compared to the control glioma cells. Investigation of the effect of glutamine or glucose deprivation conditions on the expression of cyclin A2 mRNA level has shown that the level of cyclin A2 mRNA expression is decreased both in the control and endoplasmic reticulum–nuclei-1 signaling enzyme-deficient glioma cells: -33% and -36% – in the control cells and -64% and -58% – in genetically modified cells, correspondingly.

More significant changes were found in the expression level of cyclin E2 mRNA (Fig. 4). Thus, in glioma cells with suppressed activity of signaling enzyme of endoplasmic reticulum–nuclei-1 the expression level of cyclin E2 mRNA was almost 4-fold higher than in the control cell line U87. Exposing cells to glutamine and to glucose deprivation conditions leads to a significant decrease in the expression level of cyclin E2 mRNA in both investigated cell types: -61% and -55% – in the control cells and -75% and -73% – in genetically modified cells, correspondingly.

Results of investigation of cyclin G2 mRNA expression level are shown in Fig. 5. Thus, the blockade of the activity of endoplasmic reticulum–nuclei-1 signaling enzyme leads to 2-fold induction

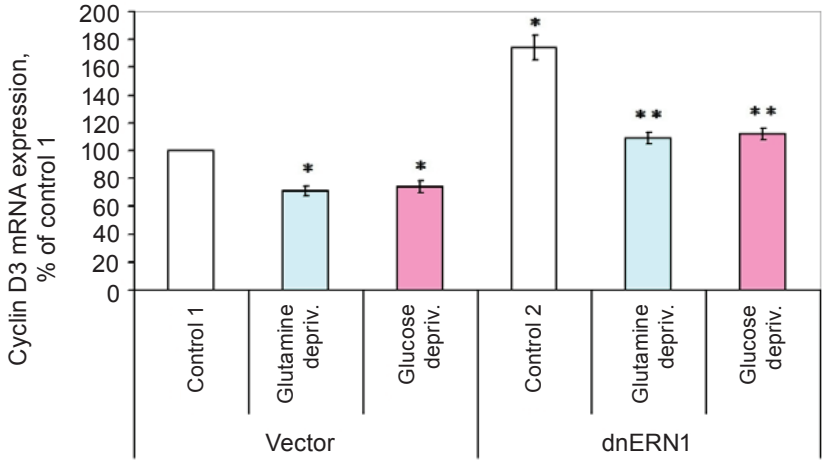

*Fig. 2. Expression of cyclin D3 mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of cyclin D3 mRNA expressions were normalized to β-actin mRNA expression and represented as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

of this mRNA expression level as compared to the control cells. Glutamine and glucose deprivation conditions significantly induce the expression level of cyclin G2 mRNA, only in control glioma cells (+98% and +71%, correspondingly). However, in genetically modified cells we observed a decrease of cyclin G2 mRNA expression level in glucose deprivation conditions (-44%).

As shown in Fig. 6 and 7, the expression level of cyclin-dependent kinase-2 mRNA is also increased (+57%) and cyclin-dependent kinase-4 is decreased in glioma cells without activity of endoplasmic reticulum–nuclei-1 signaling enzyme. However, no significant changes were found in the expression levels of cyclin-dependent kinase-5 mRNA in these genetically modified cells (Fig. 8). Exposing cells to medium without glutamine leads to significant decrease in cyclin-dependent kinase-2 and -5 mRNA expression level in both investigated cell types: -40% and -33% – in the control cells and -49% and -29% – in genetically modified cells, correspondingly. However, no significant changes were found in the expression levels of cyclin-dependent kinase-4 mRNA both

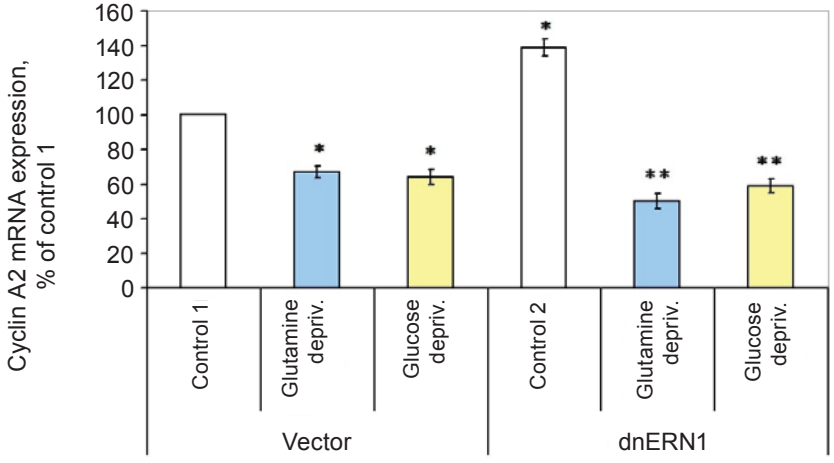

*Fig. 3. Expression of cyclin A2 mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of cyclin A2 mRNA expressions were normalized to β-actin mRNA expression and represented as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

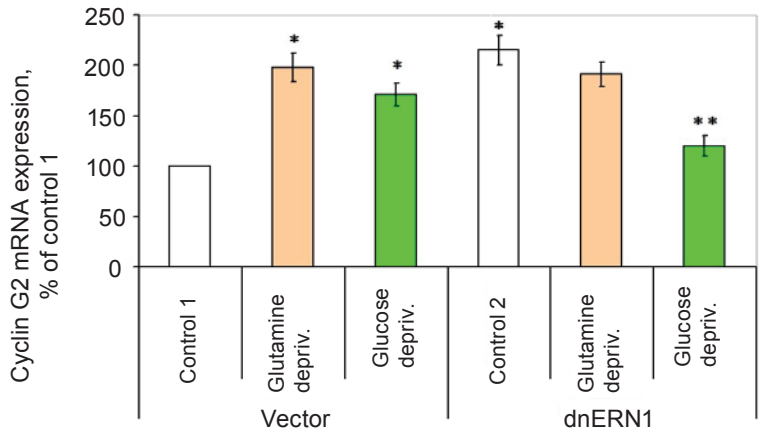

*Fig. 4. Expression of cyclin G2 mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of cyclin G2 mRNA expressions were normalized to β-actin mRNA expression and represented as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

in the control and genetically modified cells under glutamine deprivation conditions (Fig. 7). At the same time, expression levels of cyclin-dependent kinase-4 and -5 mRNA, but not cyclin-dependent kinase-2, are increased in both investigated cell types under glucose deprivation conditions. Exposing cells to the medium without glucose leads to a significant decrease in cyclin-dependent kinase-2 mRNA expression level both in control and glioma cells without activity of endoplasmic reticulum – nuclei-1 signaling enzyme: -46% in control cells and -53% in genetically modified cells (Fig. 6).

Discussion

It is known that the neovascularization process, tumor growth and cellular death processes are linked to the stress and its sensing and signal transduction pathways and endoplasmic reticulum–nuclei-1 in particular, because the complete blockade of this signaling enzyme activity had anti-tumor effects [15–17]. Moreover, the growing tumor re-

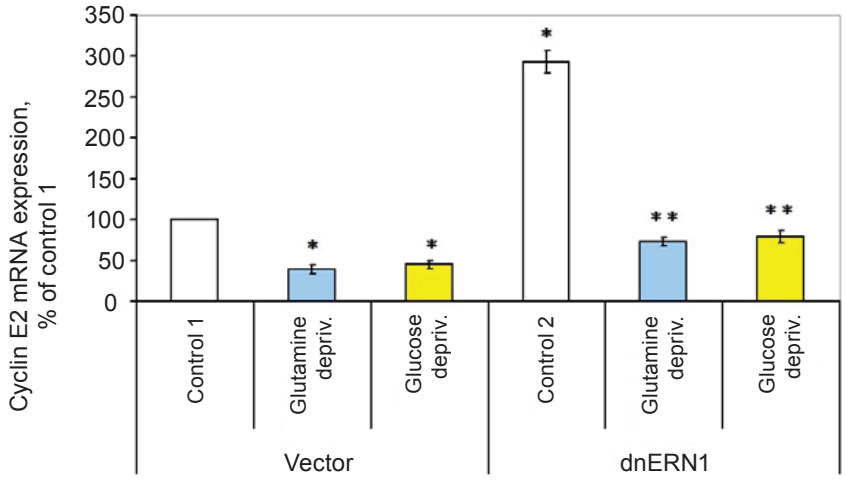

*Fig. 5. Expression of cyclin E2 mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of cyclin E2 mRNA expressions were normalized to β-actin mRNA expression and represented as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

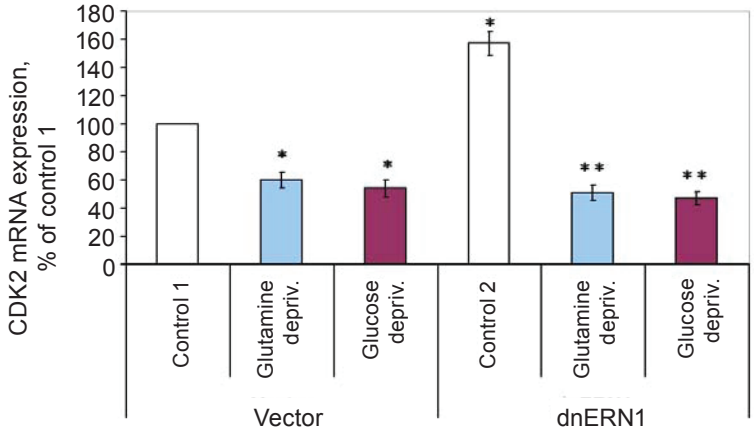

*Fig. 6. Effect of glucose and glutamine deprivation on the expression of cyclin-dependent kinase-2 (CDK2) mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of CDK2 mRNA expressions were normalized to β-actin mRNA expression and represent as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

quires ischemia and hypoxia which initiate the endoplasmic reticulum stress for own neovascularization and growth, for apoptosis inhibition [15]. It is known that many cyclins, cyclin-dependent kinases and their inhibitors, retinoblastoma proteins and E2F transcription factors are the components of endoplasmic reticulum stress system as well as participate in the control of cell cycle and proliferation processes [20, 21].

For this study we select several cyclins such as cyclin D1, D3, A2, E2 and G2 as well as three cyclin-dependent kinases – CDK2, CDK4 and CDK5 which as known are the components of endoplasmic reticulum stress system and play an important role in the control of the growth of malignant tumors, including glioblastoma [12, 20, 22, 23]. Malignant gliomas are the most frequent primary brain tumors and represent a major challenge in cancer therapy, but gliomas are not easily accessible to current therapies. 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 invasive and growth patterns in glioma cells for a comprehensive approach of these complex mechanisms. Because bifunctional transmembrane signaling enzyme of endoplasmic reticulum–nuclei-1 is a major proximal sensor of the unfolded protein response, it participates in the early cellular response to the accumulation of mis-

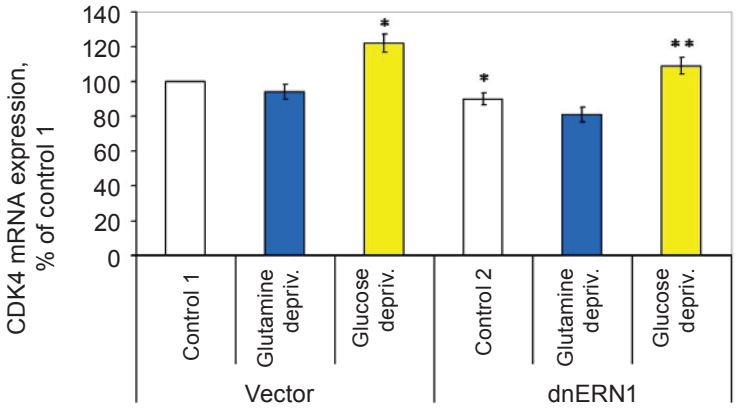

*Fig. 7. Effect of glucose and glutamine deprivation on the expression of cyclin-dependent kinase-4 (CDK4) mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of CDK4 mRNA expressions were normalized to β-actin mRNA expression and represented as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

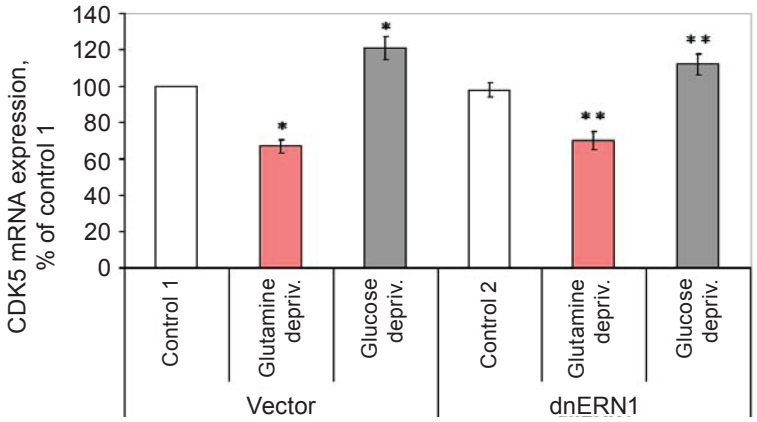

*Fig. 8. Effect of glucose and glutamine deprivation on the expression of cyclin-dependent kinase-5 (CDK5) mRNA in glioma cell line U87 (control 1) and its subline with signaling enzyme endoplasmic reticulum–nuclei-1 (ERN1)-deficiency (control 2) measured by quantitative polymerase chain reaction. Values of CDK5 mRNA expressions were normalized to β-actin mRNA expression and represent as percent for control 1 (100%); n = 3; * P < 0.05 as compared to control 1; ** P < 0.05 as compared to control 2*

folded proteins in the endoplasmic reticulum under both physiological and pathological situations and in malignant tumors, in particular [15, 17]. Therefore, in this work we studied the expression of several key cyclin and cyclin-dependent kinase genes in glioma cells without activity of endoplasmic reticulum–nuclei-1 signaling enzyme for the evaluation of cyclin and cyclin-dependent kinase genes responsibility for this signaling enzyme-1 function, because it is known that the complete blockade of this signaling enzyme activity has anti-tumor effects [15, 16].

Results of this investigation clearly demonstrated that the expression levels of cyclin-dependent kinases 4 and cyclin D1, which form a complex with and function as a regulatory subunit of this kinase and block the anti-proliferative function of RUNX3, are decreased in glioma cells without endoplasmic reticulum–nuclei-1 signaling enzyme function which is responsible for tumor growth [15, 17, 24]. This data is correlated with results of Masamha and Benbrook [25] and Courapied et al. [39]. They have shown that cyclin D1 degradation is sufficient to induce G1 cell cycle arrest despite the constitutive expression of cyclin E2 in ovarian cancer cells and that the key events associated with cyclin D1-dependent neoplastic growth (nuclear cyclin D1/CDK4 kinase complex) regulates Cullin-4 expression and triggers neoplastic growth via activation of the arginine N-methyltransferase PRMT5 [39].

Moreover, the expression levels of cyclin G2, which appears to be a negative cell-cycle regulator in cancer, is significantly increased in glioma cells with suppressed activity of endoplasmic reticulum–nuclei-1 signaling enzyme. These results are correlated with the decreased proliferative rate of genetically modified glioma cells [15, 17] and data from Kasukabe et al. [34] and Xu et al. [33]. We have also shown that the expression level of cyclin A2, D3 and especially cyclin E2 is also increased in glioma cells which have no enzyme signaling activity of endoplasmic reticulum–nuclei-1, although the biological significance of this increase is to be determined. Molecular mechanisms of regulation of different cyclin genes expression are complex and possibly include the regulatory cross talk among multiple nuclear coactivators or corepressors. Recently, it was shown that overexpression of MDM2, which has relation to oncogenesis, inhibits cyclin A mRNA expression, but does not inhibit cyclin D or E mRNA expression. Moreover, the expression of cyclin A is also controlled by transcription factor E2F via interaction with the cyclin A promoter and 5-prime UTR and down-regulation of E2F1 by siRNA leads to a decrease in the amount of cyclin A2 [35]. However, the down-regulation of cyclins E1 and E2 by siRNA shows reduction in the phosphorylation levels of the retinoblastoma protein pRB and also a decrease in the cyclin A2 mRNA expression [35]. It is possible that a significant increase in the cyclin E2 mRNA expression in our experiments with genetically modified glioma cells can induce the expression of cyclin A2.

In this study we have shown that the expression level of cyclin-dependent kinase-2 is increased, cyclin-dependent kinase-5 is not changed and cyclin-dependent kinase-4 is decreased in glioma cells without activity of endoplasmic reticulum–nuclei-1 signaling enzyme. These results are tightly correlated with data from Buecher et al.

[27]. They have shown that cyclin-dependent kinase-2 associates with and is regulated by the regulatory subunits of the complex, including cyclin A or E, but activity of cyclin-dependent kinase-4 is controlled by the regulatory subunits of D-type cyclins. Thus, the results of this investigation clearly demonstrate that in glioma cells without endoplasmic reticulum–nuclei-1 signaling enzyme function the expression levels of different cyclins which control cell cycle are decreased. It is possible that decrease of cyclin-dependent kinases-4 and cyclin D1 complex, which is responsible for blockade of the anti-proliferative function of RUNX3 and G1 cell cycle arrest despite constitutive expression of cyclin E2, leads to suppression of tumor growth [15, 17, 24, 25].In this study we have also shown that the exposure of cells to the medium without glutamine or glucose leads to a significant decrease in the expression level of cyclin-dependent kinase-2 as well as cyclin A2, D3 and E2 mRNAs both in control and endoplasmic reticulum–nuclei-1-deficient glioma cells. However, the expression level of pro-proliferative cyclin D1 is decreased and that of a negative cell-cycle regulator cyclin G2 is increased only in control glioma cells exposed in medium without glutamine or glucose. This phenomenon is probably due to suppression of cell proliferation rate via endoplasmic reticulum–nuclei-1 signaling system, because in genetically modified cells the expression level of cyclin D1 does not change significantly and that of cyclin G2 vice-versa is decreased in both ischemic conditions. Moreover, in this study we have shown that exposing cells to the medium without glutamine leads to a decrease in cyclin-dependent kinase-5 mRNA expression levels in the both investigated cell types. The expression levels of cyclin-dependent kinase-5 mRNA were also increased in both investigated cell types under glucose deprivation conditions. It is possible that these oppositely directed changes in the expression of cyclin-dependent kinase-5 in different ischemic conditions are a result of its unique properties [38, 41]. Cyclin-dependent kinase-5 is involved in the phosphorylation of p53 following the inhibition of protein phosphatase 2A, which is important for cell cycle arrest/apoptosis, inhibition of tumor progression, as well as, protein export from nucleus, and protein ubiquitination. Moreover, cyclin-dependent kinase-5 possibly participates in glucose metabolism, because cyclin-dependent kinase-5-mediated phosphorylation of PPARγ is involved in the pathogenesis of insulin-resistance [41]. These results provide, at least partly, an explanation for the oppositely directed changes in the expression of cyclin-dependent kinase-5 in different ischemic conditions: glutamine and glucose deprivation.

The major finding reported here is that the expression of most tested genes that encode cyclins and cyclin-dependent kinases are dependent on the function of endoplasmic reticulum–nuclei-1 signaling enzyme – both in normal and glutamine or glucose deprivation conditions and possibly they participate in cell adaptive response to endoplasmic reticulum stress associated with ischemia. However, the detailed molecular mechanisms of regulation of genes encoding cyclins and cyclin-dependent kinases by endoplasmic reticulum–nuclei-1 signaling system under ischemic stress conditions is complex and warrants further study.

Conclusions

Results of these investigations clearly demonstrate that the expression of different cyclin as well as cyclin-dependent kinase genes in glioma cells is regulated by glutamine and glucose deprivation and significantly depends on protein kinase and endoribonuclease activities of signaling enzyme of endoplasmic reticulum–nuclei-1 and, that it is quite possible, that they participate in cell adaptive response to endoplasmic reticulum stress associated with glutamine and glucose deprivation.

ВПЛИВ ВІДСУТНОСТІ ГЛЮТАМІНУ АБО ГЛЮКОЗИ У СЕРЕДОВИЩІ НА ЕКСПРЕСІЮ ЦИКЛІНІВ ТА ЦИКЛІНЗАЛЕЖНИХ КІНАЗ У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87 ТА ЇХНІЙ СУБЛІНІЇ ІЗ ПРИГНІЧЕНОЮ АКТИВНІСТЮ СИГНАЛЬНОГО ЕНЗИМУ ЕНДОПЛАЗМАТИЧНИЙ РЕТИКУЛУМ–ЯДРО-1

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Показано, що ішемія індукує комплекс складних внутрішньоклітинних сигнальних подій, відомих як реакція на неправильне згортання протеїнів, яка опосередковується сенсорним ензимом ендоплазматичний ретикулум–ядро-1. Ми вивчали експресію генів декількох циклінів та циклінзалежних кіназ, які беруть участь у контролі клітинного циклу та проліферації, за умов ішемії (відсутності у середовищі глюкози або глютаміну) в дефіцитних за сенсорним ензимом ендоплазматичний ретикулум–ядро-1 клітинах гліоми. Встановлено, що блокада сигнального ензиму ендоплазматичний ретикулум–ядро-1, ключового стрес-сенсора ендоплазматичного ретикулума, приводить до посилення рівня експресії генів циклінзалежної кінази-2 та циклінів A2, D3, E2 і G2, але пригнічує експресію циклiну D1. Було також показано, що рівень експресії мРНК циклінзалежної кінази 2 та циклінів A2, D3 і E2 суттєво зменшувався за відсутності у середовищі глюкози або глютаміну як у контрольних, так і в дефіцитних за ензимом ендоплазматичний ретикулум–ядро-1 клітинах гліоми. Разом з тим, в умовах відсутності у середовищі глюкози, експресія мРНК циклінзалежних кіназ-4 та -5 збільшується. Таким чином, експресія генів більшості досліджених циклінів та циклінзалежних кіназ залежить від функції сигнального ензиму ендоплазматичний ретикулум–ядро-1 як в умовах норми, так і за відсутності глютаміну та глюкози, і можливо бере участь у реакції адаптації клітин до стресу ендоплазматичного ретикулума, пов'язаного з ішемією.

Ключові слова: експресія мРНК, цикліни A2, D1, D3, E2 та G2, циклінзалежні кінази-2, -4 та -5, клітини гліоми, відсутність глюкози або глютаміну, ендоплазматичний ретикулум–ядро-1.

ВЛИЯНИЕ ОТСУТСТВИЯ ГЛЮТАМИНА ИЛИ ГЛЮКОЗЫ В СРЕДЕ НА ЭКСПРЕССИЮ ЦИКЛИНОВ И ЦИКЛИН-ЗАВИСИМЫХ КИНАЗ В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87 И ИХ СУБЛИНИИ С ПОДАВЛЕННОЙ АКТИВНОСТЬЮ СИГНАЛЬНОГО ЭНЗИМА ЭНДОПЛАЗМАТИЧЕСКИЙ РЕТИКУЛУМ–ЯДРО-1

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Показано, что ишемия индуцирует комплекс сложных внутриклеточных сигнальных событий, известных как реакция на неправильное сворачивание протеинов, которая опосредуется сенсорным энзимом эндоплазматический ретикулум–ядро-1. Мы исследовали экспрессию генов нескольких циклинов и циклинзависимых киназ, которые принимают участие в контроле клеточного цикла и пролиферации, в условиях ишемии (отсутствие в среде глюкозы или глютамина) в дефицитных по сенсорному энзиму эндоплазматический ретикулум–ядро-1 клетках глиомы. Установлено, что блокада сигнального энзима эндоплазматический ретикулум–ядро-1, ключевого стресс-сенсора эндоплазматического ретикулума, приводит к усилению уровня экспрессии генов циклинзависимой киназы-2 и циклинов A2, D3, E2 и G2, но угнетает экспрессию гена циклина D1. Было также показано, что уровень экспрессии мРНК циклинзависимой киназы-2 и циклинов A2, D3 и E2 существенно уменьшается при отсутствии в среде глюкозы или глютамина как у контрольных, так и у дефицитных по энзиму эндоплазматический ретикулум–ядро-1 клетках глиомы. Вместе с тем, при отсутствии в среде глюкозы экспрессия мРНК циклинзависимых

киназ-4 и -5 увеличивается. Таким образом, экспрессия большинства исследованных генов циклинов и циклинзависимых киназ зависит от функции сигнального энзима эндоплазматический ретикулум–ядро-1 как в нормальных условиях, так и в условиях отсутствия глютамина и глюкозы, и возможно принимает участие в реакции адаптации клеток к стрессу эндоплазматического ретикулума, связанного с ишемией.

Ключевые слова: экспрессия мРНК, циклины A2, D1, D3, E2 и G2, циклинзависимые киназы-2, -4 и -5, клетки глиомы, отсутствие глюкозы или глютамина в среде, эндоплазматический ретикулум–ядро-1.

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