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INHIBITION OF ERN1 SIGNALING ENZYME AFFECTS HYPOXIC REGULATION OF THE EXPRESSION OF *E2F8*, *EPAS1*, *HOXC6*, *ATF3*, *TBX3* AND *FOXF1* GENES IN U87 GLIOMA CELLS

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Hypoxia as well as the endoplasmic reticulum stress are important factors of malignant tumor growth and control of the expression of genes, which regulate numerous metabolic processes and cell proliferation. Furthermore, blockade of ERN1 (endoplasmic reticulum to nucleus 1) suppresses cell proliferation and tumor growth. We studied the effect of hypoxia on the expression of genes encoding the transcription factors such as E2F8 (E2F transcription factor 8), EPAS1 (endothelial PAS domain protein 1), TBX3 (T-box 3), ATF3 (activating transcription factor 3), FOXF1 (forkhead box F1), and HOXC6 (homeobox C6) in U87 glioma cells with and without ERN1 signaling enzyme function. We have established that hypoxia enhances the expression of HOXC6, E2F8, ATF3, and EPAS1 genes but does not change TBX3 and FOXF1 gene expression in glioma cells with ERN1 function. At the same time, the expression level of all studied genes is strongly decreased, except for TBX3 gene, in glioma cells without ERN1 function. Moreover, the inhibition of ERN1 signaling enzyme function significantly modifies the effect of hypoxia on the expression of these transcription factor genes: removes or introduces this regulation as well as changes a direction or magnitude of hypoxic regulation. Present study demonstrates that fine-tuning of the expression of proliferation related genes depends upon hypoxia and ERN1-mediated endoplasmic reticulum stress signaling and correlates with slower proliferation rate of glioma cells without ERN1 function.

K e y w o r d s: mRNA expression, hypoxia, endoplasmic reticulum stress, E2F8, EPAS1, HOXC6, ATF3, TBX3, FOXF1, ERN1 knockdown, glioma cells.

Malignant gliomas are highly aggressive tumors and are characterized by marked angiogenesis and extensive tumor cell invasion into the normal brain parenchyma. Moreover, hypoxia condition is associated to glioma development and locally induces an adaptive response which confers to tumor cells an enhanced survival and a more agressive behaviour. Hypoxia as well as the endoplasmic reticulum stress are important factors of malignant tumor growth and control of the expression of genes, which regulate numerous metabolic processes, cell proliferation and cancer growth [1–5]. The endoplasmic reticulum is a key organelle in the cellular response to different factors, which activate a complex set of signaling pathways named the unfolded protein response [1, 6]. 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 to Nucleus signaling 1) also known as IRE1alpha (Inositol Requiring Enzyme-1α) and ATF6 (Activating Transcription Factor 6), however, ERN1 is the dominant component of this system [1, 7-9]. The induction of endoplasmic reticulum stress is the early cell response to the accumulation of misfolded proteins in the lumen of the endoplasmic reticulum and tends to limit the de novo entry of proteins into the endoplasmic reticulum and facilitate both the endoplasmic reticulum protein folding and degradation to adapt cells for survival [10-12].

The ERN1 has two enzymatic activities: for serine/threonine kinase and endoribonuclease, which contribute to ERN1 signalling [1, 12]. The ERN1-

associated endoribonuclease is activated after autophosphorylation of this enzyme and is responsible for degradation of a specific subset of mRNA and also initiates the cytosolic splicing of the pre-XBP1 (X-box binding protein 1) mRNA whose mature transcript encodes a transcription factor that stimulates the expression of numerous unfolded protein response specific genes [8, 13-17].

At the same time, activation of ERN1 branch of the endoplasmic reticulum stress response is linked to apoptosis and to cell proliferation, and suppression of its function has been demonstrated to result in a significant decrease of tumor growth [18–20]. Thus, investigation of tumor responses to hypoxia is required for development of therapeutical strategies, based on the blockade of ERN1-mediated survival mechanisms [1, 18, 20-22].

Transcription factors responded to diverse cellular stresses to regulate expression of their target genes, thereby inducing cell cycle control, apoptosis and senescence [23-28]. E2F family of transcription factors regulates various cellular functions related to cell cycle and apoptosis and is strongly up-regulated in human hepatocellular carcinoma, thus possibly contributing to hepatocarcinogenesis [24]. Furthermore, there is data that the transcription factors E2F7 and E2F8 promote angiogenesis through transcriptional activation of vascular endothelial growth factor-A in cooperation with hypoxia inducible factor-1 [29]. Thus, E2F transcription factors, such as E2F8, are essential for orchestrating expression of genes required for cell cycle progression and proliferation [30].

The T-box transcription factor TBX3 is a transcriptional repressor and plays multiple roles in normal development and disease by either repressing or activating transcription of target genes in a context-dependent manner and control the rate of cell proliferation as well as mediate cellular signaling pathways [23]. On the other hand, overexpression of TBX3 is associated with several cancers, but it may mediate the anti-proliferative and pro-migratory role of TGF-β1 in breast epithelial and skin keratinocytes [31]. Recently it was shown that the knockdown of PLCε gene, which enhances bladder cancer cell invasion, is induced E-cadherin expression and decreased TBX3 expression, both of which were dependent on PKCα/β activity [32]. In addition, treatment of cells with TBX3-specific shorting hairpin RNA up-regulated E-cadherin expression and inhibited cell invasion/migration.

Transcription factor HOXC6 is a member of a highly conserved homeobox family of transcription factors that play an important role in proliferation as well as in morphogenesis and metastasis and regulate genes with both oncogenic and tumor suppressor activities and may contribute to the progression of gastric carcinogenesis [27, 33, 34]. Recently it was shown that the forkhead box transcription factor FOXF1 is a target of the TP53 family and its ectopic expression inhibits cancer cell invasion and migration, whereas the inactivation of FOXF1 stimulated both these processes [28, 35]. Furthermore, this transcription factor is a mesenchymal target of hedgehog signaling, known to regulate mesenchymal-epithelial interactions during lung development and contractility of fibroblasts, their production of hepatocyte growth factor and fibroblast growth factor-2 as well as their stimulation of lung cancer cell growth and migration [36].

Cyclic AMP-dependent activating transcription factor 3 binds the cAMP response element and represses transcription from promoters with ATF sites by stabilizing the binding of inhibitory cofactors at the promoter, but isoform 2 activates transcription presumably by sequestering inhibitory cofactors away from the promoters [25]. At the same time, ATF3 can suppress mutant TP53 oncogenic function, thereby contributing to tumor suppression in TP53-mutated cancer as well as promotes colon cancer metastasis and involved in the progress of laryngeal squamous cell carcinoma [37-39]. It was also found that ATF3 is strongly induced during necrosis but not apoptosis and knockdown of ATF3 by siRNA in the F28-7 cells resulted in apoptotic morphology rather than necrotic morphology [40]. These results suggest that ATF3 is also a cell-death regulator in necrosis and apoptosis.

Recently it was shown that knockdown of endothelial PAS domain protein 1, which is also known as hypoxia-inducible transcription factor-2α (HIF-2α), as well as HIF-1α in pulmonary vascular endothelial cells decreased cell proliferation under normoxic as well as hypoxic conditions and that HIF-2α and SOX9 regulate *TUBB3* gene expression and affect ovarian cancer aggressiveness [26, 41]. Moreover, the expression of *EPAS1* gene was significantly correlated with tumor size, invasion, and necrosis as well as with *VEGF* gene expression, which supported the correlation of EPAS1 up-regulation with tumor angiogenesis [42].

The aim of this study was to investigate the effects of hypoxia on the expression of genes encoded transcription factors E2F8, EPAS1, HOXC6, ATF3, TBX3, and FOXF1, which participate in the regulation of cell proliferation and apoptosis in glioma cells, and to study the contribution of endoplasmic reticulum stress sensor ERN1 through its inhibition to hypoxic regulation of their expression.

Materials and Methods

Cell Lines and Culture Conditions. The glioma cell line U87 (HTB-14) was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (DMEM; Gibco, Invitrogen, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco, USA) and streptomycin (0.1 mg/ml; Gibco) at 37 ºC in a 5% CO_2 incubator.

In this study we used two sublines of U87 glioma cell line. One subline was obtained by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dnERN1 (dominant-negative constructs of ERN1). This subline of glioma cells was used as control (control glioma cells) in the study of the effects of hypoxia as well as inhibition of ERN1 enzymatic activities on the expression level of transcription factor *E2F8*, *TBX3*, *EPAS1*, *ATF3*, *FOXF1* and *HOXC6* genes. Second subline was obtained by selection of stable transfected clones with overexpression of dnERN1 and has suppressed both protein kinase and endoribonuclease activities of this bifunctional signaling enzyme of endoplasmic reticulum stress [19, 20]. Previously it was shown that these cells have low proliferation rate and do not express XBP1 alternative splice variant, a key transcription factor in ERN1 signaling, after induction of endoplasmic reticulum stress by tunicamycin [21]. For creation of hypoxic conditions, the culture plates were exposed in a special incubator with 3% oxygen, 5% CO_2, and 92 % nitrogen mix for 16 h.

RNA isolation. Total RNA was extracted from glioma cells using Trizol reagent according to manufacturer protocols (Invitrogen, USA). The RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water. For additional purification RNA samples were re-precipitated with 95% ethanol, redissolved again in nuclease-free water and used for reverse transcription.

Reverse transcription and quantitative PCR analysis. QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis according to manufacturer protocol. The expression levels of E2F8, TBX3, EPAS1, ATF3, FOXF1, HOXC6 and ACTB mRNA were measured in U87 glioma cells by real-time quantitative polymerase chain reaction (qPCR) using Mx 3000P QPCR (Stratagene, USA) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, ABgene House, UK) or semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) using "MasterCycler Personal" (Eppendorf, Germany). Quantitative polymerase chain reaction was performed in triplicate using specific primers, which were received from Sigma-Aldrich (USA).

For amplification of HOXC6 (homeobox C6 transcription factor) cDNA we used next forward and reverse primers: 5′–AAAAGAGGAAAAGCGGGAAG–3′ and (5′–GGTCCACGTTTGACTCCCTA–3′, correspondingly, for real time RCR as well as RT-PCR analysis. The nucleotide sequences of these primers correspond to sequences 772–791 and 963–944 of human HOXC6 cDNA (GenBank accession number NM_004503). The size of amplified fragment is 192 bp.

The amplification of ATF3 (activating transcription factor 3), also known as cyclic AMP-dependent transcription factor ATF-3, cDNA was performed using forward primer (5′–CAAGTGCATCTTTGCCTCAA–3′) and reverse primer (5′–CCACCCGAGGTACAGACACT–3′). These oligonucleotides correspond to sequences 1024–1043 and 1190–1171 of human ATF3 cDNA (GenBank accession number NM_004024). The size of amplified fragment is 167 bp.

The amplification of transcription factor EPAS1 (endothelial PAS domain protein 1), also known as hypoxia-inducible factor 2α (HIF-2α), cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5′–AAGCCTTGGAGGGTTTCATT–3′ and reverse – 5′–TCATGAAGAAGTCCCGCTCT–3′. The nucleotide sequences of these primers correspond to sequences 788–807 and 1021–1002 of human EPAS1 cDNA (GenBank accession number NM_001430). The size of amplified fragment is 234 bp.

For amplification of E2F8 (E2F transcription factor 8) cDNA we used next forward and reverse primers: 5′–CCACCACAGCAAATATCGTG–3′

and 5′–CTTTGGCCTCAGGTAATCCA–3′, correspondingly. The nucleotide sequences of these primers correspond to sequences 596–615 and 805–786 of human E2F8 cDNA (GenBank accession number NM_024680). The size of amplified fragment is 210 bp.

The amplification of FOXF1 (forkhead box F1 transcription factor) cDNA for real time RCR and RT-PCR analysis was performed using two oligonucleotides primers: forward – 5′–AAGCCGCCCTATTCCTACAT–3′ and reverse – 5′–TGATGAAGCACTCGTTGAGC–3′. The nucleotide sequences of these primers correspond to sequences 185–204 and 365–346 of human FOXF1 cDNA (GenBank accession number NM_001451). The size of amplified fragment is 181 bp.

For amplification of TBX3 (T-box 3 transcriptional repressor) cDNA we used forward (5′–ACTGGGGAACAGTGGATGTC–3′ and reverse (5′–TTCGGGGAACAAGTATGTCC–3′) primers. The nucleotide sequences of these primers correspond to sequences 1551–1570 and 1729–1710 of human TBX3 cDNA (GenBank accession number NM_005996). The size of amplified fragment is 179 bp.

The amplification of β-actin (ACTB) cDNA was performed using forward – 5′–GGACTTCGAGCAAGAGATGG–3′ and reverse – 5′–AGCACTGTGTTGGCGTACAG–3′ primers. These primer nucletide sequences correspond to 747–766 and 980–961 of human ACTB cDNA (GenBank accession number NM_001101). The size of amplified fragment is 234 bp. The expression of β-actin mRNA was used as control of analyzed RNA quantity.

An analysis of quantitative PCR was performed using special computer program Differential Expression Calculator. The values of the expression of E2F8, TBX3, EPAS1, ATF3, FOXF1, HOXC6, and ACTB mRNA were normalized to β-actin mRNA expressions and represent as percent of control (100%).

Western blot analysis. E2F8 protein in hypoxia-treated U87 glioma cells was measured by Western blot analysis using polyclonal anti-E2F8 antibody (H00079733-M01) from NOVUS Biologicals. ACTB (β-actin) from Santa Cruz Biotechnology was used as control of analyzed protein quantity in extracts of glioma cells. Western blot analysis was performed as described previously [34].

Statistical analysis. Statistical analysis was performed according to Student's test using Origin-Pro 7.5 software. All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments.

Results and Discussion

To test the effect of hypoxia on the expression levels of transcription factor genes, strongly related to control of cell proliferation, we used the U87 glioma cell subline, which constitutively expresses dominant-negative mutant of ERN1 and has an inhibitory effect on ribonuclease and kinase activity of endogenous ERN1 [21, 44]. Figure 1, *A* and *B* demonstrates that hypoxia significantly increased the expression of HOXC6 gene in control U87 glioma cells measured by RT-PCR as well as quantitative PCR analysis. It was also shown that the level of HOXC6 mRNA is significantly decreased (close to 2-fold) in glioma cells expressing dnERN1. Moreover, inhibition of ERN1 removes hypoxic regulation of the expression of this transcription factor gene (Fig. 1).

We next tested whether hypoxia also participates in regulation of activating transcription factor 3 gene expression in relation of ERN1-mediated endoplasmic reticulum stress signaling using glioma cells with and without enzymatic activities of ERN1. We have found that hypoxia strongly induces this gene expression (close to 2.4-fold) in control glioma cells and that blockade of ERN1 signaling enzyme function leads to more robust (4-fold) induction of *ATF3* gene expression (Fig. 2). At the same time, the expression levels of *ATF3*, that regulates transcription of numerous proliferation and apoptosis related genes, are significantly decreased (9-fold) in glioma cells stably transfected with dnERN1 (Fig. 2).

We have also found that the mRNA level of transcription factor EPAS1 is up-regulated by hypoxia both in control glioma cells and cells overexpressed dnERN1, being more profound in cells harboring dnERN1 (without protein kinase and endoribonuclease of ERN1): +20% and + 83%, correspondingly (Fig. 3). Moreover, results presented in this figure demonstrate that the expression level of EPAS1 mRNA is also significantly less (20-fold) in glioma cells with suppressed function of ERN1 signaling enzyme as compared to control glioma cells.

As shown in Fig. 4, *A*, hypoxia significantly (close to three fold) decreases the expression level of E2F transcription factor 8 (E2F8) mRNA in control glioma cells. Moreover, we observed similar effect

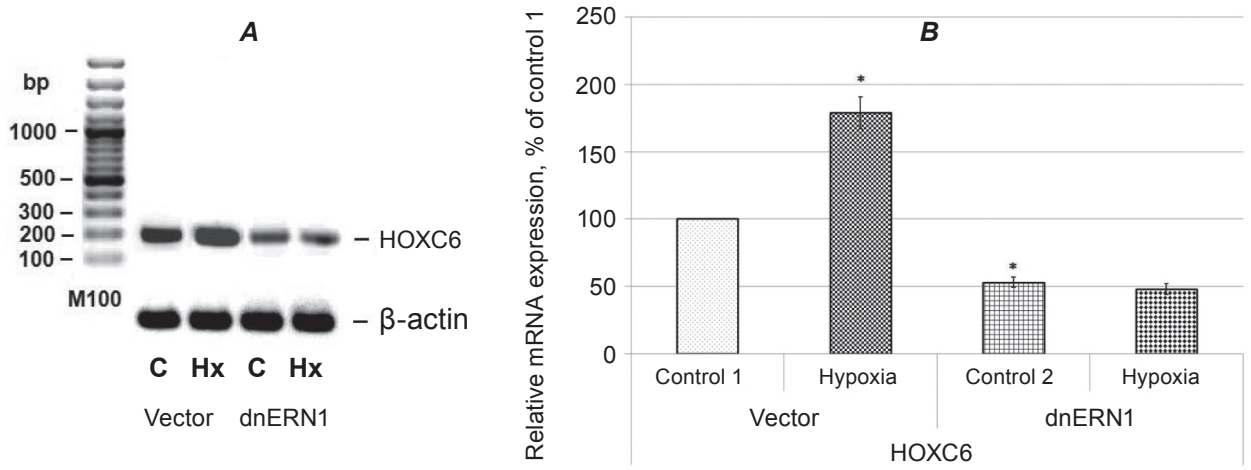

*Fig. 1. Effect of hypoxia on the expression of HOXC6 (homeobox C6 transcription factor) mRNA in glioma cell line U87, stable transfected by vector (Vector), and its sublines with a deficiency of both protein kinase and endoribonuclease of the signaling enzyme ERN1, stable transfected by dominant-negative ERN1 (dnERN1) measured by RT-PCR (A) and qPCR (B). Values of HOXC6 mRNA expressions were normalized to β-actin mRNA expression and represented as percent of control (vector, 100%); mean ± SEM; n = 4; * P < 0.05 as compared to control 1 (vector); C – control; Hx – hypoxia*

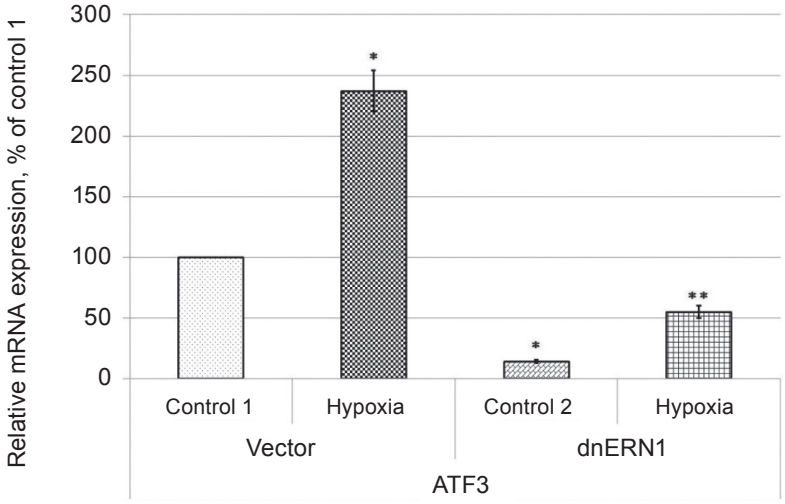

*Fig. 2. Effect of hypoxia on the expression of ATF3 (activating transcription factor mRNA (qPCR) in glioma cell line U87 (Vector) and its sublines with a deficiency of both protein kinase and endoribonuclease of the signaling enzyme ERN1 (dnERN1). Values of ATF3 mRNA expressions were normalized to β-actin mRNA expression and represented as percent of control (vector, 100 %); mean ± SEM; n = 4; * P < 0.05 as compared to control 1 (vector); ** P < 0.05 as compared to control 2 (dnERN1)*

of hypoxia on E2F8 transcription factor at protein level in these glioma cells (Fig. 4, *B*). At the same time, effect of hypoxia on the expression of this transcription factor in glioma cells harboring dnERN1 is significantly less to show that ERN1 blockade modulates the hypoxic regulation of the expression of E2F8 gene (Fig. 4, *A*). Moreover, results presented in this figure clearly demonstrate that the expression level of E2F8 mRNA is strongly suppressed (more than 16-fold) in glioma cells with suppressed function of ERN1 signaling enzyme as compared to control glioma cells and that changes in E2F8 protein level correlates with that of mRNA (Fig. 4, *A*, *B*).

We also analyzed the expression of transcription factor forkhead box F1 (FOXF1), another transcription factor, which controls the expression of

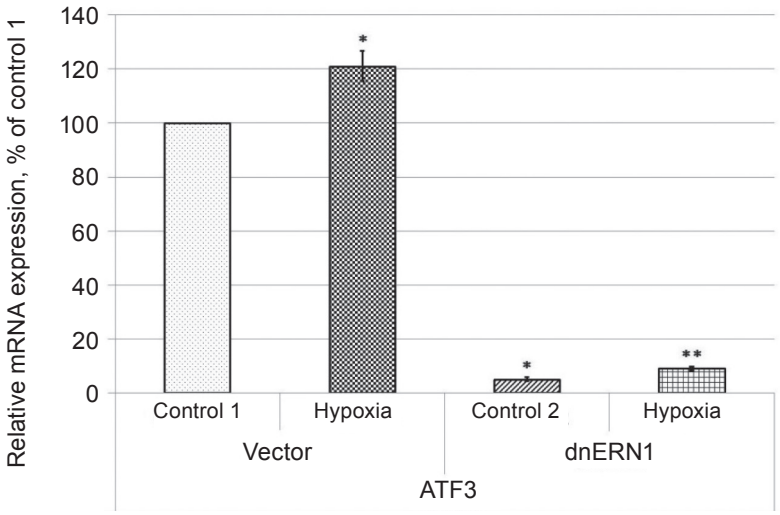

*Fig. 3. Effect of hypoxia on the expression of EPAS1 (endothelial PAS domain protein 1), also known as hypoxia-inducible factor-2α, mRNA (qPCR) in glioma cell line U87 (Vector) and its sublines with a deficiency of both protein kinase and endoribonuclease of the signaling enzyme ERN1 (dnERN1). Values of EPAS1 mRNA expressions were normalized to β-actin mRNA expression and represented as percent of control (vector, 100%); mean ± SEM; n = 4; * P < 0.05 as compared to control 1 (vector); ** P < 0.05 as compared to control 2 (dnERN1)*

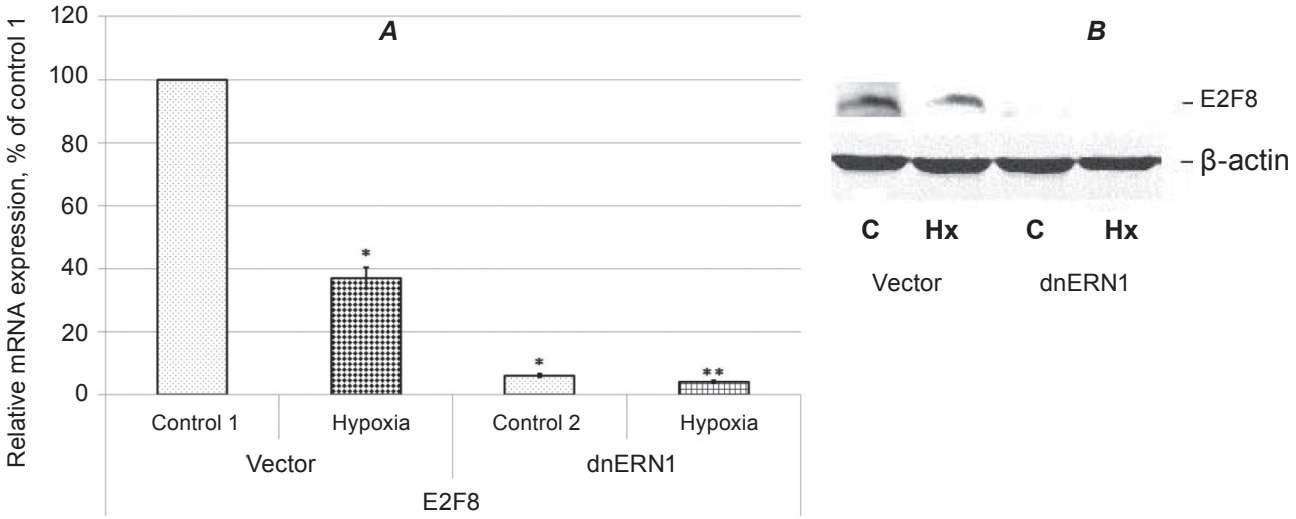

*Fig. 4. Effect of hypoxia on the expression of E2F8 (E2F transcription factor 8) mRNA in glioma cell line U87 (Vector) and its sublines with a deficiency of both protein kinase and endoribonuclease of the signaling enzyme ERN1 (dnERN1) measured by qPCR (A) and Western blot analysis of E2F8 protein (B). Values of E2F8 mRNA expressions were normalized to β-actin mRNA expression and represented as percent of control (vector, 100 %); mean ± SEM; n = 4; * P < 0.05 as compared to control 1 (vector); ** P < 0.05 as compared to control 2 (dnERN1); C – control; Hx – hypoxia*

some growth factors as well as tumor growth, in glioma cells upon hypoxia. Results presented in Fig. 5, *A*, *B*, clearly demonstrated that hypoxia does not change significantly mRNA level of transcription factor FOXF1 in control glioma cells and decreases (two-fold) in glioma cells harboring dnERN1 to show that hypoxic regulation of FOXF1 mRNA expression is controlled by ERN1-mediated endoplasmic reticulum stress signaling. It is interesting to note that mRNA level of this transcription fac-

tor is significantly decreased (more than two-fold) in control glioma cells with suppressed function of ERN1 signaling enzyme (Fig. 5).

Transcription factor TBX3 (T-box 3) is a transcription repressor and expression level of its mRNA is strongly induced in glioma cells after blockade of signaling enzyme ERN1 function (close to 2.6-fold); however, hypoxia does not affect the expression level of this gene in control glioma cells (Fig. 6). At the same time, the inhibition of signaling enzyme ERN1 in U87 glioma cells modifies the responsibility of these cells to hypoxic regulation of the expression of transcription repressor TBX3.

In this study we have shown that hypoxia increases the expression level of gene encoded transcription factors HOXC6, ATF3, and EPAS1 in control glioma cells (Fig. 1-3) and that blockade of ERN1 signaling enzyme function strongly decreases the level of these gene expressions as well as modifies its expression level. Transcription factor HOXC6 predominantly has pro-proliferative functions, plays an important role in proliferation as well as in morphogenesis and metastasis and its up-regulation upon hypoxia may contribute to tumor growth [27, 34]. At the same time, blockade of ERN1 signaling enzyme function decreases *HOXC6* gene expression as well as removes its hypoxic regulation (Fig. 1). These results completely correlate with data that ERN1 knockdown suppresses proliferation rate of these cells as well as glioma growth from these cells [19, 21]. Thus, transcription factor HOXC6 may contribute to suppression of proliferation rate of glioma cells without ERN1 signaling enzyme function. Moreover, the decreased expression level of *ATF3* and *EPAS1* genes in ERN1 knockdown glioma cells may also contribute to suppression of proliferation, because these transcription factors play an important role in the control of tumor growth [25, 26, 37-39, 42]. At the same time, blockade of ERN1 signaling enzyme function enhances the hypoxic regulation of *ATF3* and *EPAS1* gene expressions, but prospective studies are still needed to clarify the significance of these results.

We have also shown that hypoxia affects the expression of *E2F8* gene in control and ERN1 knockdown glioma cells at mRNA and protein levels and that blockade of ERN1 strongly decreases this gene expression as well as a magnitude of hypoxic regulation (Fig. 4). Thus, strong down-regulation of E2F8 in glioma cells is argued with anti-proliferative effect of ERN1 knockdown, because there is data that the transcription factor E3F8 plays an important role in various cellular functions related to cell cycle and apoptosis and is strongly up-regulated in human hepatocellular carcinoma [24]. It is possible that down-regulation of the expression of *E2F8* gene upon hypoxia plays a specific role in orchestrating expression of genes required for hypoxic adaptation

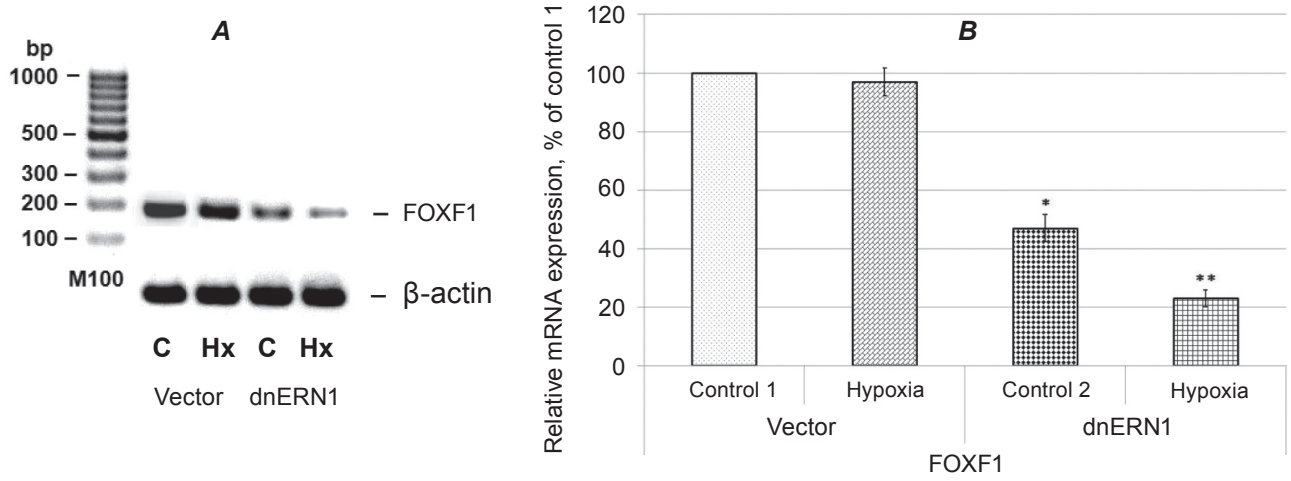

*Fig. 5. Effect of hypoxia on the expression of FOXF1 (forkhead box F1 transcription factor) mRNA (qPCR) in glioma cell line U87 (Vector) and its sublines with a deficiency of both protein kinase and endoribonuclease of the signaling enzyme ERN1 (dnERN1) by RT-PCR (A) and qPCR (B). Values of FOXF1 mRNA expressions were normalized to β-actin mRNA expression and represented as percent of control (vector, 100%); C – control; mean ± SEM; n = 4; * P < 0.05 as compared to control 1 (vector); ** P < 0.05 as compared to control 2 (dnERN1)*

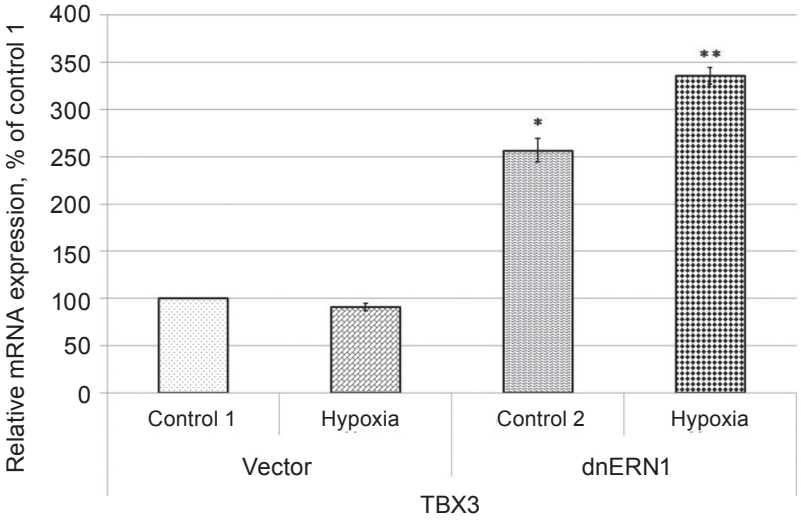

*Fig. 6. Effect of hypoxia on the expression of TBX3 (T-box 3 transcriptional repressor) mRNA (qPCR) in glioma cell line U87 (Vector) and its sublines with a deficiency of both protein kinase and endoribonuclease of the signaling enzyme ERN1 (dnERN1). Values of TBX3 mRNA expressions were normalized to β-actin mRNA expression and represented as percent of control (vector, 100%); mean ± SEM; n = 4; * P < 0.05 as compared to control 1 (vector); ** P < 0.05 as compared to control 2 (dnERN1)*

and is dependent on ERN1 signaling enzyme function.

We have also demonstrated that hypoxia does not change the expression of T-box transcription factor TBX3 and FOXF1 in control glioma cells, but the inhibition of ERN1 signaling enzyme introduces hypoxic regulation of these genes (Fig. 5 and 6). It is possible that the expression of *TBX3* and *FOXF1* genes is controlled by ERN1 signaling. Moreover, we have found that blockade of ERN1 in glioma cells down-regulates pro-proliferative *FOXF1* gene expression and up-regulates the expression of transcriptional repressor TBX3. These results correlate with data that ERN1 knockdown suppresses cell proliferation as well as glioma growth from these cells [19, 21]. Thus, the induction of *TBX3* gene expression may contribute to the suppression of cell proliferation and glioma growth from these cells, because TBX3 is a transcriptional repressor, which controls cell proliferation as well as mediates cellular signaling pathways [23, 31]. It is interesting to note that the transcription factor TBX3 has pleiotropic functions and plays multiple roles in normal development and disease by either repressing or activating transcription of target genes in a context-dependent manner, and it may mediate the antiproliferative role of TGFB1 [31]. Thus, the increased expression of TBX3 can mediate the inhibition of cell proliferation upon ERN1 inhibition.

In conclusion, the inhibition of ERN1 endoribonuclease affects growth regulation, lowering expression levels of transcription factors E2F8, HOXC6, EPAS1, and FOXF1, which have pro-proliferative properties, and up-regulate the expression of transcriptional repressor TBX3 to the level of these transcription factors in normal human astrocytes. Thus, the changes in studied transcription factor gene expressions correlate well with slower cell proliferation in cells harboring dnR-ERN1 (without endoribonuclease activity of ERN1), because endoplasmic reticulum stress is a necessary component of malignant tumor growth and cell survival [2, 3, 6, 11].

In conclusion, we have demonstrated that hypoxia up-regulates the expression level of transcription factors HOXC6, ATF3, and EPAS1 and down-regulates E2F8, but has no effect on the expression level of FOXF1 and TBX3 and that ERN1-mediated endoplasmic reticulum stress signaling participates in fine-tuning of mRNA levels as well as hypoxic regulation of the subset of transcription factor genes important for the control of cell proliferation as well as tumor growth, because endoplasmic reticulum stress as well as hypoxia is a necessary component of malignant tumor growth.

БЛОКАДА СИГНАЛЬНОГО ЕНЗИМУ ERN1 ЗМІНЮЄ ГІПОКСИЧНУ РЕГУЛЯЦІЮ ЕКСПРЕСІЇ ГЕНІВ *E2F8*, *EPAS1*, *HOXC6*, *ATF3*, *TBX3* ТА *FOXF1* У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87

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Гіпоксія, як і стрес ендоплазматичного ретикулума, є важливими факторами росту злоякісних пухлин і контролю експресії генів, що регулюють численні метаболічні процеси та проліферацію клітин. Більше того, блокада ERN1 (сигналювання від ендоплазматичного ретикулума до ядра 1) призводить до пригнічення проліферації клітин та росту пухлин. Ми вивчали ефект гіпоксії на експресію генів, що кодують транскрипційні фактори, такі як E2F8 (E2F transcription factor 8), EPAS1 (endothelial PAS domain protein 1), TBX3 (T-box 3), ATF3 (activating transcription factor 3), FOXF1 (forkhead box F1), і HOXC6 (homeobox C6) у клітинах гліоми лінії U87 з нормальною та пригніченою функцією ERN1. Встановлено, що гіпоксія посилює експресію генів *HOXC6*, *E2F8*, *ATF3* та *EPAS1*, але не змінює експресію генів *TBX3* і *FOXF1* у клітинах гліоми з нормальною функцією ERN1. У той же час у клітинах гліоми із пригніченою функцією ERN1 рівень експресії всіх досліджених генів, за винятком гена *TBX3*, істотно знижується. Більше того, пригнічення функції сигнального ензиму ERN1 модифікує ефект гіпоксії на експресію генів цих транскрипційних факторів: знімає або індукує цю регуляцію, а також впливає на напрямок та величину ефекту гіпоксії. Таким чином, у цій роботі показано, що точно відрегульована експресія генів, які контролюють процеси проліферації, залежить від гіпоксії та стресу ендоплазматичного ретикулума, опосередкованого ERN1-сигналюванням, і що одержані результати корелюють зі зниженою проліферацією клітин гліоми із пригніченою функцією ERN1.

Ключові слова: експресія мРНК, гіпоксія, стрес ендоплазматичного ретикулума, *E2F8*, *EPAS1*, *HOXC6*, *ATF3*, *TBX3*, *FOXF1*, виключення функції ERN1, клітини гліоми.

БЛОКАДА СИГНАЛЬНОГО ЭНЗИМА ERN1 ИЗМЕНЯЕТ РЕГУЛЯЦИЮ ГИПОКСИЕЙ ЭКСПРЕССИИ ГЕНОВ *E2F8*, *EPAS1*, *HOXC6*, *ATF3*, *TBX3* И *FOXF1* В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87

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Гипоксия, как и стресс эндоплазматического ретикулума, являются важными факторами роста злокачественных опухолей и контроля экспрессии генов, которые регулируют многочисленные метаболические процессы и пролиферацию клеток. Более того, блокада ERN1 (сигналинг от эндоплазматического ретикулума до ядра 1) приводит к угнетению пролиферации клеток и роста опухолей. Мы изучали эффект гипоксии на экспрессию генов, которые кодируют транскрипционные факторы, такие как E2F8 (E2F transcription factor 8), EPAS1 (endothelial PAS domain protein 1), TBX3 (T-box 3), ATF3 (activating transcription factor 3), FOXF1 (forkhead box F1), и HOXC6 (homeobox C6) в клетках глиомы линии U87 с нормальной и угнетенной функцией ERN1. Установлено, что гипоксия усиливает экспрессию генов *HOXC6*, *E2F8*, *ATF3* и EPAS1, но не изменяет экспрессию генов *TBX3* и *FOXF1* в клетках глиомы с нормальной функцией ERN1. В то же время, в клетках глиомы с угнетенной функцией ERN1 уровень экспрессии всех исследованных генов, за исключением гена *TBX3*, существенно снижается. Более того, угнетение функции сигнального энзима ERN1 моди-

фицирует эффект гипоксии на экспрессию генов этих транскрипционных факторов: снимает или индуцирует эту регуляцию, а также влияет на направление и величину эффекта гипоксии. Таким образом, в этой работе показано, что точно отрегулированная экспрессия генов, которые контролируют процессы пролиферации, зависит от гипоксии и стресса эндоплазматического ретикулума, опосредованного ERN1-сигналингом, и что полученные результаты коррелируют со сниженной пролиферацией клеток глиомы с угнетенной функцией ERN1.

Ключевые слова: экспрессия мРНК, гипоксия, стресс ендоплазматического ретикулума, *E2F8*, *EPAS1*, *HOXC6*, *ATF3*, *TBX3*, *FOXF1*, выключение функции ERN1, клетки глиомы.

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