

HYPOXIC REGULATION OF THE EXPRESSION OF CELL PROLIFERATION RELATED GENES IN U87 GLIOMA CELLS UPON INHIBITION OF IRE1 SIGNALING ENZYME

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We have studied the effect of inhibition of IRE1 (inositol requiring enzyme 1), which is a central mediator of endoplasmic reticulum stress and a controller of cell proliferation and tumor growth, on hypoxic regulation of the expression of different proliferation related genes in U87 glioma cells. It was shown that hypoxia leads to up-regulation of the expression of IL13RA2, CD24, INGI, ING2, ENDOG, and POLG genes and to down-regulation – of KRT18, TRAPPC3, TSFM, and MTIF2 genes at the mRNA level in control glioma cells. Changes for INGI and CD24 genes were more significant. At the same time, inhibition of IRE1 modifies the effect of hypoxia on the expression of all studied genes. In particular, it increases sensitivity to hypoxia of the expression of IL13RA2, TRAPPC3, ENDOG, and PLOG genes and suppresses the effect of hypoxia on the expression of INGI gene. Additionally, it eliminates hypoxic regulation of KRT18, CD24, ING2, TSFM, and MTIF2 genes expressions and introduces sensitivity to hypoxia of the expression of BET1 gene in glioma cells. The present study demonstrates that hypoxia, which often contributes to tumor growth, affects the expression of almost all studied genes. Additionally, inhibition of IRE1 can both enhance and suppress the hypoxic regulation of these gene expressions in a gene specific manner and thus possibly contributes to slower glioma growth, but several aspects of this regulation must be further clarified.

Key words: mRNA expression, endoplasmic reticulum stress, IL13RA2, KRT18, CD24, INGI, ING2, MYL9, BET1, TRAPPC3, ENDOG, POLG, TSFM, MTIF2, IRE1 inhibition, hypoxia, glioma cells.

The IL13RA2 (interleukin 13 receptor, α 2), CD24 (signal transducer CD24 molecule), INGI (inhibitor of growth family, member 1), ING2, ENDOG (endonuclease G) and many others play an important role in the regulation of numerous metabolic and proliferative processes [1-5]. It is well known that these genes play important roles in control of tumorigenesis [3, 5-7]. The *IL13RA2* gene is often overexpressed in glioma and other tumors [1, 3]. CD24 encodes a sialoglycoprotein that modulates growth and differentiation signals [4]. The *ING1* and *ING2* genes encode tumor suppressor proteins that can induce cell growth arrest and apoptosis [5, 7]. The *ING1* is a nuclear protein that physically interacts with the tumor suppressor protein TP53 and is a component of its signaling pathway. Reduced expression and rearrangement of this gene have been detected in various cancers [8, 9]. Moreover, INGI stabilizes p53 by inhibiting polyubiquitination [10].

KRT18 (keratin 18) contributes to decreased malignancy of non-small cell lung carcinoma and is directly regulated by EGR1 [11]. There is data that the decreased expression of MYL9 (myosin, light chain 9, regulatory) may play an important role in tumor progression of prostate cancer [6]. BET1 (Bet1 Golgi vesicular membrane trafficking protein) and TRAPPC3 (trafficking protein particle complex 3) participate in vesicular transport from the endoplasmic reticulum to the Golgi complex and are also possibly involved in the endoplasmic reticulum stress responsible transport of unfolded proteins [12]. The *ENDOG*, *POLG* (DNA directed polymerase gamma), *TSFM* (Ts mitochondrial translational elongation factor), and *MTIF2* (mitochondrial translational initiation factor 2) genes encode mitochondrial proteins, which are related to the control of mitochondrial genome function as well as to cell proliferation [13-17]. Moreover, ENDOG regulates an integral

network of apoptotic endonucleases, which appear to act simultaneously before and after cell death by destroying the host cell DNA [13,18].

Tumor growth is tightly associated with the endoplasmic reticulum stress response-signalling pathway and hypoxia, which are linked to the neo-vascularization and cell death processes [19-23]. The endoplasmic reticulum has an essential position as a signal integrator in the cell and is instrumental in the different phases of tumor progression because the signaling pathways elicited by endoplasmic reticulum stress sensors are connected to metabolic pathways and to other plasma membrane receptor signaling networks [24-26]. Multiple studies have clarified the link between cancer and endoplasmic reticulum stress, which controls different processes including cell proliferation and surviving as well as circadian rhythms [20, 21, 27, 28]. The IRE1 (inositol requiring enzyme 1) is a central mediator of the unfolded protein response and its inhibition leads to suppression of tumor growth through down-regulation of the angiogenesis and proliferation processes [29-31]. It contributes to changes in the expression profile of many regulatory genes resulting in proliferation, angiogenesis, and apoptosis [30, 31, 42]. The endoribonuclease of IRE1 is responsible for degradation of a specific subset of mRNAs and splicing of the XBP1 (X-box binding protein 1) transcription factor mRNA for control of the expression of unfolded protein response-specific genes [32-34].

Ablation of IRE1 function results in a significant anti-proliferative effect in glioma growth through down-regulation of key pro-angiogenic and pro-proliferative factors and up-regulation of tumor suppressor genes as well as through modification of hypoxic regulation of these genes [30-31, 36-38]. However, the executive mechanisms of the exhibited anti-proliferative effects of IRE1 inhibition are not yet known. It is possible that this anti-proliferative effect is also mediated by *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYRL2*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, and *MTIF2* proteins, which are integrated into the unfolded protein response signaling pathways and regulate cell proliferation [3-6, 9, 11, 15]. Possible involvement of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYRL2*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, and *MTIF2* genes was made evidently pertinent through transcriptomic analysis of U87 glioma cells expressing the dominant-negative mutant of IRE1 [30].

The main goal of this study was to investigate the expression levels of proliferation related genes

(*IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYRL2*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, and *MTIF2*) in U87 glioma cell line and its subline without signaling enzyme IRE1 function under hypoxic conditions for evaluation of their possible significance for the control of glioma cell proliferation through IRE1 mediated endoplasmic reticulum stress signaling.

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 °C in a 5% CO₂ incubator.

In this study we used sublines of U87 glioma cells, which were described previously [9, 40, 41, 48]. One subline was obtained by selection of stable transfected clones with overexpression of vector pcDNA3.1, which was used for creation of dnIRE1 (dominant/negative IRE1). This untreated subline of glioma cells (control glioma cells) was used in the study of the effect of hypoxia on the expression level of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *BET3/TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, and *MTIF2* genes. Second subline was obtained by selection of stable transfected clone with overexpression of IRE1 dominant/negative construct (dnIRE1) and consequent inhibition of both protein kinase and endoribonuclease activities of this signaling enzyme (clone 1C5) [40]. The expression levels of studied genes in these two sublines of glioma cells were compared with corresponding levels in cells, transfected by vector or by dnIRE1. The efficiency of IRE1 suppression in this glioma cell subline was estimated previously [30, 31] by determining the expression level of spliced XBP1, a key transcription factor in the IRE1 signaling, and the level of the phosphorylated IRE1 isoform in cells treated by tunicamycin (0.01 mg/ml during 2 h). Both sublines of glioma cells used in this study were grown with addition of geneticin (G418) while these cells carry an empty pcDNA3.1 vector or dnIRE1 construct.

To model hypoxia the culture plates were incubated in special chamber with 3% oxygen, 92% nitrogen, and 5% carbon dioxide for 16 h.

RNA isolation. Total RNA was extracted from glioma cells using Trizol reagent according to man-

ufacturer protocol (Invitrogen, USA) as described previously [31]. The RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water. For additional purification, RNA samples were re-precipitated with 95% ethanol and re-dissolved again in nuclease-free water. RNA concentration and spectral characteristics were measured using NanoDrop spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. QuantiTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis according to manufacturer protocol. The expression levels of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYRL2*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM* and *MTIF2* mRNAs as well as *ACTB* mRNA were measured in U87 glioma cells by real-time quantitative polymerase chain reaction using Mx 3000P QPCR (Stratagene, USA) or „RotorGene RG-3000” qPCR (Corbett Research, Germany) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, ABgene House, UK). Polymerase chain reaction was performed in triplicate using specific primers, received from Sigma-Aldrich, USA.

For amplification of *IL13RA2* (interleukin 13 receptor, $\alpha 2$) cDNA we used forward (5'-TCTTG-GAAACCTGGCATAGG-3' and reverse (5'-TCT-GATGCCTCCAAATAGGG-3') primers. The nucleotide sequences of these primers correspond to sequences 591–610 and 742–723 of human *IL13RA2* cDNA (NM_000640). The size of amplified fragment is 152 bp. The amplification of *KRT18* (keratin 18, type I) cDNA was performed using two oligonucleotides primers: forward – 5'-CACAGTCTGCT-GAGGTTGGA-3' and reverse – 5'-GAGCTGCTC-CATCTGTAGGG-3'. The nucleotide sequences of these primers correspond to sequences 966–985 and 1129–1110 of human *KRT18* cDNA (NM_000224). The size of amplified fragment is 164 bp.

The amplification of *CD24* (CD24 molecule; Signal Transducer CD24) cDNA was performed using two oligonucleotides primers: forward – 5'-AACTAATGCCACCACCAAGG-3' and reverse – 5'-CCTGTTTTTCTTGCCACAT-3'. The nucleotide sequences of these primers correspond to sequences 590–609 and 777–758 of human *CD24* cDNA (NM_013230). The size of amplified fragment is 169 bp. For amplification of *MYL9* (myosin, light chain 9, regulatory), also known as *MYRL2* (myosin regulatory light chain 2) cDNA we used forward (5'-ACCCACAGACGAATACCTG-3' and reverse

(5'-CCGGTACATCTCGTCCACTT-3') primers. The nucleotide sequences of these primers correspond to sequences 285–304 and 526–507 of human *MYL9* cDNA (NM_006097). The size of amplified fragment is 242 bp.

For amplification of *ING1* (inhibitor of growth family, member 1) cDNA we used forward (5'-CCAAGGGCAAGTGGTACTGT-3' and reverse (5'-CTGCCATCCCTATGAAAGGA-3') primers. The nucleotide sequences of these primers correspond to sequences 1601–1620 and 1845–1826 of human *ING1* cDNA (NM_005537). The size of amplified fragment is 245 bp. The amplification of *ING2* (inhibitor of growth family, member 2) cDNA was performed using two oligonucleotides primers: forward – 5'-ACGTCTACAGCAGCTTCTCC-3' and reverse – 5'-TGCGGGGTCTTCTTGAAGAT-3'. The nucleotide sequences of these primers correspond to sequences 369–388 and 589–570 of human *ING2* cDNA (NM_001564). The size of amplified fragment is 221 bp.

For amplification of *BET1* (Bet1 Golgi vesicular membrane trafficking protein) cDNA we used forward (5'-AGAAGTTGGTGTTCGCTGG-3' and reverse (5'-AGTCCCATAGTTGCCAGGA-3') primers. The nucleotide sequences of these primers correspond to sequences 49–68 and 214–195 of human *BET1* cDNA (NM_005868). The size of amplified fragment is 166 bp. The amplification of *TRAPPC3* (trafficking protein particle complex 3), also known as *BET3*, cDNA was performed using two oligonucleotides primers: forward – 5'-GGCAC-CGAGAGCAAGAAAAT-3' and reverse – 5'-CCAACATTTGACCGAGCCAA-3'. The nucleotide sequences of these primers correspond to sequences 158–177 and 333–314 of human *TRAPPC3* cDNA (NM_014408). The size of amplified fragment is 176 bp.

The amplification of *ENDOG* (endo-nuclease G) cDNA was performed using two oligonucleotides primers: forward – 5'-GTTCTACCTGAGCAACGTCG-3' and reverse – 5'-TGCCGATGACCTGGTACTTT-3'. The nucleotide sequences of these primers correspond to sequences 659–678 and 843–824 of human *ENDOG* cDNA (NM_004435). The size of amplified fragment is 185 bp. For amplification of *POLG* (DNA directed polymerase gamma) cDNA we used forward (5'-GATCTGGCCAATGATGCCTG-3' and reverse (5'-AAACTCCTCCTCCTCACTGC-3') primers. The nucleotide sequences of these primers

correspond to sequences 1675–1694 and 1899–1880 of human *POLG* cDNA (NM_002693). The size of amplified fragment is 225 bp.

The amplification of TSFM (Ts Mitochondrial Translational Elongation Factor) cDNA was performed using two oligonucleotides primers: forward – 5'–AAACCTTGAAGACGTTGGCC–3' and reverse – 5'–CTGCCTCTTCACCTTCTCCA–3'. The nucleotide sequences of these primers correspond to sequences 798–817 and 1021–1002 of human *TSFM* cDNA (NM_005726). The size of amplified fragment is 224 bp. For amplification of *MTIF2* (mitochondrial translational initiation factor 2) cDNA we used forward (5'–TGTGGAAGAGACCCAGTAG–3') and reverse (5'–AGACCACAATCCATTCCCGT–3') primers. The nucleotide sequences of these primers correspond to sequences 2167–2186 and 2397–2378 of human *MTIF2* cDNA (NM_002453). The size of amplified fragment is 231 bp.

The amplification of β -actin (*ACTB*) cDNA was performed using forward – 5'–GGAAGATCGAGCAAGAGATGG–3' and reverse – 5'–AGCACTGTGTTGGCGTACAG–3' primers. These primer nucleotide sequences correspond to 747–766 and 980–961 of human *ACTB* cDNA (NM_001101). The size of amplified fragment is 234 bp. The expression of β -actin mRNA was used as control of analyzed RNA quantity. The primers were received from Sigma-Aldrich (St. Louis, MO, USA).

Quantitative PCR analysis was performed using Differential Expression Calculator software. The values of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYRL2*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, *MTIF2*, and *ACTB* gene expressions were normalized to the expression of β -actin mRNA and represented as percent of control (100%). All values are expressed as mean \pm SEM from triplicate measurements performed in 4 independent experiments. The amplified DNA fragments were also analyzed on a 2% agarose gel and visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

Statistical analysis. Statistical analysis was performed according to Student's *t*-test using Excel program as described previously [39]. All values are expressed as mean \pm SEM from triplicate measurements performed in 4 independent experiments.

Results and Discussion

To determine if hypoxia affects the expression of a subset of genes encoding different proliferation related factors through the IRE1 branch of endoplas-

mic reticulum stress response, we investigated the effect of hypoxic condition on the expression levels of these genes in control glioma cells (transfected by vector) and cells without both enzymatic activities of this signaling enzyme. As shown in Fig. 1, the expression levels of *IL13RA2*, *CD24*, *ING1*, *ING2*, *ENDOG*, and *POLG* mRNAs are significantly up-regulated upon hypoxia. Thus, hypoxia leads to up-regulation of the expression level of *CD24* mRNA by 85%, *ING1* – by 146%, and *ENDOG* – by 58% in glioma cells, but smaller changes were observed for *ING2*, *POLG*, and *IL13RA2* genes (+20, +15, and +15%, respectively). At the same time, the expression levels of *KRT18*, *TRAPPC3*, *TSFM*, and *MTIF2* mRNA are down-regulated by hypoxia in control glioma cells in comparison to normoxic cells (Fig. 1). The expression levels of *TRAPPC3*, *TSFM*, and *MTIF2* mRNA are decreased by hypoxia by 24, 25, and 21%, respectively. However, no significant changes were observed for *BET1* and *MYL9* genes in control glioma cells treated by hypoxia in comparison to normoxic cells (Fig. 1).

To investigate a possible role of endoplasmic reticulum stress signaling mediated by IRE1 signaling enzyme in regulation of the expression of studied proliferation related genes by hypoxia, we investigated the effect of hypoxic condition on gene expressions in glioma cells without both enzymatic activities of this signaling enzyme. As shown in Fig. 2, inhibition of the signaling enzyme IRE1 by dnIRE1 significantly modifies the effect of hypoxia on the expression level of half of the studied genes as compared to control glioma cells (Fig. 1). Thus, hypoxia up-regulates the levels of *IL13RA2* (+35%), *ENDOG* (+110%), *POLG* (+32%), and *BET1* (+17%) genes expression in glioma cells with knockdown of signaling enzyme IRE1 in comparison to normoxic cells. At the same time, inhibition of IRE1 enzyme leads to down-regulation of the expression levels of two other genes, *ING1* and *TRAPPC3* (Fig. 2). Furthermore, no significant changes were observed for next six genes (*KRT18*, *CD24*, *ING2*, *MYL9*, *TSFM*, and *MTIF2*) in glioma cells with knockdown of signaling enzyme IRE1 treated by hypoxia in comparison to normoxic cells (Fig. 2).

As a next step we tested the effect of inhibition of signaling enzyme IRE1 on a sensitivity of studied proliferation related genes expression to hypoxia (Fig. 3 and 4). For this, we analyzed the effect of hypoxic condition on the expression levels of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*,

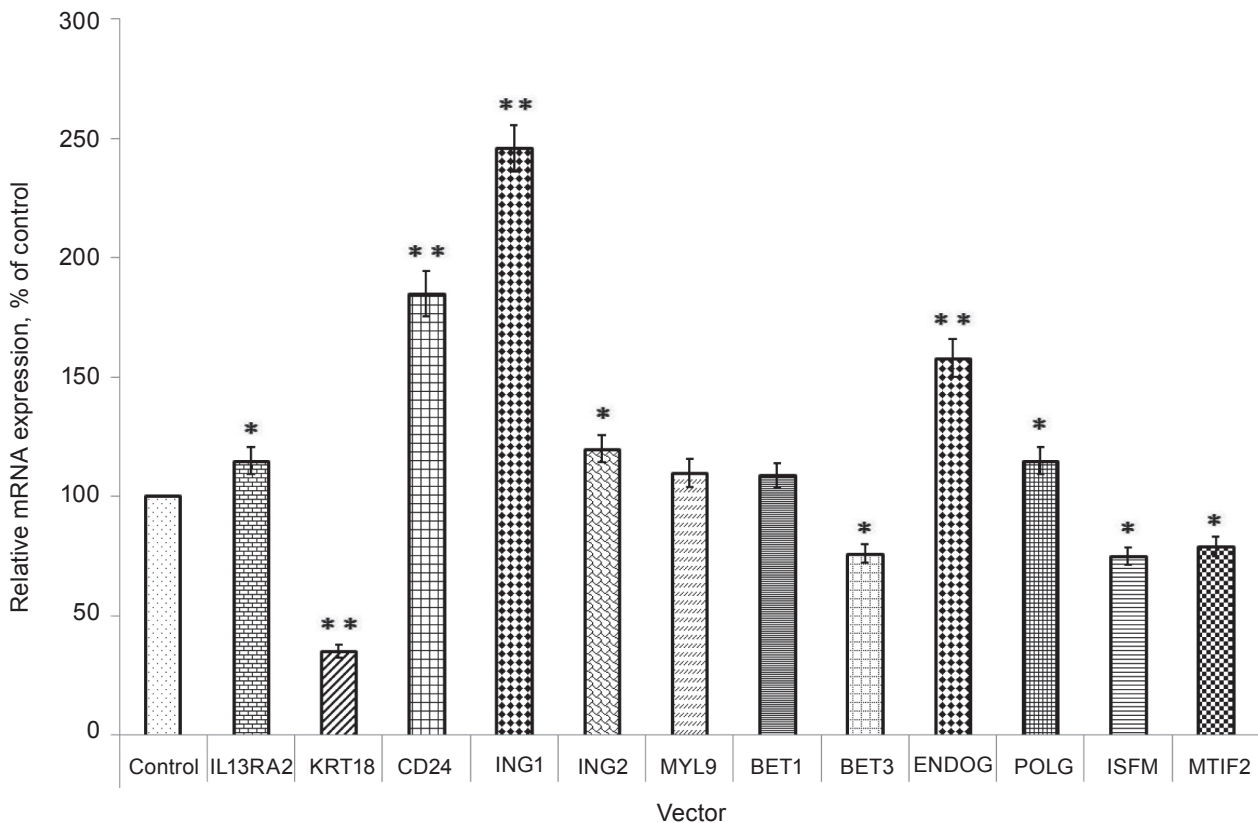


Fig. 1. Effect of hypoxia (3% oxygen – 16 h) on the expression levels of proliferation related genes in control U87 glioma cells (Vector) measured by qPCR. The values of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *BET3/TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* mRNA expressions were normalized to β -actin mRNA level and presented as percent of control (100%); $n = 4$; * $P < 0.05$ versus control; ** $P < 0.01$ versus control

BET1, *BET3/TRAPPC3*, *ENDOG*, *POLG*, *TSFM*, and *MTIF2* mRNA in glioma cells with functional IRE1 enzyme and inhibited IRE1 function in conditions when both controls (control glioma cells stable transfected by vector and glioma cells stable transfected by dnIRE1) are established as 100% in order to clarify the difference in the sensitivity of these gene expressions to hypoxia in respect to inhibition of IRE1 more precisely. As shown in Fig. 3, inhibition of the signaling enzyme IRE1 by dnIRE1 significantly modifies the effect of hypoxia on the expression levels of *IL13RA2*, *KRT18*, *CD24*, *ING1*, and *MYL9* genes as compared to control glioma cells transfected by vector. Moreover, stronger changes in sensitivity to hypoxia were observed for *KRT18*, *CD24*, and *ING1* genes: IRE1 inhibition almost completely eliminates the effect of hypoxia on these genes expression (Fig. 3).

Smaller but statistically significant changes in sensitivity to hypoxia of *MYL9* gene expression

were shown between control glioma cells and cells without signaling enzyme IRE1 function (Fig. 3). Moreover, inhibition of IRE1 enhances the effect of hypoxia on the expression of *IL13RA2* gene and eliminates hypoxic regulation of *MYL9* gene expression.

As shown in Fig. 4, inhibition of the signaling enzyme IRE1 by dnIRE1 also modifies the effect of hypoxia on the expression level of *BET3/TRAPPC3*, *ENDOG*, *POLG*, *ISFM*, and *MTIF2* genes in U87 glioma cells as compared to control glioma cells transfected by vector. It was also shown that inhibition of IRE1 slightly modifies the effect of hypoxia on the expression of *BET3/TRAPPC3* gene. At the same time, effect of hypoxia on the expression of *ENDOG* and *POLG* genes in glioma cells with IRE1 knockdown is greater than in control glioma cells: +110% versus +58% for *ENDOG* gene and +32% versus +15% for *POLG* gene (Fig. 4). It is also shown in Fig. 4 that inhibition of IRE1 signaling enzyme

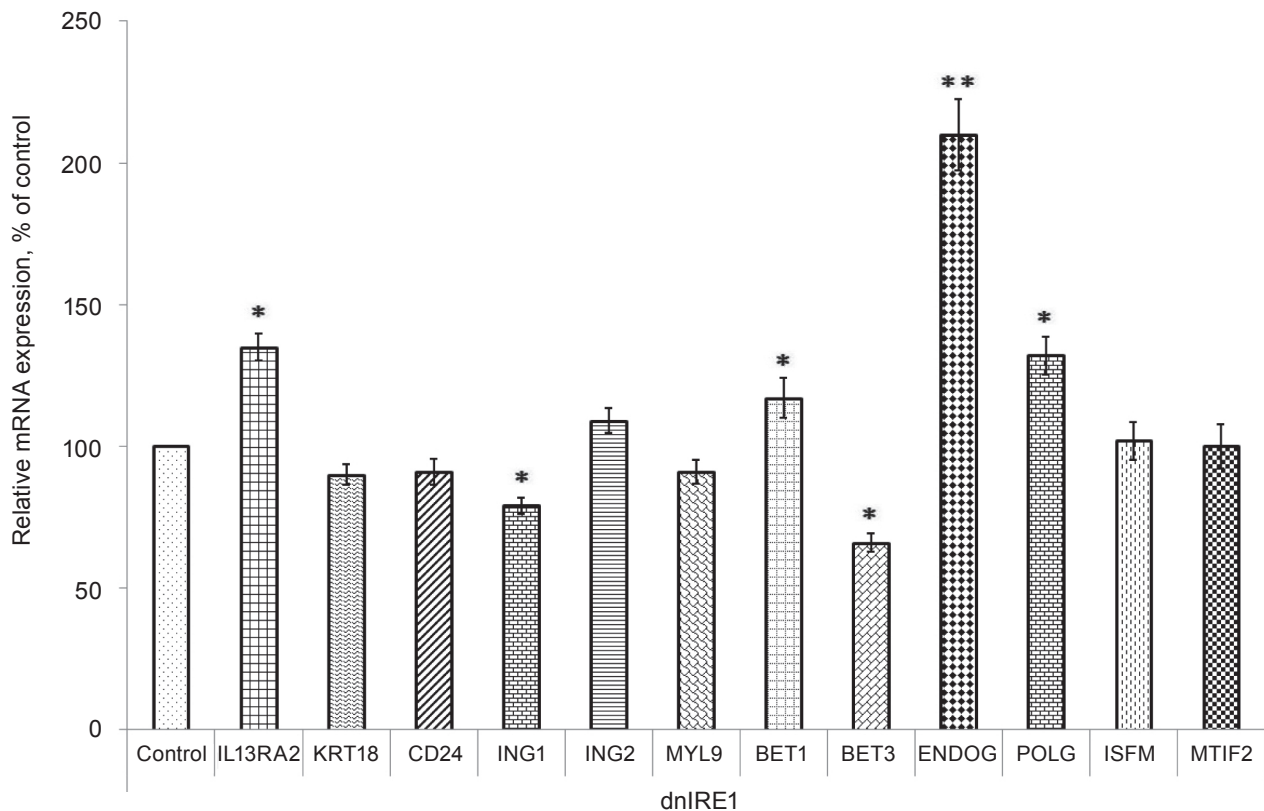


Fig. 2. Effect of hypoxia on the expression levels of proliferation related genes in U87 glioma cells with a blockade of the IRE1 by dnIRE1 (dnIRE1) measured by qPCR. The values of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *BET3/TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, and *MTIF2* mRNA expressions were normalized to β -actin mRNA level and presented as percent of control (100%); $n = 4$; * $P < 0.05$ versus control; ** $P < 0.01$ versus control

completely eliminates hypoxic regulation of *TSMF* and *MTIF2* gene expressions.

In this work we studied the expression of a subset of genes encoding different proliferation related factors in U87 glioma cells with functionally active signaling enzyme IRE1 and cells with IRE1 knockdown upon hypoxia for evaluation of possible significance of these genes in the control of glioma growth through endoplasmic reticulum stress signaling mediated by IRE1 and hypoxia. Investigation of the expression of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *BET3/TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, and *MTIF2* genes in glioma cells upon hypoxia in respect to inhibition of IRE1 signaling is important for understanding of malignant tumor growth mechanisms, because hypoxia as well as endoplasmic reticulum stress play an essential role in the control of tumor progression [22, 35-38]. The growing tumor requires the endoplasmic reticulum stress and hypoxia for its own neovascularization and growth, for apoptosis inhibition [29,

30, 38]. Cell proliferation is strongly dependent on hypoxia and glycolysis because there is the molecular connection between cell cycle progression and the provision of substrates essential for this purpose [35, 37, 38]. In this study we demonstrated that the expression of most studied genes in control glioma cells is affected by hypoxia as compared to normoxic condition. The expression levels of *IL13RA2*, *CD24*, *ING1*, *ING2*, *ENDOG*, and *POLG* genes are increased upon hypoxia. To the contrary, the expression levels of several other genes (*KRT18*, *TRAPPC3*, *TSMF*, and *MTIF2*) are decreased. Our results are mostly consistent with numerous data [22, 23, 36-38] that hypoxia associated with malignant progression through the endoplasmic reticulum unfolded protein response, but mechanisms through which malignant cells cope with potentially lethal metabolic stress induced by hypoxia remain poorly understood.

We have shown that hypoxia significantly down-regulates the expression of anti-proliferative gene *KRT18*, which contributes to decreased ma-

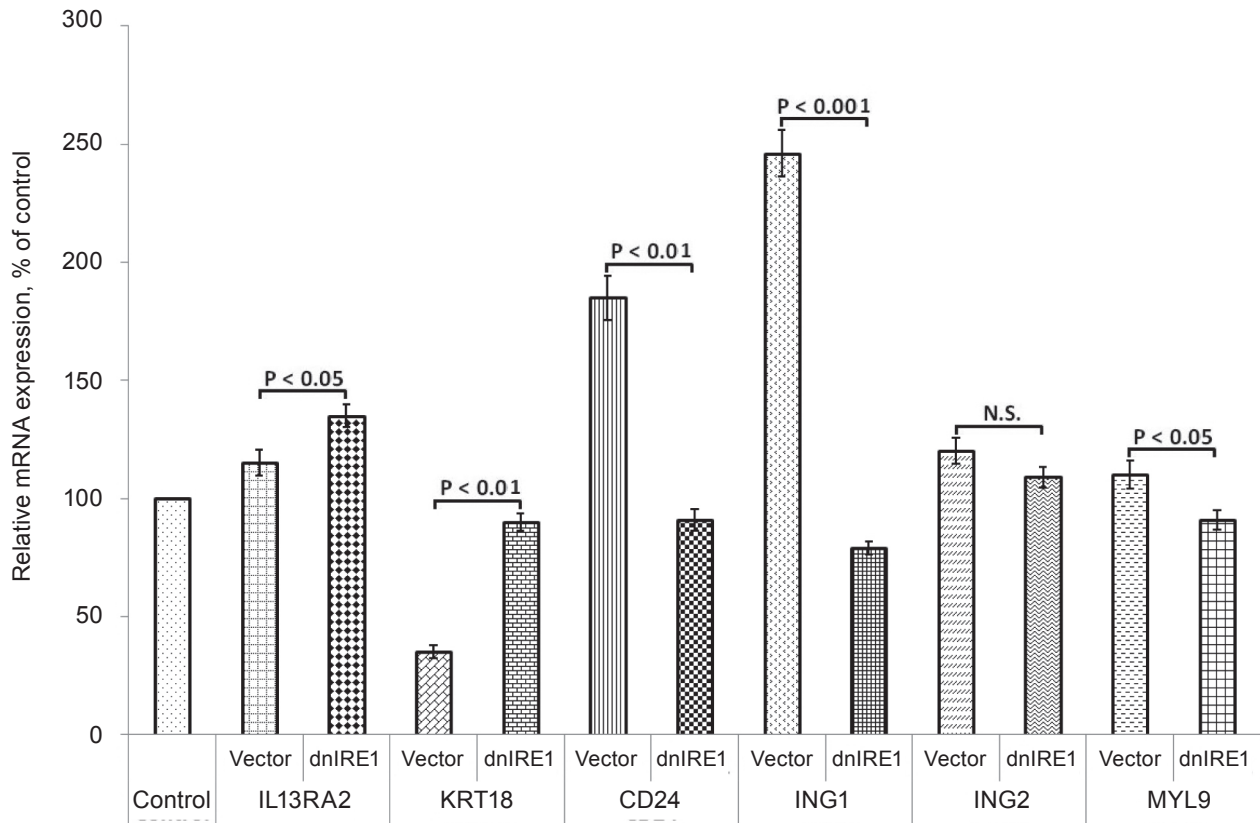


Fig. 3. Effect of hypoxia on the expression levels of *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, and *MYL9* mRNA in control U87 glioma cells (Vector) and cells with a blockade of the IRE1 by *dnIRE1* (*dnIRE1*) measured by qPCR. Values of these mRNA expressions were normalized to β -actin mRNA level and presented as percent of control (100%); $n = 4$

lignancy of non-small cell lung carcinoma [11], in control glioma cells and that inhibition of IRE1 signaling eliminates this effect of hypoxia. Thus, in case of *KRT18* gene, hypoxia may reveal the proliferative effect and inhibition of IRE1 and consequent cell proliferation abolishes this effect of hypoxia. Furthermore, inhibition of IRE1 in glioma cells eliminates the effect of hypoxia on the expression of many other studied genes, such as *CD24*, *ING2*, *TSM*, and *MTIF2* genes and suppresses hypoxic regulation of *ING1* and *POLG* genes. Similar results were obtained previously for *TP53*, *ZMAT3*, *IGFBP6*, *IGFBP7*, *NOV*, *WISP2*, and *HOXC6* genes [36, 40, 41].

Therefore, present study demonstrates that hypoxia, which usually contributes to tumor growth, affects almost all studied genes expression and that inhibition of IRE1 can both enhance and suppress the hypoxic regulation of these gene expressions in gene specific manner and thus possibly contributes to slower glioma growth. However, the detailed molecular mechanisms of IRE1-mediated hypoxic regulation of these genes, which have a pivotal role in the control of cell proliferation, are complex and warrant further investigation.

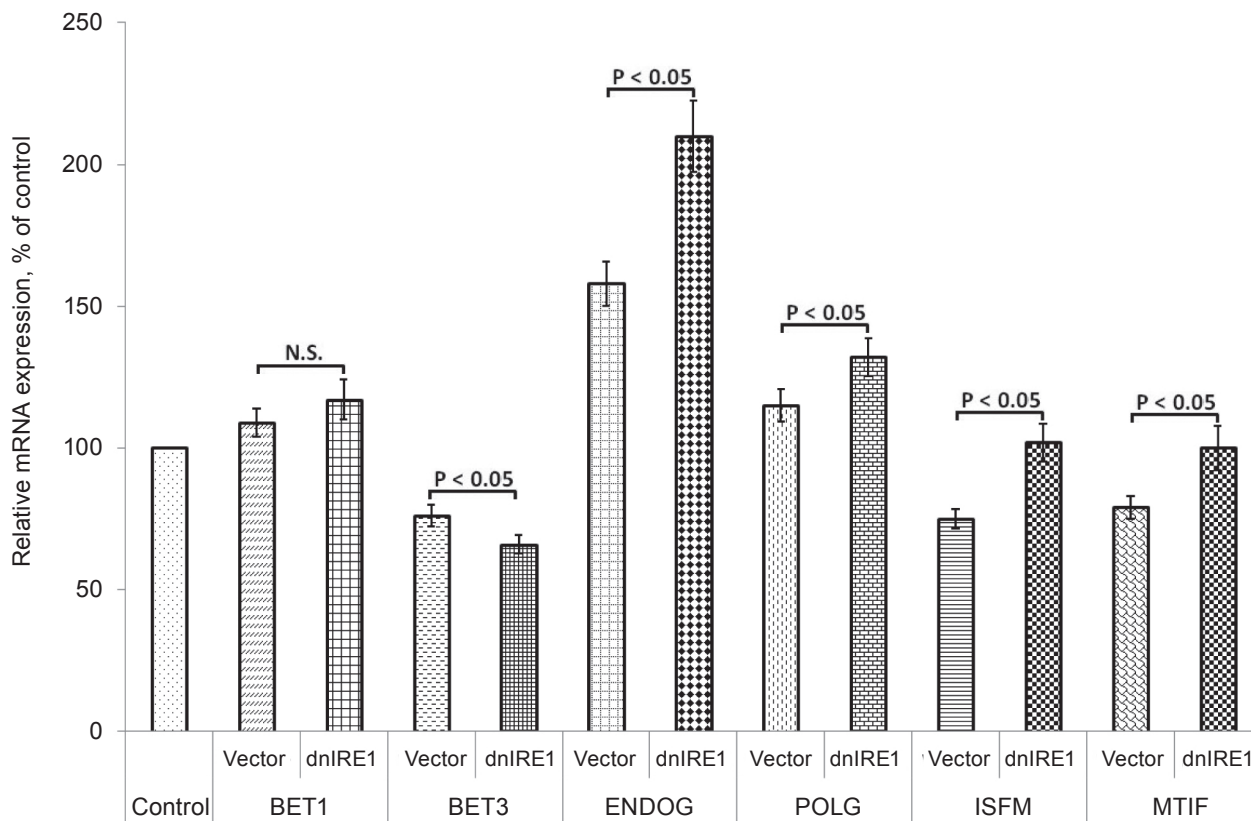


Fig. 4. Effect of hypoxia on the expression levels of *BET1*, *BET3/TRAPPC3*, *ENDOG*, *POLG*, *ISFM*, and *MTIF* mRNA in control U87 glioma cells (Vector) and cells with a blockade of the IRE1 by dnIRE1 (dnIRE1) measured by qPCR. Values of these mRNA expressions were normalized to β -actin mRNA level and presented as percent of control (100%); $n = 4$

РЕГУЛЯЦІЯ ЕКСПРЕСІЇ ГЕНІВ, ЩО МАЮТЬ ВІДНОШЕННЯ ДО ПРОЛІФЕРАЦІЇ КЛІТИН, ЗА УМОВ ГІПОКСІЇ ТА ПРИГНІЧЕННЯ СИГНАЛЬНОГО ЕНЗИМУ IRE1 У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87

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Ми вивчали ефект пригнічення IRE1 (залежного від інозитолу ензиму 1), що є центральним медіатором стресу ендоплазматичного ретикулула і контролює проліферацію клітин та ріст пухлин, на гіпоксичну регуляцію експресії різних генів, які мають відношення до

проліферації, у клітинах гліоми лінії U87. Встановлено, що за умов гіпоксії спостерігалось посилення експресії генів *IL13RA2*, *CD24*, *ING1*, *ING2*, *ENDOG* та *POLG* і зниження – генів *KRT18*, *TRAPPC3*, *TSMF* та *MTIF2* на рівні мРНК у контрольних клітинах гліоми, причому більш виражені зміни виявлені для генів *ING1* та *CD24*. Пригнічення IRE1 модифікувало ефект гіпоксії на експресію всіх досліджених генів: збільшувало чутливість до гіпоксії експресії генів *IL13RA2*, *TRAPPC3*, *ENDOG* та *PLOG*, знижувало ефект гіпоксії на ген *ING1*. Крім того, пригнічення IRE1 знімає регуляцію гіпоксією експресії генів *KRT18*, *CD24*, *ING2*, *TSMF* та *MTIF2* і ініціює чутливість до гіпоксії експресії гена *BET1* у клітинах гліоми. Результати цієї роботи продемонстрували, що гіпоксія (необхідний фактор росту пухлин), змінює рівень експресії майже всіх досліджених генів і що пригнічення IRE1 як посилює, так і знижує гіпоксичну регуляцію експресії цих генів і, та-

ким чином, можливо, є внеском у зниження росту гліоми, але деякі аспекти цієї регуляції ще необхідно з'ясувати.

Ключові слова: експресія мРНК, стрес ендоплазматичного ретикулула, *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, *MTIF2*, пригнічення IRE1, гіпоксія, клітини гліоми.

РЕГУЛЯЦИЯ ЭКСПРЕССИИ ГЕНОВ, ИМЕЮЩИХ ОТНОШЕНИЕ К ПРОЛИФЕРАЦИИ КЛЕТОК, ПРИ ГИПОКСИИ И УГНЕТЕНИИ СИГНАЛЬНОГО ЭНЗИМА IRE1 В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87

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Мы изучали эффект угнетения IRE1 (зависимого от инозитола энзима 1), который является центральным медиатором стресса эндоплазматического ретикулула, контролирует пролиферацию клеток и рост опухолей, на гипоксическую регуляцию экспрессии различных генов, имеющих отношение к пролиферации, в клетках гліомы линии U87. Установлено, что при гипоксии усиливается экспрессия генов *IL13RA2*, *CD24*, *ING1*, *ING2*, *ENDOG* и *POLG* и снижается – генов *KRT18*, *TRAPPC3*, *TSMF* и *MTIF2* на уровне мРНК в контрольных клетках гліомы, причем более выраженные изменения выявлены для генов *ING1* и *CD24*. Угнетение IRE1 модифицировало эффект гипоксии на экспрессию всех исследуемых генов, а именно увеличивало чувствительность к гипоксии экспрессии генов *IL13RA2*, *TRAPPC3*, *ENDOG* и *PLOG* и снижало эффект гипоксии на ген *ING1*. Кроме того, угнетение IRE1 снимает регуляцию гипоксией экспрессии генов *KRT18*, *CD24*, *ING2*, *TSMF* и *MTIF2* и инициирует чувствительность экспрессии гена *BET1* к гипоксии в клетках гліомы. Результаты этой работы продемонстрировали, что гипоксия (необходимый фактор роста опухолей), изменяет уровень экспрессии почти всех

исследованных генов. Угнетение IRE1 как усиливает, так и снижает регуляцию гипоксией экспрессии этих генов и, таким образом, возможно, вносит вклад в снижение роста гліомы, но некоторые аспекты этой регуляции еще необходимо объяснить.

Ключевые слова: экспрессия мРНК, стресс эндоплазматического ретикулула, *IL13RA2*, *KRT18*, *CD24*, *ING1*, *ING2*, *MYL9*, *BET1*, *TRAPPC3*, *ENDOG*, *POLG*, *TSMF*, *MTIF2*, угнетение IRE1, гипоксия, клетки гліомы.

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