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ERN1 knockdown modifies the hypoxic regulation of homeobox gene expression in U87MG glioblastoma cells

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Objective. Homeobox genes play an important role in health and disease including oncogenesis. The present investigation aimed to study ERN1-dependent hypoxic regulation of the expression of genes encoding homeobox proteins MEIS (zinc finger E-box binding homeobox 2) and LIM homeobox 1 family, SPAG4 (sperm associated antigen 4) and NKX3-1 (NK3 homeobox 1) in U87MG glioblastoma cells in response to inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1) for evaluation of their possible significance in the control of glioblastoma growth.

Methods. The expression level of homeobox genes was studied in control (transfected by vector) and ERN1 knockdown U87MG glioblastoma cells under hypoxia induced by dimethyloxalylglycine (0.5 mM for 4 h) by quantitative polymerase chain reaction and normalized to ACTB.

Results. It was found that hypoxia down-regulated the expression level of *LHX2*, *LHX6*, *MEIS2*, and *NKX3*-1 genes but up-regulated the expression level of *MEIS1*, *LHX1*, *MEIS3*, and *SPAG4* genes in control glioblastoma cells. At the same time, ERN1 knockdown of glioblastoma cells significantly modified the sensitivity of all studied genes to a hypoxic condition. Thus, ERN1 knockdown of glioblastoma cells removed the effect of hypoxia on the expression of *MEIS1* and *LHX1* genes, but increased the sensitivity of *MEIS2*, *LHX2*, and *LHX6* genes to hypoxia. However, the expression of *MEIS3*, *NKX3*-1, and *SPAG4* genes had decreased sensitivity to hypoxia in ERN1 knockdown glioblastoma cells. Moreover, more pronounced changes under the conditions of ERN1 inhibition were detected for the pro-oncogenic gene *SPAG4*.

Conclusion. The results of the present study demonstrate that hypoxia affected the expression of homeobox genes *MEIS1*, *MEIS2*, *MEIS3*, *LHX1*, *LHX2*, *LHX6*, *SPAG4*, and *NKX3-1* in U87MG glioblastoma cells in gene-specific manner and that the sensitivity of all studied genes to hypoxia condition is mediated by ERN1, the major pathway of the endoplasmic reticulum stress signaling, and possibly contributed to the control of glioblastoma growth. A fundamentally new results of this work is the establishment of the fact regarding the dependence of hypoxic regulation of SPAG4 gene expression on ER stress, in particular ERN1, which is associated with suppression of cell proliferation and tumor growth.

Key words: U87MG glioblastoma cells, ERN1 knockdown, hypoxia, homeobox genes, mRNA expression

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The homeobox genes encode an evolutionarily highly conserved family of transcriptional factors, which regulate transcription and play a very important role in normal development and tumorigenesis (Mark et al. 1997; Feng et al. 2021; de Faria Poloni and Feltes 2023). Most of the homeobox transcription factors, including MEIS1 (MEIS homeobox 1), MEIS2, and MEIS3 have carcinogenic or tumor-suppressive effects in different neoplasms (Chen et al. 2019; Cai et al. 2020; Girgin et al. 2020; Zhang et al. 2020; Wen et al. 2021; Dai et al. 2023). It has been shown that MEIS1 inhibits the renal cell carcinoma cells proliferation and that the down-regulation of MEIS1 promotes tumorigenesis and resistance to oxaliplatin in colorectal cancer (Johng et al. 2019; Jiang et al. 2021; Li et al. 2022a). It also may contribute to the cancer-promoting actions of the homeobox protein HOXB13 in cellular proliferation (Zhu et al. 2017). At the same time, the suppression of *MEIS1* gene expression by inhibitor of WNT efficiently inhibits colorectal cancer stemness and metastasis (Choi et al. 2021). Similar results have been shown for the MEIS2 transcription factor. Ectopic expression of this protein markedly enhances neuroblastoma cell survival and proliferation, anchorage-independent growth and tumorigenicity (Zha et al. 2014). Moreover, there are data indicating that MEIS2 inhibits the cell proliferation and promotes apoptosis of thyroid cancer cells (Wen et al. 2021).

LIM homeobox proteins (LHX1, LHX2, and LHX6) contain the LIM domain, a unique cysteine-rich zinc-binding domain, and have different effects on the proliferation of cancer cells and tumor growth (Liao et al. 2017; Miyashita et al. 2018; Chen et al. 2019; Liang et al. 2019; Li et al. 2022b; Wang et al. 2022; Xie et al. 2022). There are data indicating that LHX1 controls the expression of various proteins regulating cell movements and endothelial to mesenchymal transition in renal carcinoma cells and that silencing of LHX1 decreased pulmonary metastasis in the *in vivo* model (Hamaidi et al. 2019). Moreover, LHX1 is an important transcription factor for the histone *HDAC8* (deacetylase 8) gene and may play an important role in neurogenesis (Ghiaghi et al. 2019). Recently, it was shown that LHX2 acts as a transcriptional activator and is involved in controlling tumor growth and metastasis because down-regulation of LHX2 by miR-506 inhibits both tumor growth and metastasis (Liang et al. 2019). Furthermore, Song et al. (2019) have shown that the homeobox protein LHX2 promotes malignancy and inhibits autophagy in osteosarcoma via mTOR. At the same time, data indicate that this homeobox protein suppresses the proliferation of T-cell acute lymphoblastic leukemia-derived cells (Miyashita et al. 2018). Transcription factor LHX6 is a tumor suppressor and its epigenetic suppression can inhibit cell proliferation, invasion, and migration through different pathways: WNT/beta-catenin and TP53 signaling (Chen et al. 2019). Additionally, the overexpression of LHX6 inhibited tumor growth, while inhibiting LHX6 expression by miR-1290 promotes proliferation, migration, and invasion of glioblastoma cells (Yan et al. 2018).

SPAG4 (sperm associated antigen 4) has a fundamental pathological function in the migration of lung carcinoma cells as a HIF-induced gene (Knaup et al. 2014; Ji et al. 2018). Recently, it was shown that SPAG4 mRNA is overexpressed in lung squamous cell carcinoma tissue compared with their paired para-cancerous histological normal tissue samples (Wang et al. 2021). Moreover, SPAG4 could serve as an independent prognostic factor for glioblastoma patients and might be valuable for the clinical management of these patients (Zhao et al. 2019). NK3 homeobox 1 (NKX3-1) plays an important role in prostate cancerogenesis in an androgen-sensitive way (Matsuda et al. 2023).

Malignant tumors use endoplasmic reticulum (ER) stress response and its signaling pathways to adapt and enhance tumor cell proliferation under stressful environmental conditions (Bravo et al. 2013; Doultsinos et al. 2017; Papaioannou et al. 2018; Almanza et al. 2019). It is well known that activation of the ERN1 branch of the ER stress response is tightly linked to apoptosis and cell death and that suppression of its function has been demonstrated to result in a significant anti-proliferative effect in glioblastoma growth (Auf et al. 2010; Minchenko et al. 2015, 2021). Furthermore, inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1) endoribonuclease has more strong anti-proliferative effect on glioblastoma cells and leads to specific changes in the expression of genes related to ERN1 signaling pathway, the major pathway of the ER stress signaling (Auf et al. 2010, 2013; Minchenko et al. 2015, 2021; Logue et al. 2018).

Hypoxia is an essential complementary factor for the development and more aggressive behavior of solid tumors including glioblastoma (Minchenko et al. 2002; Denko 2008). Hypoxia tightly interacts with ER stress to control tumor growth (Bi et al. 2005; Fels and Koumenis 2006). It has been shown that ER stress-regulated translation through the PERK/eIF2a/ATF4 module of the unfolded protein response increases tolerance to extreme hypoxia and promotes tumor growth. Moreover, the inhibition of ER stress

signaling mediated by ERN1 significantly suppresses the glioblastoma cell proliferation and tumor growth and modifies the impact of hypoxia on the expression of numerous genes (Auf et al. 2010, 2013; Minchenko et al. 2013, 2015, 2020). The complete molecular mechanisms of the interaction of the ERN1 mediated stress signaling pathway with hypoxia are complex and warrant further study.

The current study aimed to investigate the expression of genes encoded MEIS1, MEIS2, MEIS3, LHX1, LHX2, LHX6, SPAG4, and NKX3-1 homeobox proteins in response to hypoxia in ERN1 knockdown U87MG glioblastoma cells to reveal the role of ERN1 signaling pathway in the ER stress-dependent hypoxic regulation of homeobox genes expression and tumor growth.

Material and Methods

Cell lines and culture conditions. The glioblastoma cell line U87MG was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc., USA), penicillin (100 units/ml, Gibco), and streptomycin (0.1 mg/ml, Gibco) at 37 °C in incubator with 5% CO_2. In this study, we used two sublines of U87MG glioblastoma cells. One was obtained by the selection of stable transfected clones with overexpression of vector pcDNA 3.1, which was used for the creation of dnERN1. These untreated glioblastoma cells were used as control 1 (control glioblastoma cells) in the study of the effect of hypoxia on the level of homeobox gene expression. The second subline was obtained by selection of stable transfected clones with overexpression of ERN1 dominant/negative construct (dnERN1) having suppression of both the protein kinase and endoribonuclease activities of this signaling enzyme (Auf et al. 2010, 2013). It has been shown that cells with dnERN1 have a lower proliferation rate, do not express a spliced variant of XBP1, a key transcription factor in ERN1 signaling, and do not have the phosphorylated isoform of ERN1 after induction of ER stress by tunicamycin (Auf et al. 2013; Minchenko et al. 2015). Both sublines of glioblastoma cells used in this study grew in the presence of geneticin (G418), while carrying empty vector pcDNA3.1 or dnERN1 construct. Hypoxia was created by 0.5 mM dimethyloxalylglycine as described previously (Minchenko et al. 2002) and culture plates were exposed for 4 h.

RNA isolation. Total RNA was extracted from glioblastoma cells using the Trizol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, 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 and re-dissolved again in nuclease-free water. RNA concentrations

Table 1
Characteristics of the primers used for quantitative real-time polymerase chain reaction

Gene symbol	Gene name	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
MEIS1	Meis homeobox 1	F: 5'- aaggtgatggcttggacaac R: 5'- tgtgccaactgctttttctg	1472–1491 1661–1642	NM_002398.3
MEIS2	Meis homeobox 2	F: 5'- gtcagcaacacatggggatc R: 5'- ggaccaccctgagaaacgta	1505–1524 1673–1654	NM_002399.4
MEIS3	Meis homeobox 3	F: 5'- cagcctcccagaccagaata R: 5'- tcctggtccaagtcctcatc	1028–1047 1222–1203	NM_020160.3
SPAG4	sperm associated antigen 4	F: 5'- cagcttctggaactacgcac R: 5'- gtggatgctgcagagtgatg	1042–1061 1193–1174	NM_003116.3
LHX1	LIM homeobox 1	F: 5'- atttatttccgttcccgccg R: 5'- ccaaaactcgcaccaggaaa	354–373 591–572	NM_005568.5
LHX2	LIM homeobox 2	F: 5'- tccctactacaatggcgtgg R: 5'- gtcggggttgtggttaatgg	1214–1233 1457–1438	NM_004789.4
LHX6	LIM homeobox 6	F: 5'- cggaacagctgcaggttatg R: 5'- ctgaacggggtgtagtggat	987–1006 1224–1205	NM_014368.5
NKX3-1	NK3 homeobox 1	F: 5'- aagaacctcaagctcacgga R: 5'- tgtcacctgagctggcatta	524–543 591–572	NM_006167.4
ACTB	beta-actin	F: 5'- catccgcaaagacctgtacg R: 5'- cctgcttgctgatccacatc	948–967 1165–1146	NM_001101.5

and spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. The expression levels of homeobox mRNAs as well as ACTB mRNA were measured in control U87MG glioblastoma cells and cells with a deficiency of ERN1, introduced by dnERN1, by quantitative polymerase chain reaction (PCR) using SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described (Rudnytska et al. 2021). PCR was performed in triplicate. The expression of beta-actin mRNA was used as a control of analyzed RNA quantity. The pair of primers specific for each studied gene was received from Sigma-Aldrich (St. Louis, MO, USA.) and Metabion Int. AG (Martinstried, Germany) and used for quantitative PCR (Table 1). The quantitative PCR analysis was performed using a special computer program "Differential Expression Calculator".

Statistical analysis. The Excel program and OriginPro 7.5 software were used for statistical analysis as described previously (Rudnytska et al. 2021). A comparison of two means was performed by the use of a two-tailed Student's *t*-test. A value of $p<0.05$ was considered significant in all cases. The values of studied gene expression were normalized to the expression of beta-actin mRNA and expressed as a percentage of controls (100%). All values were expressed as mean±SEM from triplicate measurements performed in four independent experiments. All experimental qPCR data were analyzed for the normality of distribution using a graphical tool (normal probability plot) and a histogram as described previously (Rudnytska et al. 2021). A normal distribution was shown for all analyzed data sets. The amplified DNA fragments were also analyzed on a 2% agarose gel and then visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA.).

Results

Examining a possible role of the ER stress signaling mediated by ERN1 in the hypoxic regulation of the expression of *MEIS1*, *MEIS2*, *MEIS3*, *LHX1*, *LHX2*, *LHX6*, *SPAG4*, and *NKX3-1* genes in control

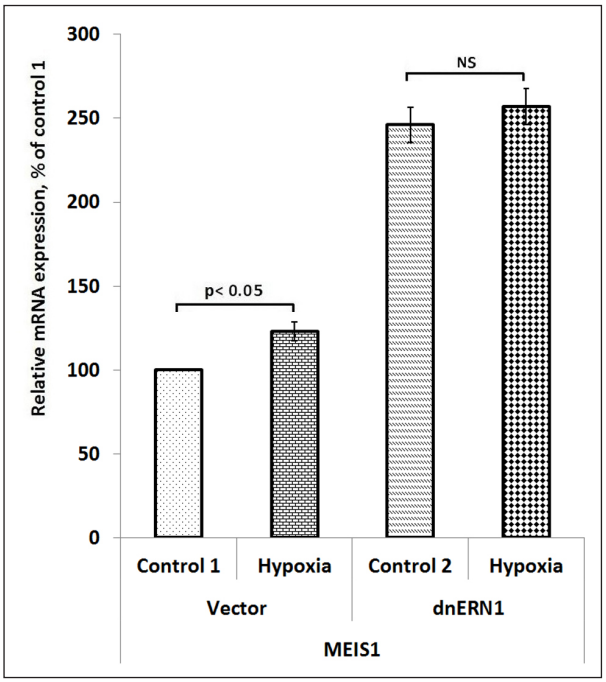


Figure 1. Impact of hypoxia on the expression level of *MEIS1* (Meis homeobox 1) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

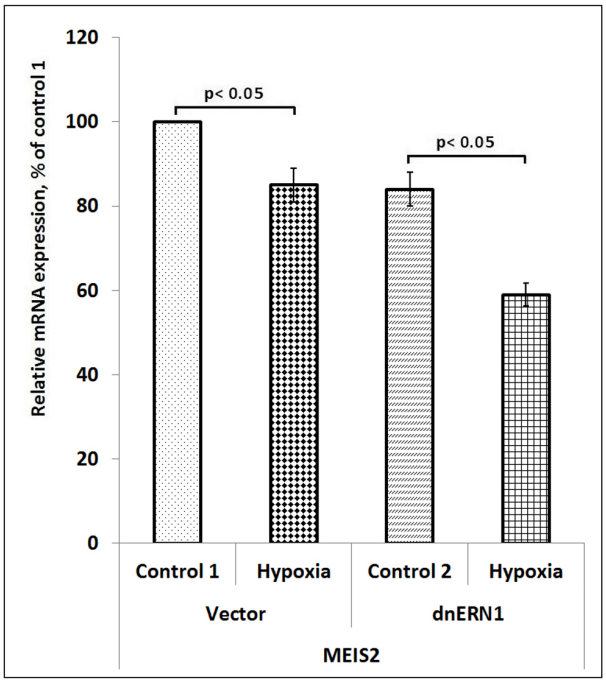


Figure 2. Impact of hypoxia on the expression level of *MEIS2* (Meis homeobox 2) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

glioblastoma cells and ERN1 knockdown cells were evaluated. As shown in Figure 1, hypoxia up-regulates the expression of MEIS1 mRNA in control glioblastoma cells (transfected by empty vector pcDNA3.1) by 23% as compared to not treated control cells (control 1). At the same time, hypoxia does not change significantly this mRNA expression in ERN1 knockdown glioblastoma cells as compared to not treated ERN1 knockdown cells (control 2). Next, we studied the impact of hypoxia on MEIS2 mRNA expression in control glioblastoma cells and ERN1 knockdown cells. Results, presented in Figure 2, demonstrate a small but statistically significant down-regulation of MEIS2 mRNA (–15%) in control glioblastoma cells but inhibition of ERN1 signaling enzyme function by dnERN1 significantly modified the sensitivity of this gene expression to hypoxia as compared to corresponding control. In this case, hypoxia suppresses the level of MEIS2 mRNA expression by 30% (Figure 2). More pronounced changes were introduced by hypoxia in the *MEIS3* gene expression. Thus, the expression of the *MEIS3* gene in control glioblastoma cells is more sensitive to hypoxia than other members of MEIS family transcription factors. As shown in Figure 3, hypoxia up-regulates the expression of MEIS3 mRNA in control glioblastoma cells by 66% compared to untreated control cells. At the same time, inhibition of ERN1 decreases the sensitivity of the *MEIS3* gene to hypoxia: by +51% compared to not treated ERN1 knockdown cells (Figure 3).

Hypoxia also affects the expression of genes encoding LHX family transcription factors. Results, presented in Figure 4, demonstrate that hypoxia up-regulates the expression of LHX1 mRNA (+19%) in control glioblastoma cells but inhibition of ERN1 by dnERN1 removed the sensitivity of this gene expression to hypoxia as compared to corresponding control. At the same time, the expression of *LHX2* and *LHX6* homeobox genes is down-regulated (–20 and –49%, correspondingly) in control glioblastoma cells as compared to not treated control cells (Figures 5 and 6). At the same time, inhibition of ERN1 leads to more pronounced down-regulation of both *LHX2* and *LHX6* gene expressions: by 29% for *LHX2* and 57% for *LHX6* (Figures 5 and 6).

Next, we studied the impact of hypoxia on the expression of *NKX3-1* homeobox gene in control and ERN1 knockdown glioblastoma cells. As shown in Figure 7, exposure of control glioblastoma cells

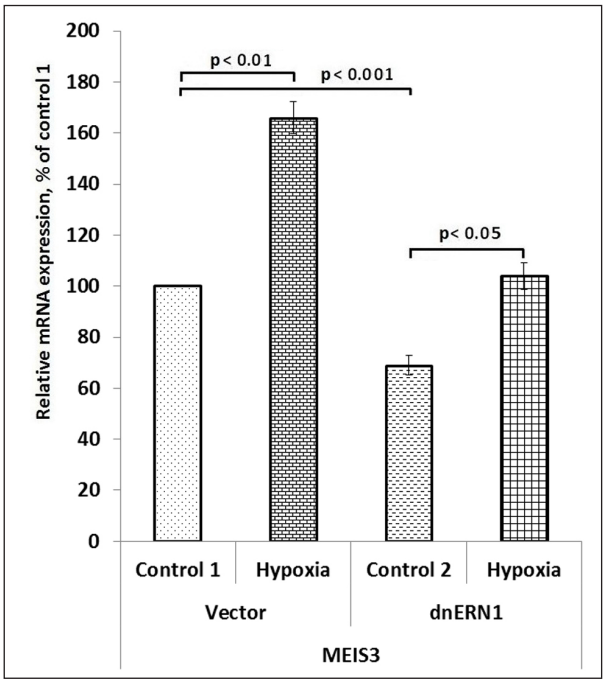


Figure 3. Impact of hypoxia on the expression level of *MEIS3* (Meis homeobox 3) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

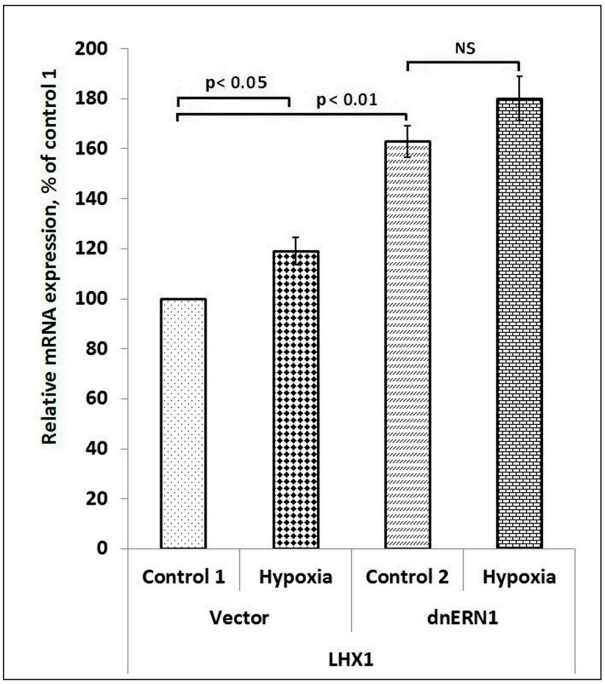


Figure 4. Impact of hypoxia on the expression level of *LIM1* (LIM homeobox 1) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

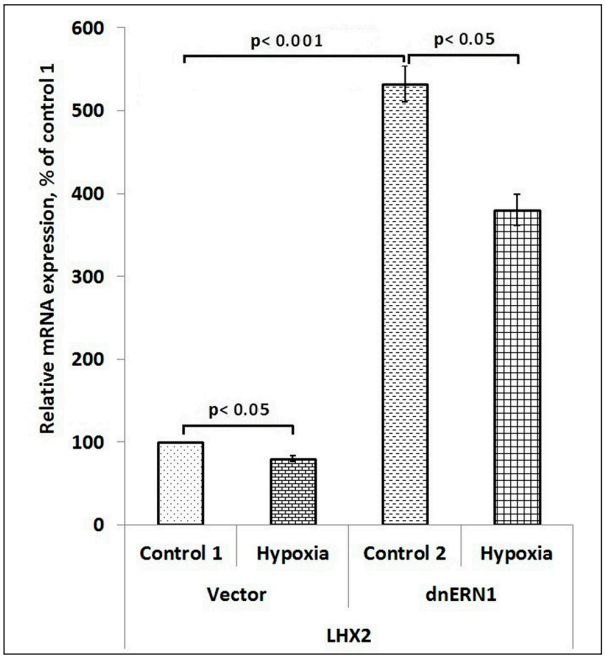


Figure 5. Impact of hypoxia on the expression level of *LIM2* (LIM homeobox 2) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

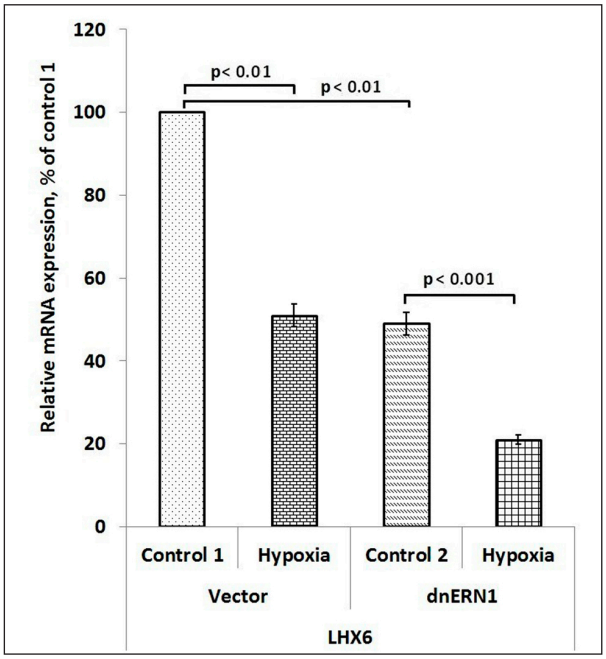


Figure 6. Impact of hypoxia on the expression level of *LIM6* (LIM homeobox 6) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

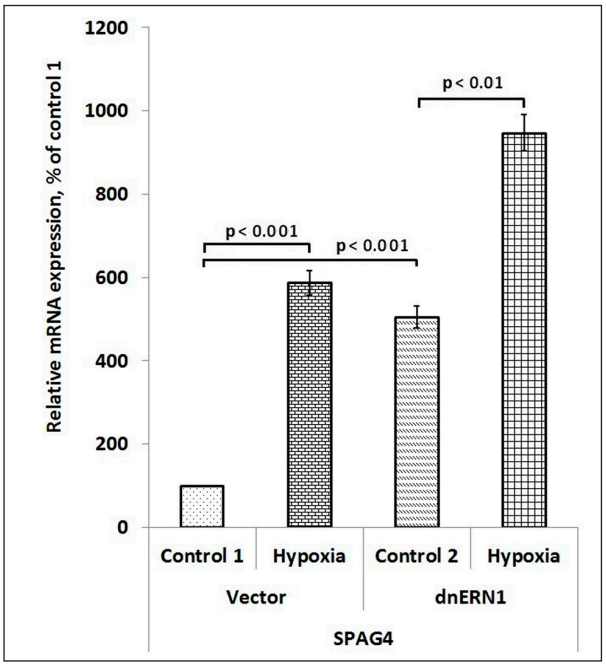


Figure 7. Impact of hypoxia on the expression level of *SPAG4* (Sperm associated antigen 4) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

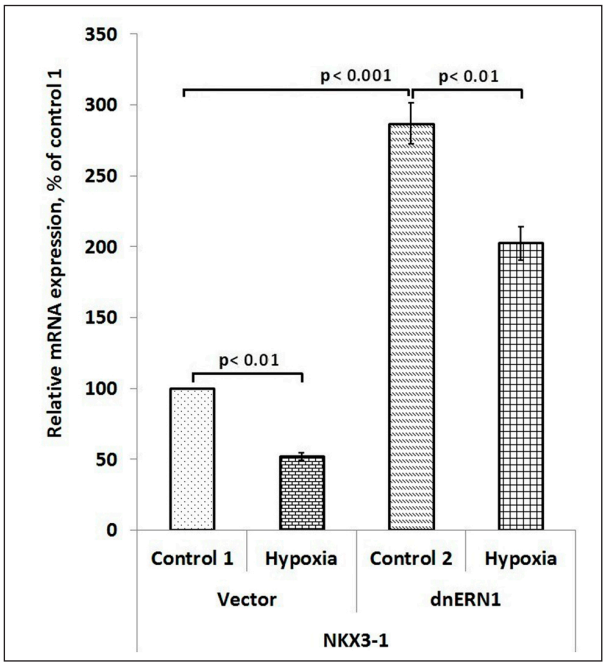


Figure 8. Impact of hypoxia on the expression level of *NKX3-1* (NK3 homeobox 1) gene in control U87MG glioblastoma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

under hypoxia strongly down-regulated (–48%) of this homeobox gene expression. Moreover, inhibition of ERN1 significantly decreased the sensitivity of the *NKX3-1* homeobox gene to hypoxia (–29%) as compared to not treated ERN1 knockdown glioblastoma cells (Figure 7). More pronounced changes in treated hypoxia control glioblastoma cells were shown for the *SPAG4* gene. Results, presented in Figure 8, demonstrate strong up-regulation of the expression of SPAG4 mRNA (+487%) in control glioblastoma cells by hypoxia. At the same time, inhibition of ERN1 by dnERN1 strongly suppressed the sensitivity of this gene expression to hypoxia (+88%) as compared to not treated ERN1 knockdown glioblastoma cells (Figure 8). Therefore, these results demonstrate that hypoxic up-regulation of SPAG4 mRNA expression in control glioblastoma cells is dependent on the functional activity of ERN1 signaling because knockdown of ERN1 strongly suppressed the sensitivity of this homeobox gene expression to hypoxia.

Thus, hypoxia affected the expression of genes encoding the homeobox family transcription factors in a gene-specific manner, and observed changes in gene expressions were preferentially controlled by ERN1 signaling because inhibition of ERN1 modified the sensitivity of these homeobox genes to hypoxia.

Discussion

In this work, the impact of hypoxia on the expression of genes encoding important homeobox proteins, which control the transcription preferentially regulatory factors, was studied in U87MG glioblastoma cells in relation to inhibition of ERN1 the major signaling pathway of the unfolded protein response. Results of this investigation summarized in Figure 9 demonstrate the gene-specific character of changes in the expression profile of studied homeobox genes introduced by hypoxia via ER stress signaling mediated by ERN1 in glioblastoma cells. The results of hypoxic regulation of SPAG4 gene expression show strong induction of its expression in glioblastoma cells, which agree well with data from Knaup et al. (2014) that hypoxia up-regulates the SPAG4 via HIF in renal clear cell carcinoma and is associated with tumor progression, cell migration, and invasion. SPAG4 is identified as a cancer biomarker for glioblastoma and controls glioblastoma progression (Ji et al. 2018; Zhao et al. 2019; Wang et al. 2021). A fundamentally new result of this work is the establishment of the fact regarding the dependence of hypoxic regulation of SPAG4 gene expression on ER stress, in particular, ERN1, which is associated with suppression of cell proliferation and tumor growth (Auf et al. 2010, 2013; Minchenko et al. 2015, 2021; Logue et al. 2018).

We also showed that hypoxic regulation of homeobox transcription factors MEIS1, MEIS2, and MEIS3 is also controlled by ER stress because inhibition of ERN1 more significantly decreased MEIS2, removed the effect of hypoxia on MEIS1, and decreased hypoxic regulation of MEIS3. It is still quite difficult to interpret the obtained results, because these homeobox transcription factors can manifest both carcinogenic and tumor-suppressive effects in different neoplasms (Cai et al. 2020; Girgin et al. 2020; Zhang et al. 2020; Wen et al. 2021; Li et al. 2022a; Dai et al. 2023), although a decrease of MEIS2 can contribute to the suppression of glioblastoma cell

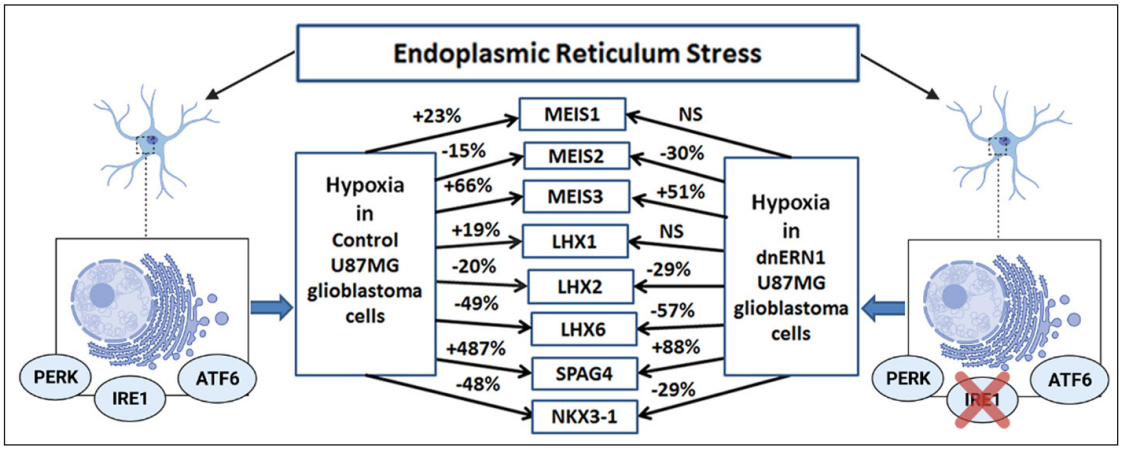


Figure 9. Schematic demonstration of *MEIS1*, *MEIS2*, *MEIS3*, *LHX1*, *LHX2*, *LHX6*, *SPAG4*, and *NKX3-1* genes expression profile in control and ERN1 knockdown glioblastoma cells under hypoxia; NS – no significant changes.

proliferation after ERN1 knockdown (Auf et al. 2010). Hypoxic regulation of LIM homeobox proteins also controls by ERN1 signaling, but it is still difficult to explain the meaning of the obtained results, because these homeobox proteins have different effects on cancer cell proliferation and tumor growth (Liao et al. 2017; Miyashita et al. 2018; Liang et al. 2019; Li et al. 2022b; Wang et al. 2022; Xie et al. 2022).

Hypoxia is an important factor in tumor growth due to the fact that ER stress induces resistance to the toxic effects of hypoxia through genome reprogramming and promotes tumor growth (Bi et al. 2005). Hypoxic regulation of gene expression is realized preferentially through transcription factor HIF but many other factors can modulate the impact of hypoxia on gene expression (Minchenko and Caro 2000; Denko 2008; Minchenko et al. 2013, 2020). At the same time, the expression of genes under hypoxia is gene-specific and is controlled by ER stress through the PERK/eIF2a/ATF4 module and ERN1 signaling of the UPR (Fels and Koumenis 2006; Minchenko et al. 2013, 2020; Sliusar et al. 2023). ERN1 inhibition may modify the hypoxic regulation of gene expression by genome reprogramming through specific changes in additional factors, which can interact with HIF and modulate its activity (Minchenko and Caro 2000).

Conclusions

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding some homeobox transcription factors, such as *MEIS1*, *MEIS2*, *MEIS3*, *LHX1*, *LHX2*, *LHX6, SPAG4*, and *NKX3-1* in glioblastoma cells in response to hypoxia and their relation to inhibition of ERN1 activity and cell proliferation attesting to the fact that hypoxia as well as ER stress are necessary components of the glioblastoma growth. Present results also validate the interaction of ER stress signaling mediated by ERN1 with hypoxia in a gene-specific manner, but the detailed molecular mechanisms of this regulation have not been yet clearly defined and warrant further investigations.

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Conflict of interest: *The authors declare no conflicts of interest.*

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