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ERN1 dependent impact of glucose and glutamine deprivations on PBX3, PBXIP1, PAX6, MEIS1, and MEIS2 genes expression in U87 glioma cells

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Objective. Homeobox genes play a fundamental role in the embryogenesis, but some of them have been linked to oncogenesis. The present study is aimed to investigate the impact of glucose and glutamine deprivations on the expression of homeobox genes such as *PAX6* (paired box 6), *PBX3* (*PBX* homeobox 3), *PBXIP1* (PBX homeobox interacting protein 1), *MEIS1* (*MEIS* homeobox 1), and *MEIS2* in ERN1 knockdown U87 glioma cells with the intent to reveal the role of ERN1 (endoplasmic reticulum to nucleus signaling 1) signaling pathway on the endoplasmic reticulum stress dependent regulation of homeobox genes.

Methods. The control (transfected by empty vector) and ERN1 knockdown (transfected by dominant-negative ERN1) U87 glioma cells were exposed to glucose and glutamine deprivations for 24 h. The cells RNA was extracted and reverse transcribed. The expression level of *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* genes was evaluated by a real-time quantitative polymerase chain reaction analysis and normalized to ACTB.

Results. It was found that glucose deprivation down-regulated the expression level of *PAX6*, *MEIS1*, and *MEIS2* genes in control glioma cells, but did not significantly alter *PBX3* and *PBXIP1* genes expression. At the same time, ERN1 knockdown significantly modified the sensitivity of all studied genes to glucose deprivation. Other changes in gene expression were detected in control glioma cells under the glutamine deprivation. The expression of *PBX3* and *MEIS2* genes was down- while *PAX6* and *PBXIP1* genes up-regulated. Furthermore, ERN1 knockdown significantly modified the effect of glutamine deprivation on the majority of studied genes expression in U87 glioma cells.

Conclusion. The results of the present study demonstrate that the exposure of U87 glioma cells under glucose and glutamine deprivations affected the expression of the majority of the studied homeobox genes and that the sensitivity of *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* genes expression under these experimental conditions is mediated by ERN1, the major pathway of the endoplasmic reticulum stress signaling.

Key words: U87 glioma cells, ERN1 knockdown, glucose and glutamine deprivations, homeobox genes, mRNA expression

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The homeobox genes encode an evolutionarily highly conserved family of transcriptional factors, which are sequence-specific DNA binding proteins. They regulate transcription and play very important role in the normal development and tumorigenesis (Li et al. 2016; Mark et al. 1997; Feng et al. 2021). Some of them have relation to β-cell development and are involved in the transcriptional regulation of insulin, glucagon, and somatostatin genes expression via binding to a common element of promoters (Sander et al. 1997; Laakso et al. 2009; Ritz-Laser et al. 2002). In addition, most of the homeobox transcription factors, including MEIS1 (MEIS homeobox 1) and MEIS2, have carcinogenic or tumor suppressive effects in different neoplasms (Wang et al. 2008; Chen et al. 2016; Girgin et al. 2020; Choi et al. 2021; Jiang et al. 2021; Li et al. 2022). 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. It also may contribute to the cancer-promoting actions of homeobox protein HOXB13 in cellular proliferation (Zhu et al. 2017; Johng et al. 2019; Li et al. 2022).

At the same time, the suppression of *MEIS1* gene expression by inhibitor of WNT efficiently inhibits the colorectal cancer stemness and metastasis (Choi et al. 2021). Similar results have been shown for MEIS2. Ectopic expression of this protein markedly enhances neuroblastoma cell proliferation, anchorage-independent growth, and tumorigenicity (Zha et al. 2014) and inhibits the proliferation and promotes apoptosis of thyroid cancer cells (Wen et al. 2021). MEIS proteins are involved in the transcriptional regulation of PAX6 (paired box 6) and PBX proteins, which also participate in the tumorigenesis (Li et al. 2016; Ooki et al. 2018; Khumukcham and Manavathi 2021; Wang et al. 2022). There are data indicating that inhibition of PAX6 transcripts leads to a decline in cell growth and survival and that the transcription factor PBX3 (PBX homeobox 3) promotes tumor cell growth through transcriptional suppression of the tumor suppressor p53 (Mascarenhas et al. 2009; Li et al. 2021). Protein PBXIP1 (PBX homeobox interacting protein 1) is a protooncogene and also promotes cancer cell proliferation (Chen et al. 2016; Khumukcham and Manavathi 2021).

Glucose and glutamine, as a substrate for glycolysis and endoplasmic reticulum stress, are very important complementary factors for the development and a more aggressive behavior of gliomas, which are highly aggressive tumors with poor prognosis (Colombo et al. 2011; Qing et al. 2012; Doultsinos et al. 2017; Zhao et al. 2017; Papaioannou and Chevet 2018; Almanza et al. 2019; Lypova et al. 2019; Alimohammadi et al. 2020). The inhibition of endoplasmic reticulum stress signaling mediated by ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1/inositol requiring enzyme 1) significantly suppresses the glioma cell proliferation and tumor growth and modifies the impact of nutrient deprivation on many genes expression (Auf et al. 2010, 2013; Minchenko et al. 2013, 2015a, 2020, 2021; Tsymbal et al. 2016, 2020; Hetz et al. 2019; Riabovol et al. 2019). Bioinformatics profiling identifies a glucose-related risk signature for the glioma malignancy (Zhao et al. 2017). It is known that glucose deprivation has a deleterious effect on cancer glycolysis, which may even conduce to cell death (Gatenby and Gillies 2007).

The complete molecular mechanisms of the interaction of ERN1 mediated stress signaling pathway with glutamine or glucose are complex and warrant further study. The nutrient starvation is a very important factor of cancer cells chemoresistance (Awale et al. 2006; Raffaghello et al. 2008; Buono and Longo 2018; Jiang et al. 2019). Arctigenin, as a natural lignan compound extracted from *Arctium lappa*, inhibits the proliferation of various cancer cells and induces tumor cell death under nutrient starvation probably by inhibiting the unfolded protein response (Awale et al. 2006; Kim et al. 2010; Gu et al. 2012; He et al. 2018). Glutamine supports the cancer cell growth and survival and influences the other nutrients on the cancer cell adaptation to glutamine starvation (Jiang et al. 2019). The transformed cells require much larger concentration of glutamine to survive than not transformed ones and deprivation of glutamine in cell culture reveals its potential for cancer treatment (Rubin 2019).

The aim of the present study was to investigate the impact of glucose and glutamine deprivations on the expression of homeobox genes such as *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* in ERN1 knockdown U87 glioma cells to reveal the role of ERN1 signaling pathway in this endoplasmic reticulum stress dependent regulation of homeobox genes expression.

Material 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, 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 U87 glioma cells. One was obtained by selection of stable transfected clones with overexpression of vector pcDNA 3.1, which was used for creation of dnERN1. These untreated glioma cells were used as control 1 (control glioma cells) in the study of the effect of glucose and glutamine deprivations on the level of homeobox genes expression. 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 and do not express spliced variant of XBP1, a key transcription factor in ERN1 signaling, and do not have the phosphorylated isoform of ERN1 after induction of endoplasmic reticulum stress by tunicamycin (Auf et al. 2010, 2013; Minchenko et al. 2015b). The impact of glucose and glutamine deprivations on the expression of *MEIS1*, *MEIS2*, *PAX6*, *PBX3*, and *PBXIP1* genes in cells with ERN1 deficiency introduced by dnERN1, was compared with cells transfected with the previously mentioned, empty vector (control glioma cells, pcDNA3.1). Both sublines of glioma cells used in this study grew in the presence of geneticin (G418), while carrying empty vector pcDNA3.1 or dnERN1 construct. Glucose and glutamine deprivations were created by changing the complete DMEM medium into culture plates on DMEM medium without glucose or glutamine. The plates were exposed to these conditions for 16 h.

RNA isolation. Total RNA was extracted from glioma cells using the Trizol reagent according to 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 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 U87 glioma cells and cells with a deficiency of ERN1, introduced by dnERN1, by quantitative polymerase chain reaction 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 (Minchenko et al. 2018). Polymerase chain reaction 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, U.S.A.) and Metabion Int. AG (Martinstried, Germany) and used for quantitative polymerase chain reaction (Table 1).

The quantitative PCR analysis was performed using a special computer program "Differential expression calculator" and statistical analysis using Excel program and OriginPro 7.5 software as described previously (Rudnytska et al. 2021). Comparison of two means was performed by the use of two-tailed Student's *t*-test. $p<0.05$ was considered significant in all cases. The values of studied genes 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

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
PAX6	Paired box 6	F: 5'- tgtccaacggatgtgtgagt R: 5'- tttcccaagcaaagatggac	466–485 627–608	NM_000280.5
PBX3	PBX homeobox 3	F: 5'- aacaaccagaccaattcgcc R: 5'- cctccaaggccatcactgta	1054–1073 1262–1243	NM_006195.6
TBXIP1	PBX homeobox interacting protein 1	F: 5'- caggatgggctaagggaaca R: 5'- caggatgggctaagggaaca	803–822 994–975	NM_020524.4
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
ACTB	beta-actin	F: 5'- ggacttcgagcaagagatgg R: 5'- agcactgtgttggcgtacag	747–766 980–961	NM_001101

triplicate measurements performed in 4 independent experiments. 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 glucose and glutamine deprivations in homeobox gene expressions in relation to suppression of endoplasmic reticulum stress signaling mediated by ERN1, the expression of *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* genes in ERN1 knockdown and control glioma cells were evaluated. As shown in Figure 1, the expression of PAX6 mRNA in control glioma cells transfected by empty vector pcDNA3.1 decreased after exposure to glucose deprivation (–28%) in comparison with the cells growing in complete DMEM medium. The inhibition of ERN1 signaling enzyme function by dnERN1 significantly modified the sensitivity of *PAX6* gene expression to this experimental condition in comparison with corresponding control (Figure 1). In this case, a significant increase at the level of this mRNA expression (+41%) was found.

Investigating the effect of glucose deprivation on the expression of genes encoding PBX3 and PBXIP1 proteins in relation to inhibition of ERN1 function, the exposure of control glioma cells under glucose deprivation did not alter significantly the expression of these homeobox genes in comparison with the control cells (Figures 2, 3). At the same time, inhibition of ERN1 signaling protein lead to down-regulation of both *PBX3* and *PBXIP1* genes expression being more significant for *PBXIP1* gene (–18% for *PBX3* and –41% for *PBXIP1*) (Figures 2, 3).

Studying the effect of glucose deprivation on the expression of gene encoding MEIS1 and MEIS2 proteins in glioma cells in relation to complete inhibition of ERN1 signaling enzyme function, a small, but statistically significant down-regulation of both *MEIS1* and *MEIS2* genes expression in control glioma cells (transfected by empty vector) under glucose deprivation in comparison with cells growing in regular medium was found (Figures 4, 5). Inhibition of ERN1 signaling enzyme function significantly

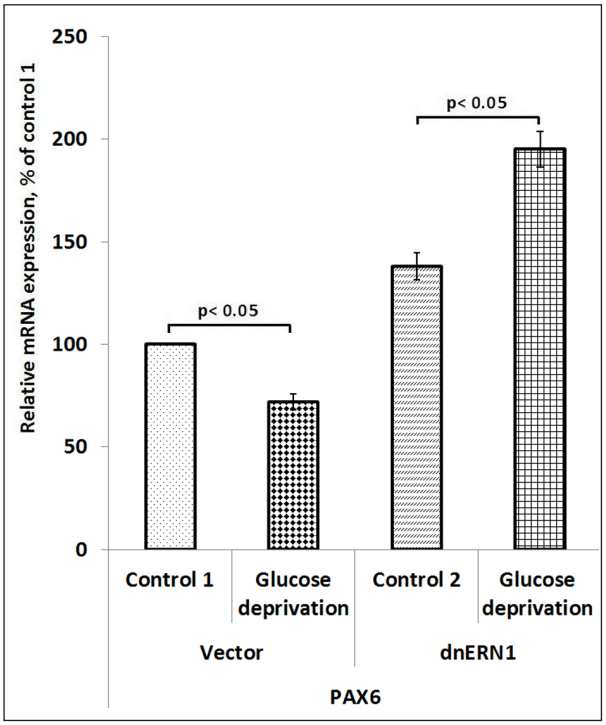


Figure 1. Effect of glucose deprivation on the paired box 6 (PAX6) mRNA expression in control U87 glioma cells (Vector) and cells with an inhibition of the ERN1 signaling protein by dnERN1 measured by qPCR. PAX6 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

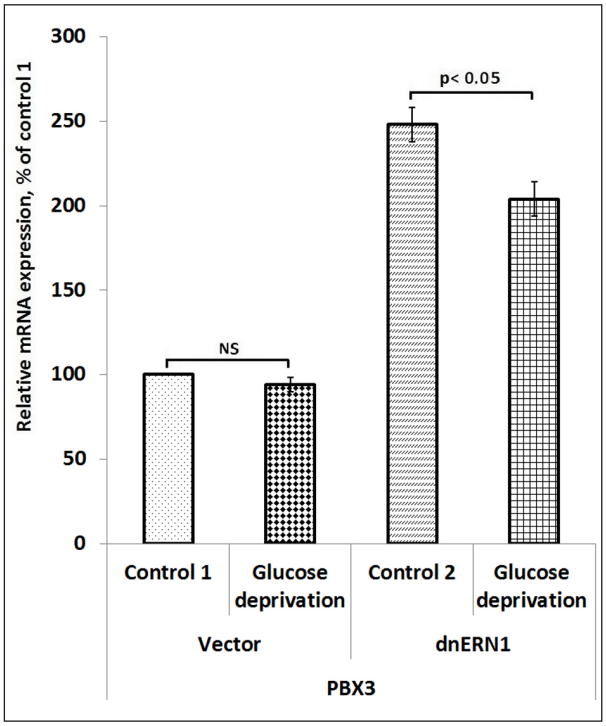


Figure 2. Effect of glucose deprivation on the PBX homeobox 3 (PBX3) mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. PBX3 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4. Abbreviations: NS – not significant changes.

enhanced the sensitivity of these genes expression to glucose deprivation. Thus, the expression of *MEIS1* and *MEIS2* genes was down-regulated on –41% and –52%, respectively, as compared to corresponding control cells (transfected by dnERN1 (Figures 4, 5).

Slightly different changes in the expression of these homeobox genes in glioma cells affected by glutamine deprivation were detected. As shown in Figure 6, exposure of control glioma cells under glutamine deprivation lead to an up-regulation of PAX6 mRNA expression (+28%) in comparison with cells growing under condition with glutamine in the medium. At the same time, inhibition of both enzymatic activities of ERN1 eliminated this impact of glutamine deprivation on PAX6 mRNA expression (Figure 6). The expression of *PBX3* homeobox gene was down-regulated (–23%) in control glioma cells treated by glutamine and inhibition of ERN1 significantly enhanced the sensitivity of this homeobox gene to glutamine deprivation (–42%) in comparison with corresponding control cells growing with glutamine presence (Figure 7). As shown in Figure 8, the expression of *PBXIP1* gene was up-regulated (+35%) in control glioma cells treated by glutamine deprivation and these changes were dependent on the functional activity of ERN1 signaling protein because knockdown of ERN1 completely removed the sensitivity of this homeobox gene expression to glutamine deprivation.

Investigating the impact of glutamine deprivation on the expression of *MEIS1* gene in relation to ERN1 function inhibition, the exposure of control glioma cells under glutamine deprivation did not show significant change in the expression of this homeobox gene in comparison with the cells growing in the presence of glutamine (Figure 9). It is possible that this resistance to glutamine deprivation is mediated by ERN1 signaling because inhibition of ERN1 lead to an up-regulation (+28%) of *MEIS1* gene expression (Figure 9). At the same time, the expression of another studied *MEIS* gene was very sensitive to glutamine deprivation. As shown in Figure 10, the expression of *MEIS2* gene was significantly down-regulated (–52%) in control glioma cells treated by glutamine deprivation. Similar effect of glutamine deprivation on *MEIS2* gene expression was also observed in ERN1 knockdown glioma cells (–50%) showing relative

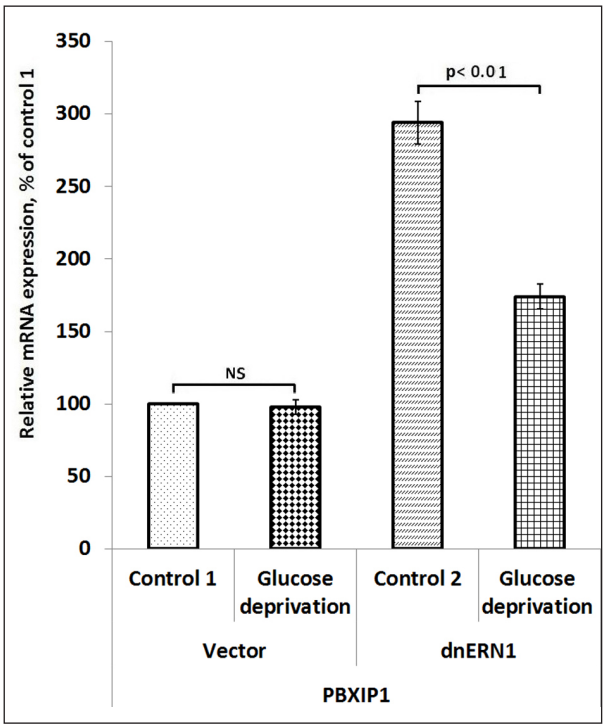


Figure 3. Effect of glucose deprivation on the PBX homeobox interacting protein 1 (PBXIP1) mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. PBXIP1 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

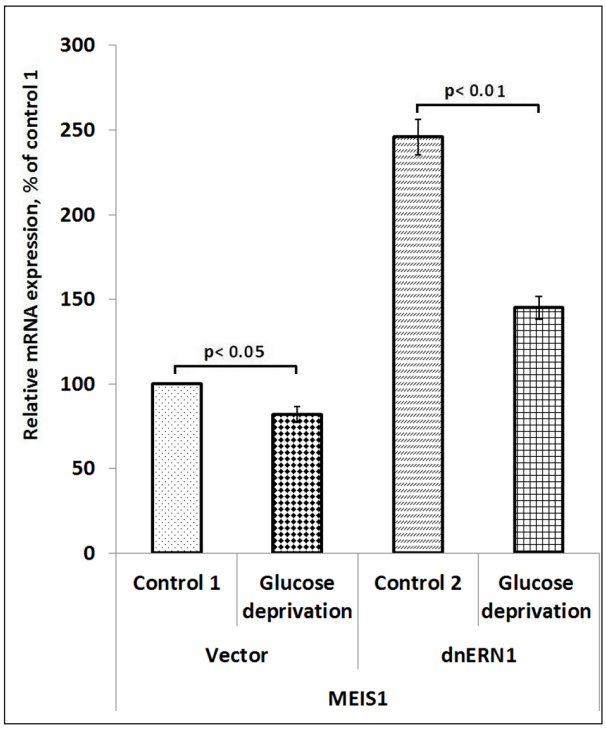


Figure 4. Effect of glucose deprivation on the MEIS homeobox 1 (MEIS1) mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. MEIS1 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

independence of this regulation from endoplasmic reticulum stress mediated by ERN1.

Both glucose and glutamine deprivations affected the expression of genes encoding the homeobox family transcription factors in 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 nutrient deprivation. Results of this investigation are summarized in Figure 11 demonstrating the gene-specific character of changes in the expression profile of studied homeobox genes in glioma cells under both glucose and glutamine deprivations via endoplasmic reticulum stress signaling mediated by ERN1.

Discussion

In this work, the effect of glucose and glutamine deprivations on the expression of genes encoding important homeobox proteins, which control transcription, preferentially the transcription or regulatory factors in U87 glioma cells in relation to inhibition of ERN1 the major signaling pathway of the unfolded protein response, was studied. The control glioma cells, transfected by empty vector pcDNA3.1 and cells with full ERN1 deficiency introduced by dnERN1 (ERN1 knockdown) were used. This was important for appraisal of a possible significance of ERN1 dependent control of glioma cell proliferation because the endoplasmic reticulum stress signaling mediated by ERN1 is involved in numerous metabolic pathways and ERN1 knockdown displays clear anti-tumor effects (Auf et al. 2010, 2013; Bravo et al. 2013; Minchenko et al. 2014, 2015b; Logue et al. 2018). Results of this study clarify possible mechanisms of influence of glucose and glutamine deprivations on the proliferation/surviving of ERN1 knockdown glioma cells through specific changes in the expression profile of genes encoding important transcription factors of homeobox family such as PAX6, PBX3, PBXIP1, MEIS1, and MEIS2.

We are showing that all studied genes have different sensitivity to glucose deprivation (Figure 11). Thus, the expression of *PAX6*, *MEIS1*, and *MEIS2* genes in control glioma cells is down-regulated by

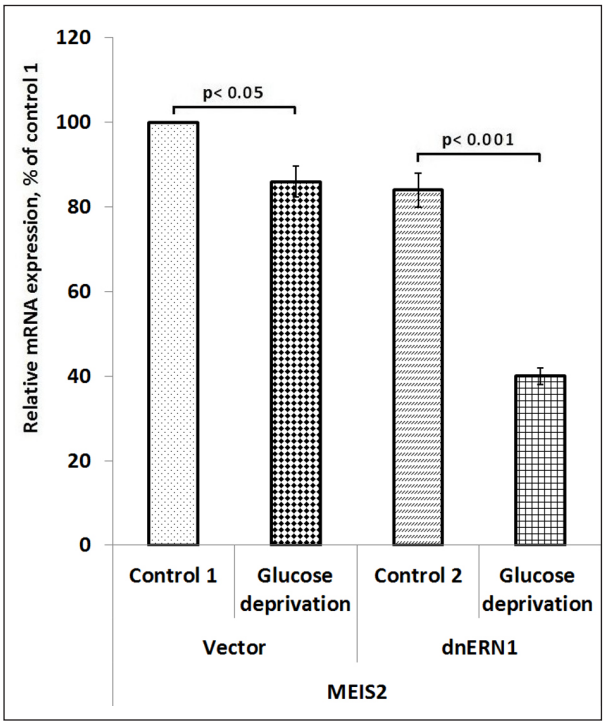


Figure 5. Effect of glucose deprivation on the MEIS homeobox 2 (MEIS2) mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. MEIS2 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

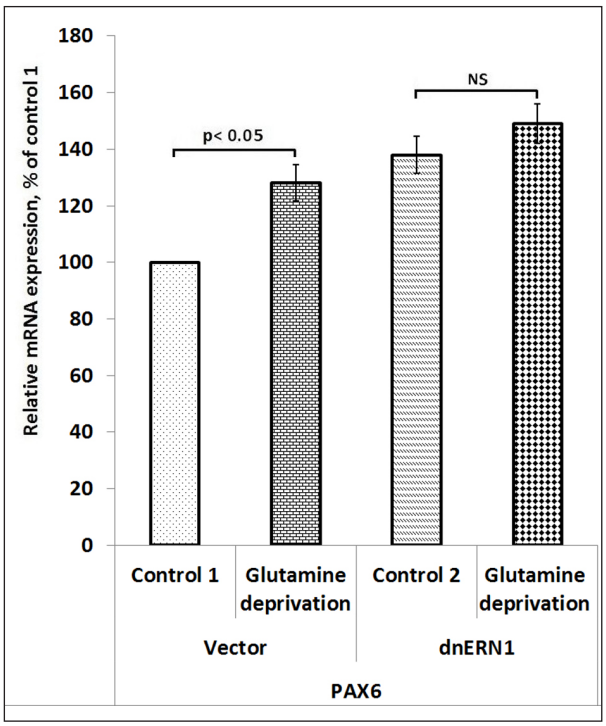


Figure 6. Effect of glutamine deprivation on the PAX6 mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 signaling protein by dnERN1 measured by qPCR. PAX6 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

glucose deprivation, while *PBX3* and *PBXIP1* gene expressions are resistant to this treatment. At the same time, ERN1 knockdown significantly modified the sensitivity of all studied genes to glucose deprivation. Thus, the expression of *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* genes significantly decreased by ERN1 knockdown, in *MEIS1* and *MEIS2* genes in greater extent than control glioma cells.

It is well known that activation of ERN1 branch of the endoplasmic reticulum stress response under stressful environmental conditions is tightly linked to apoptosis. The suppression of its function has been demonstrated to result in a significant anti-proliferative effect in glioma growth (Auf et al. 2010, 2013; Minchenko et al. 2015b; Hetz et al. 2019). ERN1 dependent suppression of *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* gene expressions in glioma cells under glucose deprivation agree well with its involving in cancer biology and decreased cell proliferation (Auf et al. 2010, 2013; Minchenko et al. 2014; Zha et al. 2014; Hetz et al. 2019; Johng et al. 2019; Choi et al. 2021; Li et al. 2021, 2022; Wen et al. 2021). The present data also indicate that the endoplasmic reticulum stress mediated by ERN1 creates the resistance to glucose deprivation and that ERN1 blockade suppresses this resistance. At the same time, we showed that glucose deprivation increases the expression of *PAX6* gene both in control and ERN1 knockdown glioma cells. It is possible that this effect of glucose deprivation on *PAX6* gene expression is due to glucose deficiency and plays an important role of PAX6 in the support of insulin expression (Sander et al. 1997).

The results of this study also demonstrate variable effects of glutamine deprivation on the expression of homeobox genes encoding proteins PAX6, PBX3, PBXIP1, MEIS1, and MEIS2, which diversely affect the tumor growth (Zha et al. 2014; Johng et al. 2019; Choi et al. 2021; Wen et al. 2021; Li et al. 2021, 2022). The exposure of control glioma cells without glutamine leads to up-regulation of *PAX6* and *PBXIP1* and down-regulation of *PBX3* and *MEIS2* genes expression (Figures 11). We also showed that ERN1 knockdown in glioma cells removes the up-regulation of *PAX6* and *PBXIP1* genes expression introduced by glutamine deprivation and enhances the sensitivity of *PBX3* gene expression to this experimental condition.

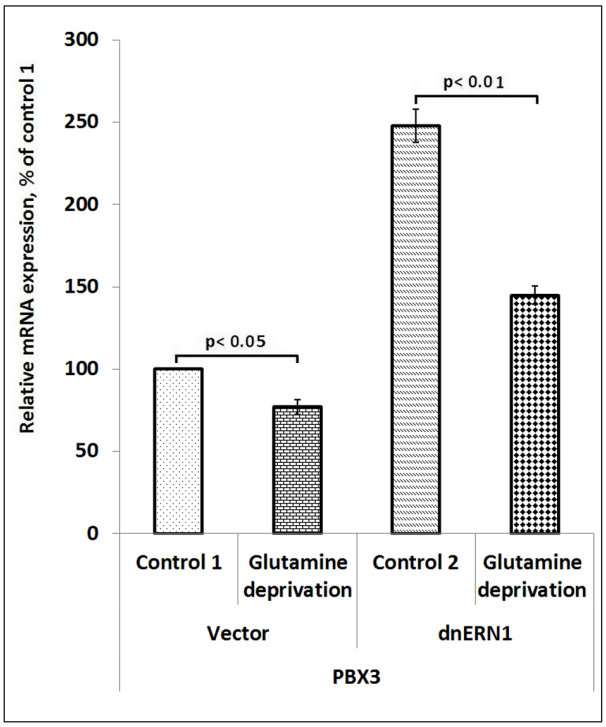


Figure 7. Effect of glutamine deprivation on the PBX3 mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. PBX3 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4. Abbreviations: NS – no significant changes.

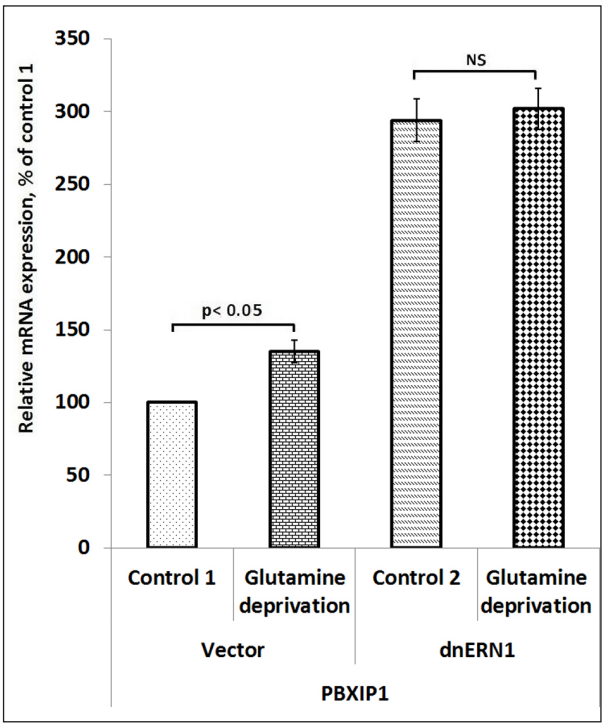


Figure 8. Effect of glutamine deprivation on the expression level of PBXIP1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. PBXIP1 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

The functional significance of these changes in the expression of *PAX6*, *PBX3*, *PBXIP1*, and *MEIS2* genes, which preferentially have diverse impacts on cell proliferation and tumor growth, is under glutamine deprivation condition possibly connected with glutamine deficiency mediated by ERN1 (Auf et al. 2010; Zha et al. 2014; Li et al. 2016, 2021; Khumukcham and Manavathi 2021; Minchenko et al. 2021; Wen et al. 2021; Wang et al. 2022). It is also possible that the endoplasmic reticulum stress mediated by ERN1 creates the resistance to glutamine deprivation and that ERN1 blockade lowers this resistance. At the same time, we showed that glutamine deprivation increases the expression of *MEIS2* gene in both control and ERN1 knockdown glioma cells independently from ERN1 knockdown. These data also agree well with the suppression of cell proliferation under glutamine deprivation because glutamine is very important factor for tumor growth (Drogat et al. 2007; Colombo et al. 2011; Cluntun et al. 2017; Jiang et al. 2019; Yoo et al. 2020).

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding some homeobox transcription factors, such as *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* in glioma cells in response to glucose and glutamine deprivations and their relation to inhibition of ERN1 activity and cell proliferation, attesting to the fact that endoplasmic reticulum stress as well as glucose and glutamine are necessary components of the glioma growth. Present results also validate the interaction of endoplasmic reticulum stress signaling mediated by ERN1 with both glucose and glutamine deprivations in 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 interests: *The authors declare no conflict of interest.*

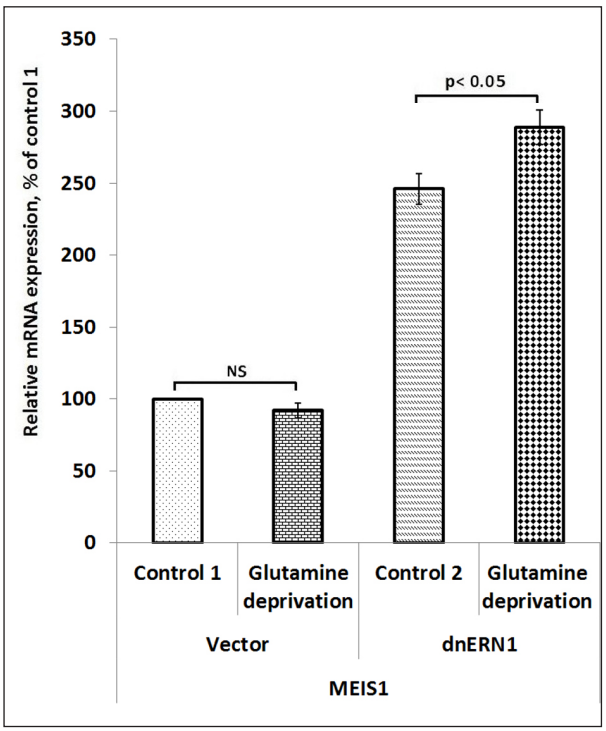


Figure 9. Effect of glutamine deprivation on the MEIS1 mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. MEIS1 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

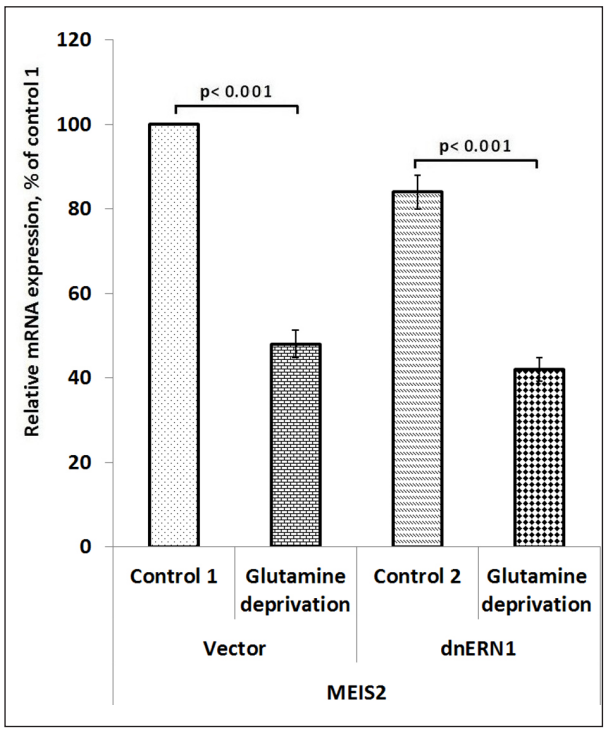


Figure 10. Effect of glutamine deprivation on the MEIS2 mRNA expression in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. MEIS2 mRNA expression values were normalized to beta-actin mRNA level as percentage of control 1 (100%); n=4.

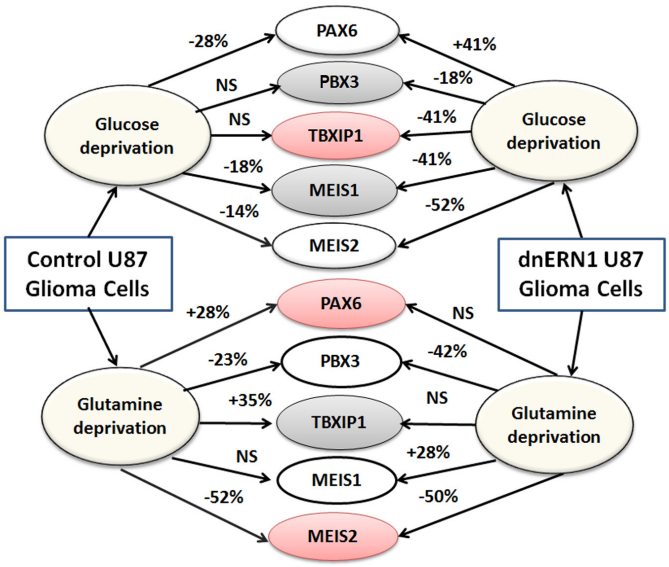


Figure 11. Schematic depiction of the changes in the expression profile of *PAX6*, *PBX3*, *PBXIP1*, *MEIS1*, and *MEIS2* genes in control and ERN1 knockdown U87 glioma cells induced by glucose and glutamine deprivation conditions.

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