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ERN1 knockdown modifies the effect of glucose deprivation on homeobox gene expressions in U87 glioma cells

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Objective. The aim of the present investigation was to study the expression of genes encoding homeobox proteins ZEB2 (zinc finger E-box binding homeobox 2), TGIF1 (TGFB induced factor homeobox 1), SPAG4 (sperm associated antigen 4), LHX1 (LIM homeobox 1), LHX2, LHX6, NKX3-1 (NK3 homeobox 1), and PRRX1 (paired related homeobox 1) in U87 glioma cells in response to glucose deprivation in control glioma cells and cells with knockdown of ERN1 (endoplasmic reticulum to nucleus signaling 1), the major pathway of the endoplasmic reticulum stress signaling, for evaluation of it possible significance in the control of glioma growth through ERN1 signaling and chemoresistance.

Methods. The expression level of homeobox family genes was studied in control (transfected by vector) and ERN1 knockdown U87 glioma cells under glucose deprivation condition by real-time quantitative polymerase chain reaction.

Results. It was shown that the expression level of *ZEB2*, *TGIF1*, *PRRX1*, and *LHX6* genes was up-regulated in control glioma cells treated by glucose deprivation. At the same time, the expression level of three other genes (*NKX3-1*, *LHX1*, and *LHX2*) was down-regulated. Furthermore, ERN1 knockdown of glioma cells significantly modified the effect glucose deprivation condition on the expression almost all studied genes. Thus, treatment of glioma cells without ERN1 enzymatic activity by glucose deprivation condition lead to down-regulation of the expression level of *ZEB2* and *SPAG4* as well as to more significant up-regulation of *PRRX1* and *TGIF1* genes. Moreover, the expression of *LHX6* and *NKX3-1* genes lost their sensitivity to glucose deprivation but *LHX1* and *LHX2* genes did not change it significantly.

Conclusions. The results of this investigation demonstrate that ERN1 knockdown significantly modifies the sensitivity of most studied homeobox gene expressions to glucose deprivation condition and that these changes are a result of complex interaction of variable endoplasmic reticulum stress related and unrelated regulatory factors and contributed to glioma cell growth and possibly to their chemoresistance.

Key words: ERN1 knockdown, homeobox genes, mRNA expression, glucose deprivation, U87 glioma cells

Malignant gliomas are highly aggressive tumors with very poor prognosis and glucose as a substrate for glycolysis is important for the glioma development and a more aggressive behavior through regulation of the cell cycle (Minchenko et al. 2002; Colombo et al. 2011; Yalcin et al. 2014; Guo et al. 2016; Zhao et al.

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2017; Alimohammadi et al. 2019). It is interesting to note that glucose deprivation as well as endoplasmic reticulum stress are very important and complementary factors for tumor growth and that ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1/inositol requiring enzyme 1) mediated stress signaling can significantly modify the effects of glucose deprivation on gene expressions (Minchenko et al. 2013, 2015a; Tsymbal et al. 2016a, b; Iurlaro et al. 2017; Riabovol et al. 2019; Teramoto and Katoh 2019). It has also been shown that ERN1/XBP1 pathway is essential for the glucose response and protection of β cells (Hassler et al. 2015). Bioinformatic profiling identifies a glucose-related risk signature for the malignancy of glioma (Zhao et al. 2017). However, the detailed molecular mechanisms of the interaction of glucose deprivation with ERN1 mediated stress signaling pathway are complex and warrant further study. Furthermore, there are data indicating that glucose is an important factor for cancer cells chemoresistance (Awale et al. 2006). Thus, arctigenin, a natural lignan compound extracted from Arctium lappa, inhibits the growth of various cancer cells and induces tumor cell death under glucose deprivation possibly by blocking the unfolded protein response (Awale et al. 2006; Kim et al. 2010; Gu et al. 2012; He et al. 2018). Moreover, this antitumor agent has ability to eliminate the tolerance of cancer cells to glucose deprivation, but glucose deprivation leads to suppression of chemoresistance through unknown mechanisms.

Transcription factors play an important role in the malignant tumor growth, preferentially through ERN1 (endoplasmic reticulum to nucleus signaling 1) signaling pathway of endoplasmic reticulum stress (Minchenko et al. 2015b, c; Tsymbal et al. 2016a). The homeobox proteins represent an important group of transcription factors, which are related to tumorigenesis and has been largely investigated (Kataoka et al. 2001; Guca et al. 2018; Le Magnen et al. 2018; Miyashita et al. 2018; Chen et al. 2019; Hamaidi et al. 2019; Li et al. 2019; Wang et al. 2019). Recently, it was shown that transcriptional inhibitor ZEB2 (zinc finger E-box binding homeobox 2) functions as oncogene in human laryngeal squamous cell carcinoma (Li et al. 2019). Furthermore, transcription factor ETS1 (ETS proto-oncogene 1) is coexpressed with ZEB2 and mediates ZEB2-induced epithelial-mesenchymal transition in human tumors (Yalim-Camci et al. 2019). Moreover, ZEB2 regulates the activity of ETS1 by direct binding to its promoter, but migration and invasion of tumor cells are regulated by ZEB2-induced ETS1 activity, where ZEB2 acts as an upstream positive regulator of ETS1. At the same time, Zhu et al. (2019) have shown that miR-138-5p inhibits epithelial-mesenchymal transition, growth and metastasis of lung adenocarcinoma cells through targeting ZEB2.

TGIF1 (TGFB induced factor homeobox 1) is a member of homeobox proteins, which are highly conserved transcription regulators. It binds to retinoid X receptor responsive element from the cellular retinol-binding protein II promoter. In addition to its role in inhibiting 9-cis-retinoic acid-dependent RXR alpha transcription activation of the retinoic acid responsive element, the protein is an active transcriptional co-repressor of SMAD2 and represses TGF-beta signaling (Guca et al. 2018). Furthermore, it has been shown that upregulation of TGIF1 by carcinogen BaP is associated with cell proliferation, cell migration, tumor invasiveness, and metastasis of lung adenocarcinoma cells (Yang et al. 2018) and that silencing of TGIF1 suppresses migration, invasion, and metastasis of human breast cancer cells (Wang et al. 2018). SPAG4 (sperm associated antigen 4) has a fundamental pathological function in the migration of lung carcinoma cells (Knaup et al. 2014; Ji et al. 2018).

Homeobox-containing transcription factor NKX3-1 (NKX3A or NK3 homeobox 1) functions as a negative regulator of epithelial cell growth in prostate tissue and its aberrant expression is associated with prostate tumor progression as a tumor suppressor, because it can inhibit proliferation and invasion activities of PC-3 prostate cancer cells (Le Magnen et al. 2018). Furthermore, NKX3-1 is required for induced pluripotent stem cell reprogramming and can replace OCT4 and generate fully pluripotent stem cells (Mai et al. 2018). There is data, which indicates the regulation of C-MYC by NKX3.1 protein (Fonseca-Alves et al. 2018). Regulation of NKX3.1 by androgens and 17beta-estradiol in prostate cancer cells suggest that it may have important regulatory roles during prostate cancer progression (Korkmaz et al. 2000).

Paired related homeobox 1 (PRRX1) as well as LIM homeobox proteins (LHX1, LHX2 and LHX6) have also relation to tumor growth (Hamaidi et al. 2019; Liang et al. 2019; Marchand et al. 2019; Wang et al. 2019). There are data that PRRX1 may be one of the main driving forces for the cellular phenotype plasticity and tumor dormancy of head and neck squamous cell carcinoma and that LHX1 is involved in the expression of various proteins regulating cell movements and EMT in renal carcinoma cells and that silencing of LHX1 decreased pulmonary metastasis in the *in vivo* model (Hamaidi et al. 2019; Jiang et al. 2019). Recently, it was shown that LHX2 acts as a transcriptional activator and is involved in the

control of cell differentiation in developing lymphoid and neural cell types and that miR-506 inhibits tumor growth and metastasis via inhibition of WNT/beta-catenin signaling by down-regulating LHX2 (Liang et al. 2019). Furthermore, there are also data indicating that LHX2 is capable of blocking proliferation of human T cell acute lymphoblastic leukemia-derived cells (Miyashita et al. 2018). LHX6 contains the LIM domain, a unique cysteine-rich zinc-binding domain. The encoded protein has tandem LIM domains as well as a DNA-binding homeodomain. Recently, it was shown that transcription factor LHX6 is tumor suppressor and its expression is regulated by DNA methylation and can inhibit the proliferation, invasion and migration through WNT/beta-catenin and TP53 signaling pathways during the Microcystin-LR-induced hepatocarcinogenesis (Nathalia et al. 2018; Chen et al. 2019). Furthermore, miR-1290 promotes proliferation, migration, and invasion of glioma cells by inhibiting LHX6, while LHX6 overexpression inhibited tumor growth (Yan et al. 2018). It is possible that these transcription factors are regulating by long noncoding RNAs in human cancers (Liu et al. 2018).

Malignant tumors use endoplasmic reticulum stress response and its signaling pathways to adapt and to enhance tumor cells proliferation under stressful environmental conditions (Huber et al. 2013; Manie et al. 2014; Papaioannou and Chevet 2018; Almanza et al. 2019). It is well known that activation of ERN1 branch of the endoplasmic reticulum stress response is tightly linked to apoptosis and to cell death, and suppression of its function has been demonstrated to result in significant anti-proliferative effect in glioma growth (Auf et al. 2010; Minchenko et al. 2014; Hetz et al. 2019). Furthermore, inhibition of ERN1 endoribonuclease has more strong anti-proliferative effect on glioma cells and leads to specific changes in the expression of genes related to ERN1 signaling pathway (Auf et al. 2013; Minchenko et al. 2015c).

The aim of this study was to examine the expression of genes encoding homeobox proteins in response to glucose deprivation condition in control U87 glioma cells and cells with complete inhibition of ERN1 enzymatic activity for evaluation of possible significance of glucose deprivation in the control of homeobox gene expressions and cell proliferation through IRE1 mediated endoplasmic reticulum stress signaling.

Materials and methods

Cell lines and culture conditions. The glioma cell line U87 was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (Gibco, Invitrogen, 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 work we used two sublines of these cells. One subline was obtained by selection of stable transfected clones with overexpression of vector pcDNA3.1, which was used for creation of dnERN1. This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of the effect of glucose deprivation on the level of gene expressions. 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). It has been shown that cells with dnERN1 have a lower proliferation rate, do not express spliced XBP1, a key transcription factor in ERN1 signaling, and have not the phosphorylated isoform of ERN1 after induction of endoplasmic reticulum stress by tunicamycin (Auf et al. 2010, 2013; Minchenko et al. 2015c). The expression of the studied genes in cells with a deficiency of ERN1, introduced by dnERN1, was compared with cells transfected with the previously mentioned, empty vector (control glioma cells, pcDNA3.1). Both used in this study sublines of glioma cells are grown in the presence of geneticin (G418) while these cells carrying empty vector pcDNA3.1 or dnERN1 construct. Glucose deprivation condition were created by changing the complete DMEM medium into culture plates on DMEM medium without glucose and plates were exposed to this condition 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 concentration 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

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
ZEB2	zinc finger E-box binding homeobox 2	F: 5'- actcctgtctgtctcgcaaa R: 5'- gctcgataaggtggtgcttg	3198–3217 3383–3364	NM_014795.4
TGIF1	TGFB induced factor homeobox 1	F: 5'- acaagttacgggagagtcgg R: 5'- gttgccccttctccttctct	208–227 441–422	NM_003244.3
SPAG4	sperm associated antigen 4	F: 5'- cagcttctggaactacgcac R: 5'- gtggatgctgcagagtgatg	1042–1061 1193–1174	NM_003116.3
PRRX1	paired related homeobox 1	F: 5'- cgtacagatcctcgtccctc R: 5'- tccttggccttcagtctcag	681–700 858–839	NM_006902.5
NKX3-1	NK3 homeobox 1	F: 5'- aagaacctcaagctcacgga R: 5'- tgtcacctgagctggcatta	524–543 591–572	NM_006167.4
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
ACTB	beta-actin	F: 5'- ggacttcgagcaagagatgg R: 5'- agcactgtgttggcgtacag	747–766 980–961	NM_001101

Biosystems, USA). Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described previously (Minchenko et al. 2019). Polymerase chain reaction was performed in triplicate. The expression of beta-actin mRNA was used as 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 used for quantitative polymerase chain reaction (Table 1).

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 (Minchenko et al. 2015c). 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 gene expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100%). All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments. The amplified DNA fragments were also analyzed on a 2% agarose gel and that visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

Results

To investigate a possible role of glucose deprivation and endoplasmic reticulum stress signaling mediated by ERN1 protein in the expression level of homeobox genes in U87 glioma cells we studied the effect of glucose deprivation on gene expressions in cells with and without ERN1 functional activity. As shown in Figure 1, the expression of zinc finger E-box binding homeobox 2 (ZEB2) mRNA in control glioma cells, transfected by empty vector pcDNA3.1, is increased after exposure under glucose deprivation condition (+19%) in comparison with the cells growing in complete DMEM medium. Furthermore, inhibition of ERN1 signaling enzyme function by dnERN1 is significantly modified the sensitivity of *ZEB2* gene expression to this experimental condition (Figure 1). Thus, the level of ZEB2 mRNA expression is strongly decreased (–52%) in cells without ERN1 signaling enzyme function. Next, we investigated the effect of glucose deprivation on the expression of gene encoding TGFB induced factor homeobox 1 (TGIF1) in relation to inhibition of ERN1 function. As shown in Figure 2, glucose deprivation condition results in significant induction of this homeobox gene expression (+57%) in comparison with control glioma cells, transfected by empty vector. At the same time, inhibition of ERN1 signaling protein function leads to more strong changes in the expression level of this gene (+132%; Figure 2).

We also studied the effect of glucose deprivation on the expression of gene encoding sperm associated antigen 4 (SPAG4) in control U87 glioma cells (Vector) and cells with ERN1 knockdown. As shown

in Figure 3, the expression of SPAG4 mRNA is resistant to glucose deprivation condition in control glioma cells but inhibition of ERN1 signaling protein leads to significant down-regulation of this mRNA expression (–49%) in comparison with dnERN1 cells growing with glucose.

We also investigated the effect of glucose deprivation condition on the expression of gene encoding paired related homeobox 1 (PRRX1) protein in glioma cells in relation to complete inhibition of ERN1 signaling enzyme function. It was shown that the expression level of *PRRX1* gene is up-regulated (+17%) in control glioma cells (transfected by empty vector) under glucose deprivation condition in comparison with cells growing in regular medium (Figure 4). Furthermore, inhibition of ERN1 signaling enzyme function significantly enhances the effect of glucose deprivation on this homeobox gene expression (+35%) as compared to corresponding control cells (transfected by dnERN1; Figure 4). At the same time, of control glioma cells under glucose deprivation condition leads to small but statistically significant down-regulation (–15%) of the expression of NK3 homeobox 1 (*NKX3-1*) gene, but suppression of ERN1 signaling eliminates the sensitivity of this gene expression to glucose deprivation (Figure 5).

We have also studied the effect of glucose deprivation on the expression of genes encoding LIM homeobox proteins with different properties (LHX1, LHX2, and LHX6) in relation to ERN1 knockdown. As shown in Figure 6 and Figure 7, exposure glioma cells under glucose deprivation condition leads to significant down-regulation of LHX1 and LHX2 mRNA expressions (–37 and –67%, respectively) in comparison with control cells growing under condition with glucose in the medium. Furthermore, inhibition of both enzymatic activities of ERN1 does not significantly modify the effect of glucose deprivation condition on the expression of LIM homeobox 1, but decreases the sensitivity of LIM homeobox 2 gene expression to this experimental condition. At the same time, the expression of LIM homeobox 6 gene is up-regulated (+33%) in control glioma cells treated by glucose deprivation condition, but ERN1 knockdown eliminates the sensitivity of this LIM homeobox gene to glucose deprivation in comparison with corresponding control cells growing under condition with glucose (Figure 8).

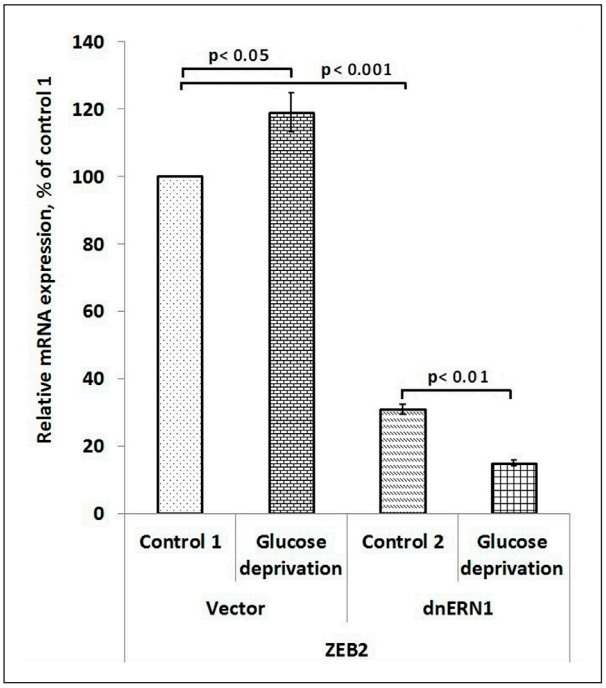

Figure 1. Effect of glucose deprivation on the expression level of zinc finger E-box binding homeobox 2 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of ZEB2 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

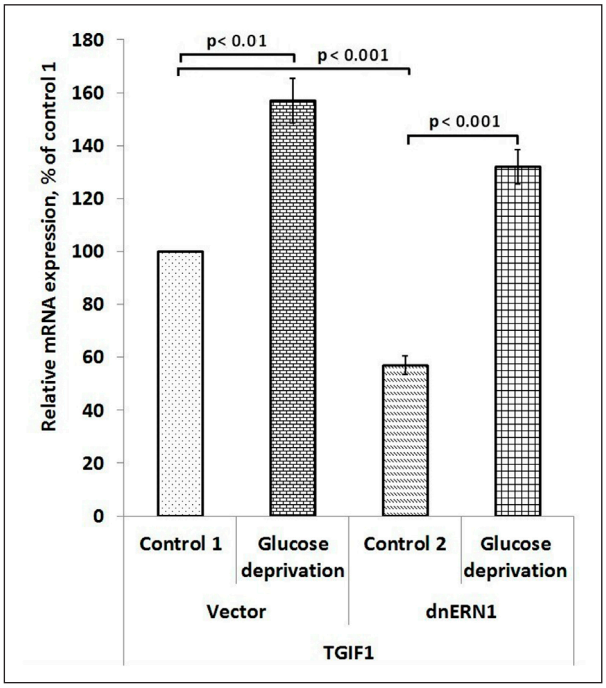

Figure 2. Effect of glucose deprivation on the expression level of TGFB induced factor homeobox 1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of TGIF1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

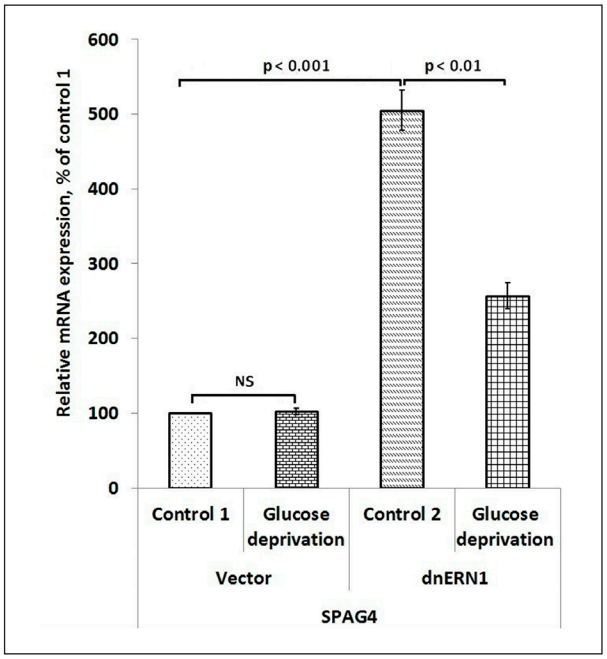

Figure 3. Effect of glucose deprivation on the expression level of sperm associated antigen 4 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of SPAG4 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

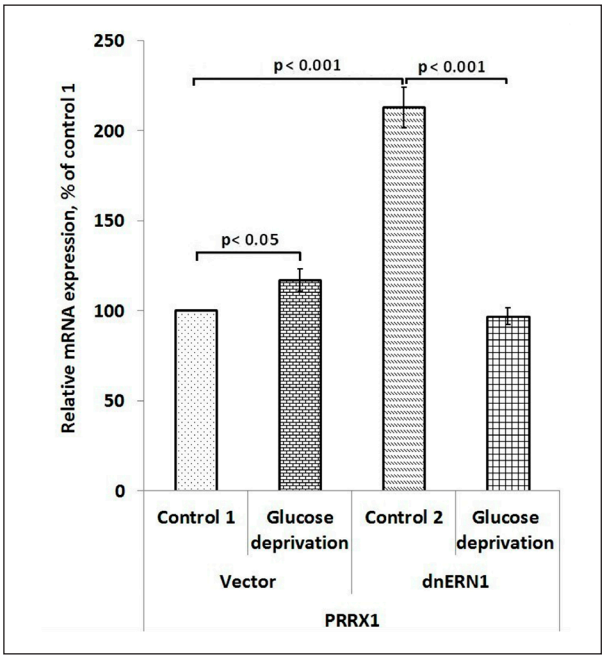

Figure 4. Effect of glucose deprivation on the expression level of paired related homeobox 1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PRRX1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

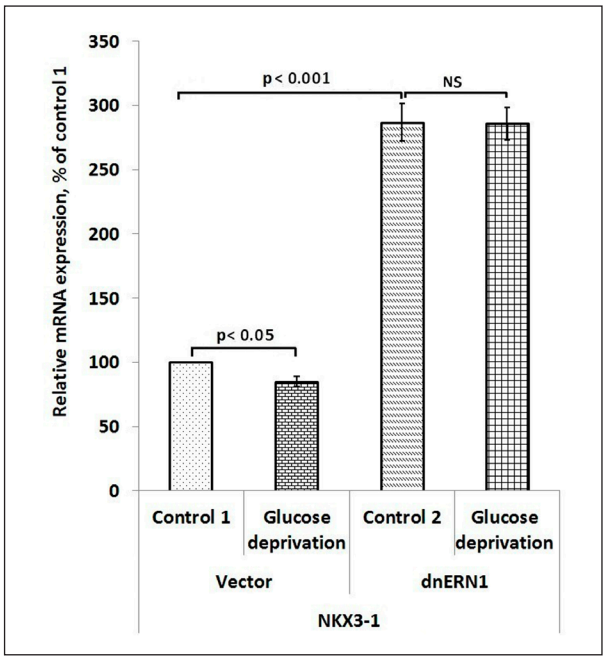

Figure 5. Effect of glucose deprivation on the expression level of NK3 homeobox 1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of NKX3-1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

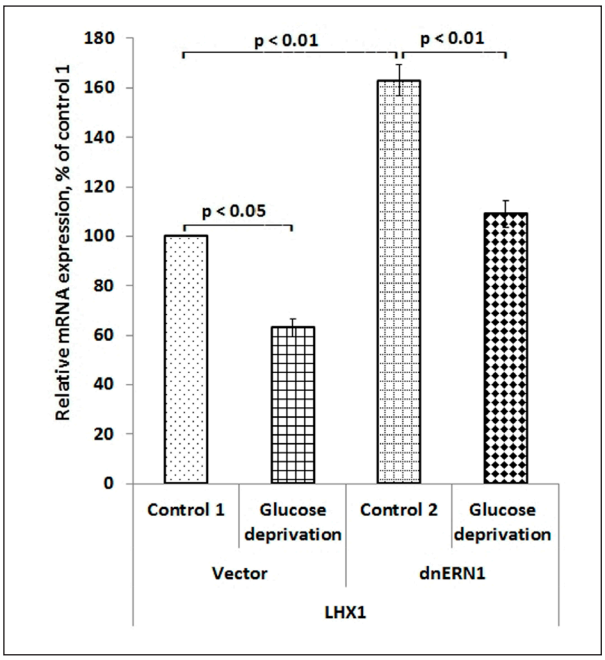

Figure 6. Effect of glucose deprivation on the expression level of LIM homeobox 1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of LHX1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

Thus, glucose deprivation condition affects the expression of different homeobox genes in gene-specific manner and these effects of glucose deprivation condition on gene expressions preferentially depends on ERN1 signaling. Results of this investigation are summarized in Figure 9, which clearly demonstrated the ERN1 dependent character of changes in the expression profile of most homeobox family genes in glioma cells under glucose deprivation.

Discussion

In this work, we studied the effect of glucose deprivation on the expression of genes encoding important homeobox proteins, which control transcription preferentially as a transcription factors, in U87 glioma cells in relation to inhibition of ERN1, the major signaling pathway of the unfolded protein response. We used control glioma cells, transfected by empty vector pcDNA3.1 and cells with full ERN1 deficiency introduced by dnERN1. This is important for the evaluation of possible significance of in ERN1 dependent control of glioma cell proliferation because endoplasmic reticulum stress signaling mediated by ERN1 is involved in numerous metabolic pathways and ERN1 knockdown has clear anti-tumor effects (Auf et al. 2010, 2013; Bravo et al. 2013; Manie et al. 2014; Minchenko et al. 2014, 2015a, c; Logue et al. 2018). Furthermore, there are data that glucose deprivation can enhance the sensitivity of cancer cells to anti-cancer drugs, particularly arctigenin, which inhibits the growth of various cancer cells and induces tumor cell death under glucose deprivation condition possibly by blocking the unfolded protein response and by inhibiting cellular energy metabolism (Awale et al. 2006; Kim et al. 2010; Gu et al. 2012; He et al. 2018). Results of our study clarify possible mechanisms of glucose deprivation on the proliferation/surviving of ERN1 knockdown glioma cells through specific changes in the expression of genes encoding important homeobox proteins.

We showed that ERN1 knockdown of glioma cells leads to a strong down-regulation of the expression of *ZEB2* gene under glucose deprivation. Homeobox protein ZEB2 is functioning as an oncogene in human laryngeal squamous cell carcinoma and acts as an upstream positive regulator of ETS1 (ETS proto-oncogene 1) (Li et al. 2019; Yalim-Camci et al. 2019). Furthermore, transcription factor ETS1 is

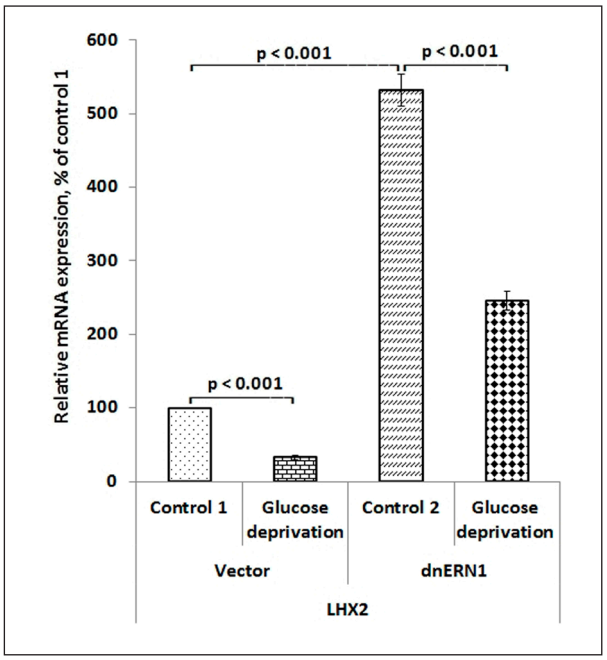

Figure 7. Effect of glucose deprivation on the expression level of LIM homeobox 2 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of LHX2 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

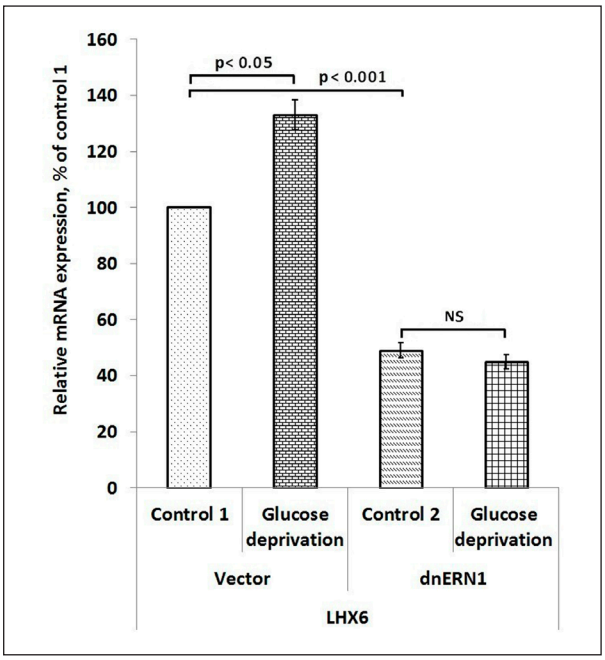

Figure 8. Effect of glucose deprivation on the expression level of LIM homeobox 6 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of LHX6 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

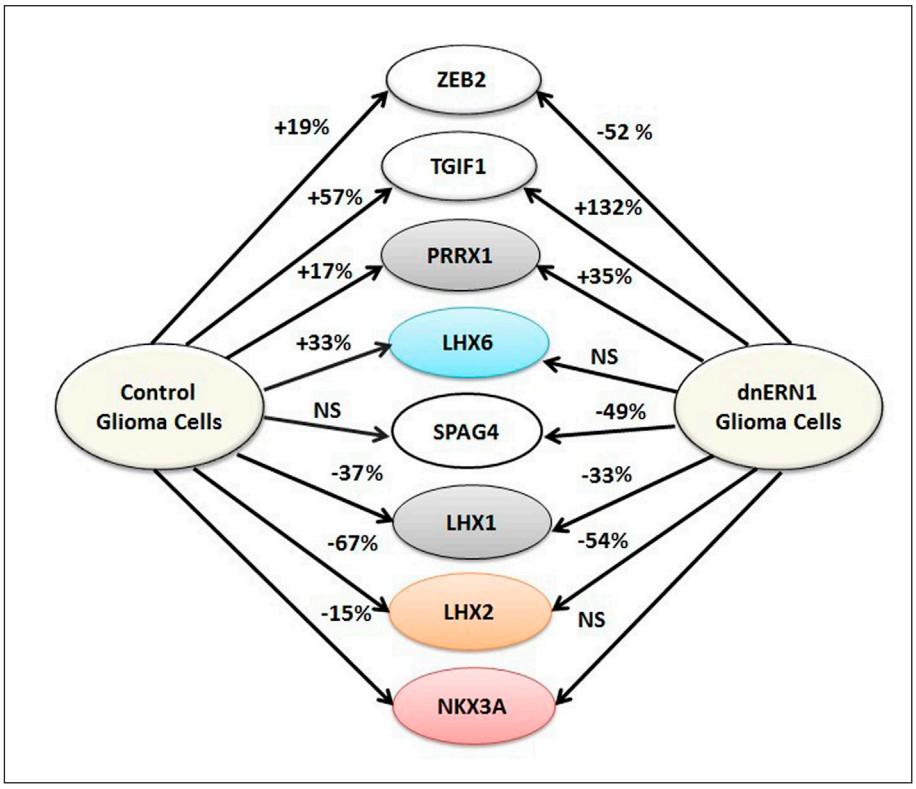

Figure 9. Schematic demonstration of changes in the expression profile of homeobox family genes in the control and ERN1 knockdown glioma cells under glucose deprivation; NS – no significant changes.

coexpressed with ZEB2 and mediates ZEB2-induced epithelial-mesenchymal transition in human tumors (Yalim-Camci et al. 2019). At the same time, transcription factor ZEB2 regulates the activity of ETS1 by directly binding to its promoter. Therefore, down-regulation of *ZEB2* gene expression under glucose deprivation may contribute to decreased proliferation potential of these cells (Auf et al. 2010; Minchenko et al. 2015c) and according to Kim et al. (2010) and Gu et al. (2012) decrease the chemoresistance of ERN1 knockdown glioma cells. We found both glucose deprivation and inhibition of endoplasmic reticulum stress.

Furthermore, we showed that the expression of *TGIF1* gene in glioma cells with inhibited ERN1 signaling is strongly up-regulated under glucose deprivation, while in control glioma cells the effect of glucose deprivation was significantly lower (Figure 9). TGFB induced factor homeobox 1 is a member of the atypical homeobox proteins, which are highly conserved transcription regulators. It is an active transcriptional co-repressor of SMAD2 and represses TGF-β signaling (Guca et al. 2018). At the same time, TGF-β signaling can suppresses tumor formation by inhibiting cell growth and apoptosis and can also promote cancer growth. It is possible that more significant induction of *TGIF1* gene expression in glioma cells with inhibited ERN1 signaling under glucose deprivation condition is also contributed to suppression of glioma cells proliferation by ERN1 knockdown. We also showed that the expression level of *SPAG4* gene is significantly suppressed under glucose deprivation only in glioma cells with ERN1 knockdown. Encoded by this gene protein has a fundamental pathological function in the migration of lung carcinoma cells (Knaup et al. 2014; Ji et al. 2018) and down-regulation of its expression under glucose deprivation condition in ERN1 knockdown glioma cells possibly contribute to decreased proliferation potential of these cells. At the same time, we showed that ERN1 knockdown eliminates the sensitivity of LHX6 and NKX3-1 gene expressions to glucose deprivation conditions and does not modulate effect of glucose deprivation on LHX1 gene expression in glioma cells. Functional significance of ERN1 knockdown mediated elimination of down-regulated expression of NKX3-1 under glucose deprivation conditions is possibly connected

with suppression of ERN1 knockdown glioma cells proliferation (Auf et al. 2010) because this transcription factor functions as a tumor suppressor and can inhibits proliferation as well as invasion (Le Magnen et al. 2018).

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding homeobox proteins in glioma cells in response to glucose deprivation and their correlation with inhibition of ERN1 activity and reduced cell proliferation in cells harboring dnERN1, attesting to the fact that endoplasmic reticulum stress as well as glucose is a necessary component of malignant tumor growth and cell survival. Furthermore, our results validate tight interaction of endoplasmic reticulum stress signaling pathway ERN1 with glucose deprivation in the regulation of the expression of genes encoding homeobox proteins, but the detailed molecular mechanisms of this regulation have not been yet clearly defined and requires further investigation.

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