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Effect of glucose deprivation on the expression of genes encoding glucocorticoid receptor and some related factors in ERN1-knockdown U87 glioma cells

Olena O. Riabovol[1], Dariia O. Tsymbal[1], Dmytro O. Minchenko[1,2], Kateryna M. Lebid-Biletska[1], Myroslava Y. Sliusar[1], Olha V. Rudnytska[1], Oleksandr H. Minchenko[1]

[1]*Department of Molecular Biology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine;* [2]*Department of Pediatrics, National Bohomolets Medical University, Kyiv, Ukraine*
E-mail: ominchenko@yahoo.com

Objective. The aim of the present study was to examine the effect of glucose deprivation on the expression of genes encoded glucocorticoid receptor (NR3C1) and some related proteins (NR3C2, AHR, NRIP1, NNT, ARHGAP35, SGK1, and SGK3) in U87 glioma cells in response to inhibition of endoplasmic reticulum stress signaling mediated by ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1/inositol requiring enzyme 1) for evaluation of their possible significance in the control of glioma growth through endoplasmic reticulum stress signaling mediated by IRE1 and glucose deprivation.

Methods. The expression of *NR3C1*, *NR3C2*, *AHR*, *NRIP1*, *NNT*, *ARHGAP35*, *SGK1*, and *SGK3* genes in U87 glioma cells transfected by empty vector pcDNA3.1 (control cells) and cells without ERN1 signaling enzyme function (transfected by dnERN1) under glucose deprivation was studied by real time quantitative polymerase chain reaction.

Results. It was shown that the expression level of *NR3C2*, *AHR*, *SGK1*, *SGK3*, and *NNT* genes was up-regulated in control U87 glioma cells under glucose deprivation condition in comparison with the control cells growing with glucose. At the same time, the expression of *NRIP1* gene is down-regulated in these glioma cells under glucose deprivation, but *NR3C1* and *ARHGAP35* genes was resistant to this experimental condition. We also showed that inhibition of ERN1 signaling enzyme function significantly modified the response of most studied gene expressions to glucose deprivation condition. Thus, effect of glucose deprivation on the expression level of *NR3C2*, *AHR*, and *SGK1* genes was significantly stronger in ERN1 knockdown U87 glioma cells since the expression of *NNT* gene was resistant to glucose deprivation condition. Moreover, the inhibition of ERN1 enzymatic activities in U87 glioma cells led to up-regulation of ARHGAP35 gene expression and significant down-regulation of the expression of *SGK3* gene in response to glucose deprivation condition.

Conclusions. Results of this study demonstrated that glucose deprivation did not change the expression level of *NR3C1* gene but it significantly affected the expression of *NR3C2*, *AHR*, *NRIP*, *SGK1*, *SGK3*, and *NNT* genes in vector-transfected U87 glioma cells in gene specific manner and possibly contributed to the control of glioma growth since the expression of most studied genes in glucose deprivation condition was significantly dependent on the functional activity of IRE1 signaling enzyme.

Key words: glucose deprivation, mRNA expression, NR3C1, NR3C2, AHR, NRIP1, SGK1, SGK3, ARHGAP35, NNT, ERN1 inhibition, U87 glioma cells

Corresponding author: Dmytro O. Minchenko, MD, PhD, Department of Molecular Biology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovycha 9, Kyiv 01601, Ukraine; e-mail: ominchenko@yahoo.com.

Malignant gliomas are highly aggressive tumors with very poor prognosis and to date, there is no efficient treatment available (Nayak and Reardon 2017; Alimohammadi et al. 2019). Therefore, the moderate efficacy of conventional clinical approaches underlines the need for new therapeutic strategies. Cell proliferation is strongly dependent on the glycolysis and glucose level, because there is the molecular connection between the cell cycle progression and the provision of nutrients essential for this purpose. Glucose as well as glutamine are important substrates for glycolysis and glutaminolysis, which are important to glioma development and a more aggressive behavior through regulation of the cell cycle at distinct stages (Colombo et al. 2011; Yalcin et al. 2014; Zhao et al. 2017). It has been shown that down-regulation of glucose transporter 1 by microRNA miR-451 inhibits the glucose metabolism and proliferation as well as invasion of glioma cells (Guo et al. 2016). Glucose shortage associated with malignant progression triggers apoptosis through the endoplasmic reticulum unfolded protein response, particularly by inducing the unfolded protein response transcription factors ATF4 and CHOP as well as through the cystine/glutamate transporter SLC7A11 (solute carrier family 7 member 11) as a key regulator of EPH Receptor A2 (EPHA2) (Huber et al. 2013; Iurlaro et al. 2017; Teramoto and Katoh 2019). Furthermore, a better knowledge of tumor responses to glucose deprivation conditions is required to elaborate therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms (Huber et al. 2013; Tsymbal et al. 2016; Iurlaro et al. 2017; Teramoto and Katoh 2019).

The growing tumor requires the endoplasmic reticulum stress for own neovascularization and growth and apoptosis inhibition because it has an important position as a signal integrator in both the normal and malignant cells (Drogat et al. 2007; Auf et al. 2010). The endoplasmic reticulum stress signaling pathways have connections with other plasma membrane receptor signaling networks and numerous metabolic pathways (Bravo et al. 2013; Minchenko et al. 2013; Chevet et al. 2015). Malignant tumors use endoplasmic reticulum stress response and its signaling pathways to adapt and enhance tumor cells proliferation under stressful environmental conditions (Manie et al. 2014; Dejeans et al. 2015). It is well known that activation of IRE1/ERN1 (inositol requiring enzyme 1/endoplasmic reticulum to nucleus signaling 1) branch of the endoplasmic reticulum stress response is tightly linked to apoptosis and cell death and suppression of its function has been demonstrated to result in a significant antiproliferative effect in glioma growth (Moenner et al. 2007; Auf et al. 2010, 2013; Minchenko et al. 2014, 2015a, 2015b).

Receptor for glucocorticoids (nuclear receptor subfamily 3, group C, member 1; NR3C1) has a dual mode of action: 1) as a transcription factor that binds to glucocorticoid response elements and 2) as a modulator of other transcription factors. It plays an important role in the regulation of numerous metabolic and proliferative processes, including tumorigenesis and metabolic diseases, such as obesity and diabetes, preferentially as a transcription factor that binds to glucocorticoid response element in the target genes, both for the nuclear and mitochondrial DNA (de Guia and Herzig 2015; Pufall 2015; Thomas et al. 2015; Liang et al. 2017). It affects inflammatory responses, cellular proliferation, and differentiation in target tissues. Unlike other steroid hormone receptors, the glucocorticoid receptor is not considered to be an oncogene. There are data indicating that miR-124 might contribute to the glucocorticoid resistance by promoting the proliferation and inhibiting the apoptosis (Vonlanthen et al. 2014; Kim et al. 2015; Huang et al. 2016; Liang et al. 2017). Furthermore, it has also been shown that NR3C1 is a key component of the regulatory pathway for signal transmission in mitochondria and that glucocorticoids can stimulate directly the mitochondrial transcription by the mitochondrially localized glucocorticoid receptors (Minchenko and Germanyuk 1984; Minchenko 1988; Minchenko and Tronjko 1988; Psarra and Sekeris 2011; Qi and Ding, 2017). Moreover, glucocorticoid receptors can also be a signal by binding to other transcription factors and modulate the transcriptional regulation of target genes (Khan et al. 2011; Li et al. 2012; Dasgupta et al. 2015).

It has been shown that human mineralocorticoid receptor (NR3C2) has functional kinship with the glucocorticoid receptor and shows high affinity for glucocorticoids as well as ability to stimulate a glucocorticoid-responsive promoter (Arriza et al. 1987). The mineralocorticoid receptors together with glucocorticoid ones provide unexpected functional diversity, in which hormone-binding properties, target gene interactions, and patterns of tissue-specific expression may be used in a combinatorial fashion to achieve complex physiologic control (Arriza et al. 1987; Le Billan et al. 2018). Thus, both receptors dynamically and cyclically interact at the same promoter of period circadian protein 1 (PER1), a target gene in a specific and distinct transcriptional signature (Le Billan et al. 2018). Furthermore, during

this process, both receptors regulate PER1 gene by binding as homo- or heterodimers to the same promoter region. There are also data demonstrating that epidermal growth factor (EGF) administration increases the mineralocorticoid receptor transcriptional activity through EGFR/ERK pathway and that targeting of NR3C2 by microRNA-766 promotes cancer progression in hepatocellular carcinoma (Mitsuishi et al. 2018; Yang et al. 2019).

There are data indicating that ARHGAP35 (Rho GTPase Activating Protein 35), also known as glucocorticoid receptor DNA binding factor 1 (GRF-1), acts as a tumor suppressor gene, associates with the promoter region of the glucocorticoid receptor gene and represses transcription of the glucocorticoid receptor by binding to the cis-acting regulatory sequence (Organ et al. 2014; Zhao et al. 2014; Choi et al. 2018; Onodera et al. 2018). Moreover, there is a regulatory cross-talk between the glucocorticoid receptor and aryl hydrocarbon receptor (AHR) in HepG2 cells because cellular signaling by these receptors shares several functional and regulatory features (Dvorak et al. 2018). There are data showing that the glucocorticoids increase AHR levels in hepatoma cells via a glucocorticoid receptor-dependent transcriptional mechanism (Dvorak et al. 2018). Furthermore, it has been shown that AHR controls redox homeostasis and shapes the tumor microenvironment in BRCA1-associated breast cancer and that the chemical inhibition of AHR activity sensitizes human breast cancer models to Erlotinib, a selective EGFR tyrosine kinase inhibitor, suggesting a promising combinatorial anticancer effect of AHR and EGFR pathway inhibition (Kubli et al. 2019).

Nuclear receptor interacting protein 1 (NRIP1) is a nuclear protein that is expressed in a circadian manner and specifically interacts with the hormone-dependent activation domain AF2 of nuclear receptors NR3C1, NR3C2, and ESR1 (estrogen receptor) and modulates their transcriptional activity. It is a positive regulator of the circadian clock genes and its expression is altered in breast cancers (Jalaguier et al. 2017; Muller et al. 2019). Moreover, NRIP1 plays a critical role in promoting the progression and development of breast cancer because suppressing of NRIP1 inhibits growth of breast cancer cells both *in vitro* and *in vivo* (Aziz et al. 2015). There are data demonstrating that mutations in *NNT* (nicotinamide nucleotide transhydrogenase) gene cause familial glucocorticoid deficiency because it is a key regulator of adrenal redox homeostasis and steroidogenesis (Meimaridou et al. 2012, 2018; Weinberg-Shukron et al. 2015). The NNT is an integral protein of the inner mitochondrial membrane and functions as a proton pump across the membrane. It has also been shown that NNT plays a crucial role in the tumor progression and that knockdown of NNT significantly reduced NADPH, increased the level of reactive oxygen species and cell apoptosis under oxidative stress conditions, such as glucose deprivation (Li et al. 2018a).

Glucocorticoids are responsible for the regulation of SGK1 and SGK3 (serum/glucocorticoid regulated kinases 1 and 3), which also are induced by a very large spectrum of stimuli distinct from glucocorticoids and serum (Liu et al. 2015; Xiaobo et al. 2016; Cao et al. 2019; Fan et al. 2019). It has been shown that Src (SRC proto-oncogene, non-receptor tyrosine kinase) positively regulates SGK1 expression in triple negative breast cancer cells, which exhibit a prominent signaling network governed by Src family kinases (Ma et al. 2019).

The aim of the present study was to examine the effect of glucose deprivation on the expression of genes encoded glucocorticoid receptor (NR3C1) and some related proteins (NR3C2, AHR, NRIP1, NNT, ARHGAP35, SGK1, and SGK3) in U87 glioma cells in response to inhibition of ERN1 for evaluation of their possible significance in the control of glioma growth through endoplasmic reticulum stress signaling mediated by IRE1 and glucose deprivation.

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 this glioma 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 expression level of genes encoding glucocorticoid receptor (NR3C1) and some related proteins (NR3C2, AHR, NRIP1, NNT, ARHGAP35, SGK1, and SGK3. Second subline was obtained by selection of stable transfected clone with overexpression of ERN1 dominant/negative construct (dnERN1) and has suppressed both protein kinase and endoribonuclease activities of this signaling enzyme

(Auf et al. 2010). The expression level of studied genes in dnERN1 cells exposure under glucose deprivation condition was compared with these cells growing with glucose (Control 2). The efficiency of ERN1 suppression in this glioma cell subline was estimated previously (Auf et al. 2010, 2013) by determining the expression level of the XBP1 alternative splice variant, a key transcription factor in the ERN1 signaling, and the level of the phosphorylated isoform ERN1 using cells treated by tunicamycin (0.01 mg/ml during 2 h). 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 dn-ERN1 construct.

Glucose deprivation condition was created by changing the complete DMEM medium into culture plates on DMEM medium without glucose (Gibco) and plates were exposed to this condition for 16 hrs.

RNA isolation. Total RNA was extracted from glioma cells using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). 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 effect of glucose deprivation on the expression levels of glucocorticoid receptor (NR3C1) and related proteins (NR3C2, ARHGAP35, AHR, NRIP1, NNT, SGK1, and SGK3 mRNAs as well as ACTB mRNA were measured in control U87 glioma cells and cells with a deficiency of ERN1 by quantitative polymerase chain reaction using SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) and qPCR „RotorGene RG-3000" (Corbett Research, Germany) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). QuaniTect Reverse Transcription Kit (QIAGEN, Hilden, Germany) and Thermo Scientific Verso cDNA Synthesis Kit was used for reverse transcription as described previously (Minchenko et al. 2015, 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 – as described previously (Bochkov et al. 2006). The values of *NR3C1, NR3C2, ARHGAP35, AHR, NRIP1, NNT, SGK1,* and *SGK3* gene expressions were normalized

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
NR3C1	nuclear receptor subfamily 3 group C member 1 (glucocorticoid receptor)	F: 5'-tttccctcctgctccttctg R: 5'-tcacatctcccctctcctga	382–401 571–552	NM_000176
NR3C2	nuclear receptor subfamily 3 group C member 2 (mineralocorticoid receptor 1)	F: 5'-tcctgcttgcagacttcaga R: 5'-gaggaaccagtgctgtgttg	2222–2241 2433–2414	NM_000901
ARHGAP35	Rho GTPase activating protein 35 (glucocorticoid receptor DNA binding factor 1)	F: 5'-ggcaacctgggagagtaact R: 5'-agtctttctctgccaggtcc	3717–3736 3934–3915	NM_004491
AHR	aryl hydrocarbon receptor (class E basic helix-loop-helix protein 76)	F: 5'-cttccaagcggcatagagac R: 5'-agttatcctggcctccgttt	717–736 914–895	NM_001621
NRIP1	nuclear receptor interacting protein 1	F: 5'-ctccggatgacatcagagct R: 5'-cgcaaggaggaggagaagaa	180–199 390–371	NM_003489
NNT	nicotinamide nucleotide transhydrogenase (NAD(P) transhydrogenase, mitochondrial)	F: 5'-gtctcctgaaatctgcccct R: 5'-cagcacagtgataacgacgg	2549–2568 2769–2750	NM_012343
SGK1	serum/glucocorticoid regulated kinase 1	F: 5'-gcagaaggacaggacaaagc R: 5'-tcggtaaactcggggtcaaa	1085–1104 1261–1242	NM_005627
SGK3	serum/glucocorticoid regulated kinase family member 3	F: 5'-attcccagctccgatgaaca R: 5'-tcgtttagtcctgctcgtct	312–331 547–528	NM_013257
ACTB	beta-actin	F: 5'-ggacttcgagcaagagatgg R: 5'-agcactgtgttggcgtacag	747–766 980–961	NM_001101

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 bifunctional enzyme in the expression level of mRNA for glucocorticoid receptor and related proteins, we studied the effect of glucose deprivation on expression of these genes in U87 glioma cells with and without ERN1 functional activity. As shown in Figure 1, the expression of NR3C1 mRNA in control, transfected by empty vector pcDNA3.1, U87 glioma cells is resistant to glucose deprivation in comparison with the cells growing in complete DMEM medium. Furthermore, inhibition of ERN1 signaling enzyme function does not significantly change the sensitivity of glucocorticoid receptor gene expression to this experimental condition (Figure 1). We next investigated the effect of glucose deprivation on the expression of gene encoding mineralocorticoid receptor (NR3C2) in relation to inhibition of ERN1 function. As shown in Figure 2, the expression of NR3C2 mRNA is strongly up-regulated under glucose deprivation (+75%) in transfected by empty vector pcDNA3.1 U87 glioma cells in comparison with these cells growing in complete DMEM medium. More significant changes were observed in glioma cells with ERN1 knockdown (+109%) as compared to cells growing in glucose-containing medium (Figure 2).

We also studied the effect of glucose deprivation on the expression glucocorticoid receptor DNA binding factor 1 (GRLF1), also known as Rho GTPase Activating Protein 35 (ARHGAP35), in relation to functional activity of ERN1 signaling enzyme. As also shown in Figure 3, the expression level of ARHGAP35 mRNA is resistant to glucose deprivation condition in glioma cells transfected by empty vector. At the same time, inhibition of ERN1 signaling enzyme function leads to up-regulation of *ARHGAP35* (+24%) gene expression in cells exposure under glucose deprivation condition.

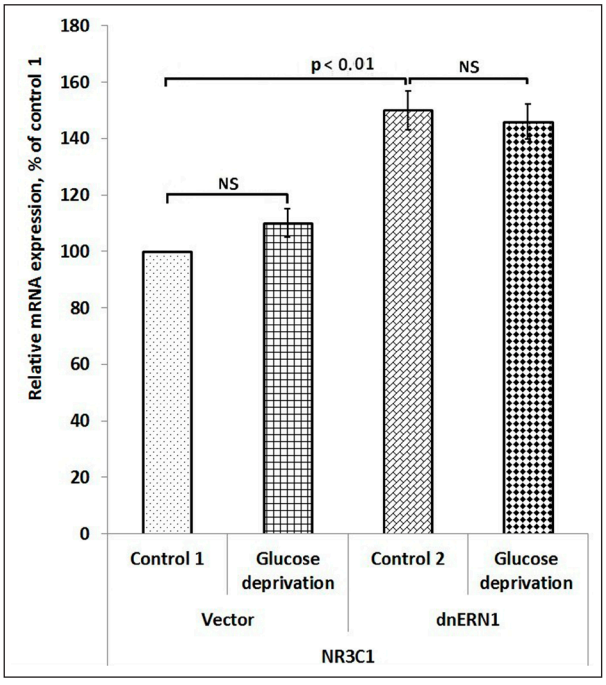

Figure 1. Effect of glucose deprivation on the expression level of glucocorticoid receptor (nuclear receptor subfamily 3, group C, member 1; NR3C1) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of NR3C1 mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

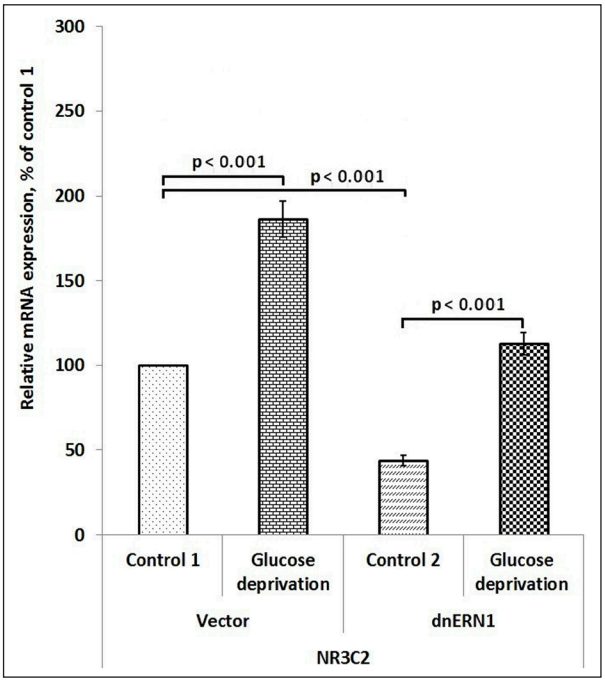

Figure 2. Effect of glucose deprivation on the expression level of mineralocorticoid receptor (nuclear receptor subfamily 3, group C, member 2; NR3C2) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of NR3C2 mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

Investigation of AHR (aryl hydrocarbon receptor) gene encoding poly-functional protein, which can interact with glucocorticoid receptor in many essential physiological processes, showed its sensitivity to glucose deprivation (Figure 4). Thus, the expression level of *AHR* gene is significantly up-regulated (+47%) by glucose deprivation in glioma cells transfected by empty vector in comparison with cells growing with complete DMEM medium. Moreover, inhibition of ERN1 signaling enzyme function by dnERN1 strongly enhances effect of glucose deprivation on this gene expression (+124%; Figure 4). At the same time, glucose deprivation leads to down-regulation of the expression level of *NRIP1* gene in glioma cells with functional ERN1 (–29%) in comparison with cells growing in complete DMEM medium (Figure 5). It was also shown that inhibition of ERN1 signaling enzyme function does not significantly change the effect of glucose deprivation on the expression level of *NRIP1* gene in glioma cells (–26%). We also studied the effect of glucose deprivation on the expression of *NNT* gene in glioma cells in relation to inhibition of ERN1 signaling enzyme function. As shown in Figure 6, exposure glioma cells transfected by empty vector under glucose deprivation condition leads to small but statistically significant up-regulation of the expression *NNT* gene (+17%) in comparison with control cells growing in complete medium. We also found that inhibition of ERN1 signaling eliminates sensitivity of *NNT* gene expression to glucose deprivation condition (Figure 6).

We next investigated the effect of glucose deprivation on the expression of gene encoding serum/glucocorticoid regulated kinases 1 and 3 (SGK1 and SGK3) in glioma cells in relation to inhibition of ERN1 signaling enzyme function. It was shown that in control glioma cells (transfected by empty vector) the expression level of *SGK1* gene is up-regulated (+32%) under glucose deprivation in comparison with cells growing in complete medium and similar but slightly lesser effect was observed on *SGK3* gene expression (+24%) (Figure 7 and Figure 8). At the same time, inhibition of ERN1 signaling enzyme function significantly enhances effect of glucose deprivation on *SGK1* gene expression (+90%; Figure 7), on the other hand, the expression of *SGK3* gene in glioma

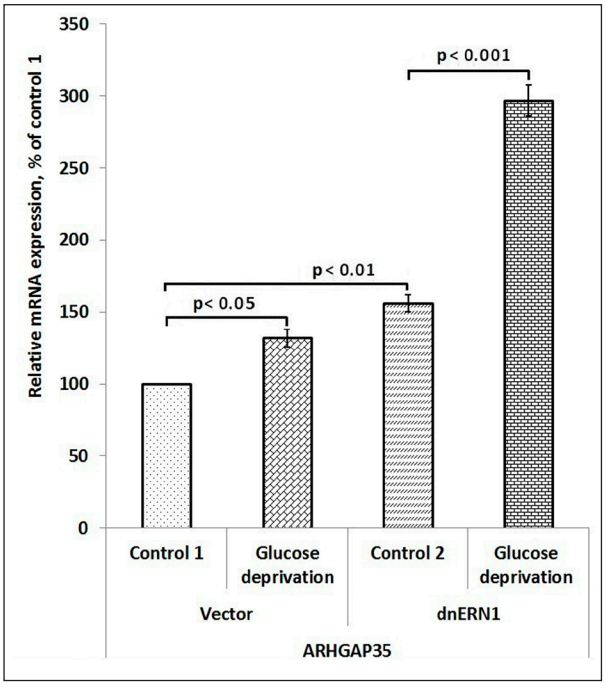

Figure 3. Effect of hypoxia on the expression level of Rho GTPase Activating Protein 35 (ARHGAP35), also known as glucocorticoid receptor DNA binding factor 1 (GRLF1), mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of ARHGAP35 mRNA expressions were normalized to beta-actin mRNA level and represent as percent for control 1 (100%); n=4.

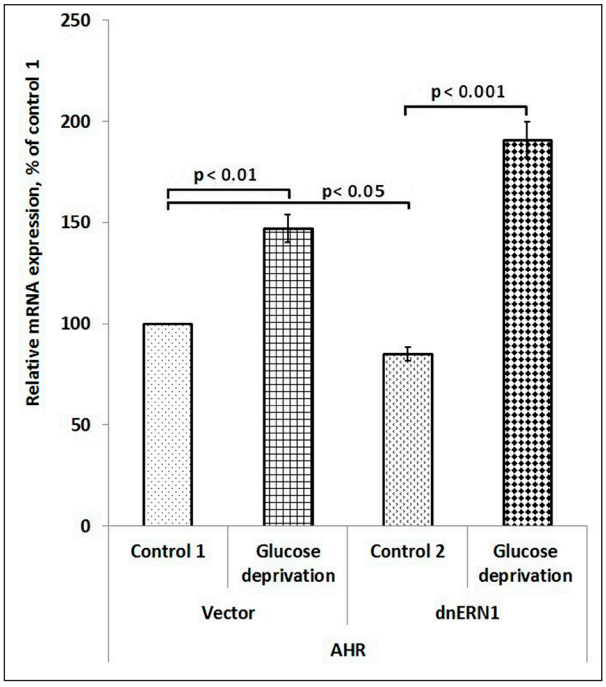

Figure 4. Effect of hypoxia on the expression level of aryl hydrocarbon receptor (AHR), also known as class E basic helix-loop-helix protein 76, mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of AHR mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

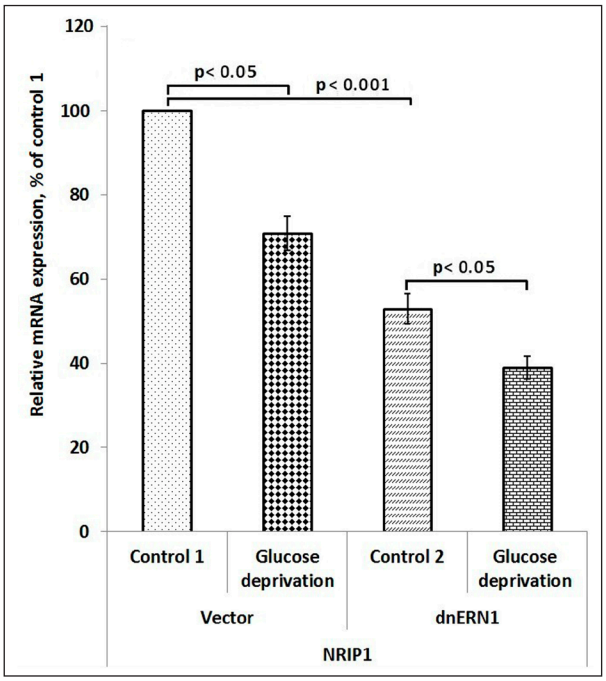

Figure 5. Effect of hypoxia on the expression level of nuclear receptor interacting protein 1 (NRIP1) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of NRIP1 mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

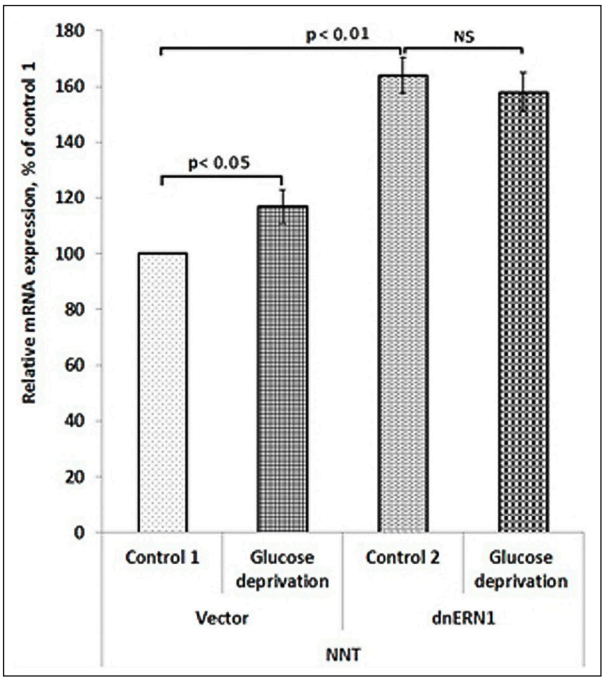

Figure 6. Effect of hypoxia on the expression level of nicotinamide nucleotide transhydrogenase (NNT), also known as NAD(P) transhydrogenase, mitochondrial, mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of NNT mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100 %); n=4.

cells with inhibited ERN1 signaling enzyme function is down-regulated (–39%) in comparison to cells growing with glucose (Figure 8).

Thus, glucose deprivation affects the expression of most studied genes and inhibition of endoplasmic reticulum stress signaling mediated by ERN1 signaling enzyme modifies the effect of glucose deprivation on the expression of almost all glucocorticoid receptor related factors in gene-specific manner: enhances effect of glucose deprivation on *NR3C2, AHR,* and *SGK1* genes in U87 glioma cells, introduces sensitivity of *ARHGAP35* gene, eliminates sensitivity of *NNT* gene, and suppresses the expression of *SGK3* gene.

Discussion

In this work, we studied the effect of glucose deprivation on the expression of genes encoded glucocorticoid receptor and a subset of related factors, which have relation to its functional activity, in relation to inhibition of ERN1, the major signaling pathway of the unfolded protein response, in U87 glioma cells. It is important to evaluate the significance of these genes in the control of glioma growth through endoplasmic reticulum stress signaling mediated by ERN1 and glucose level because stress signaling pathways is involved in numerous metabolic pathways and inhibition of the activity of ERN1 signaling enzyme in glioma cells had anti-tumor effects (Auf et al. 2010, 2013; Bravo et al. 2013; Manie et al. 2014; Minchenko et al. 2013, 2014, 2015a,b). In addition, glucose is an important indicator of the cancer cells chemoresistance (Awale et al. 2006a,b).

Results of this study demonstrate that growing of glioma cells in glucose-deprived medium affects the expression of most genes studied in a gene specific manner. Thus, glucose deprivation leads to the up-regulation of the expression of *NR3C2*, *AHR*, *NNT*, *SGK1*, and *SGK3* genes and down-regulation of *NRIP1* gene (Figure 9). At the same time, the expression of genes encoding glucocorticoid receptor and glucocorticoid receptor DNA binding factor 1 (ARHGAP35) is resistant to glucose deprivation. Furthermore, inhibition of ERN1 signaling enzyme function significantly modifies the effect of glucose deprivation on the expression level of all genes studied, except the *NR3C1* and *NRIP1* genes (Figure 9), indi-

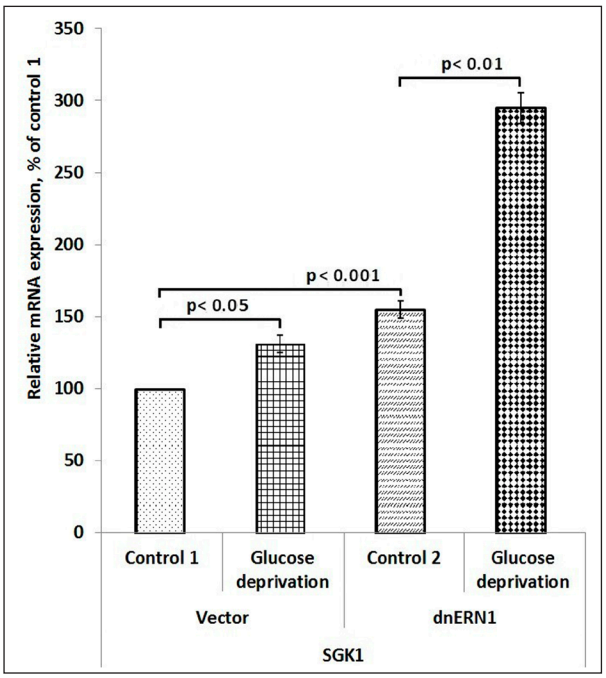

Figure 7. Effect of hypoxia on the expression level of serum/glucocorticoid regulated kinase 1 (SGK1) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of SGK1 mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

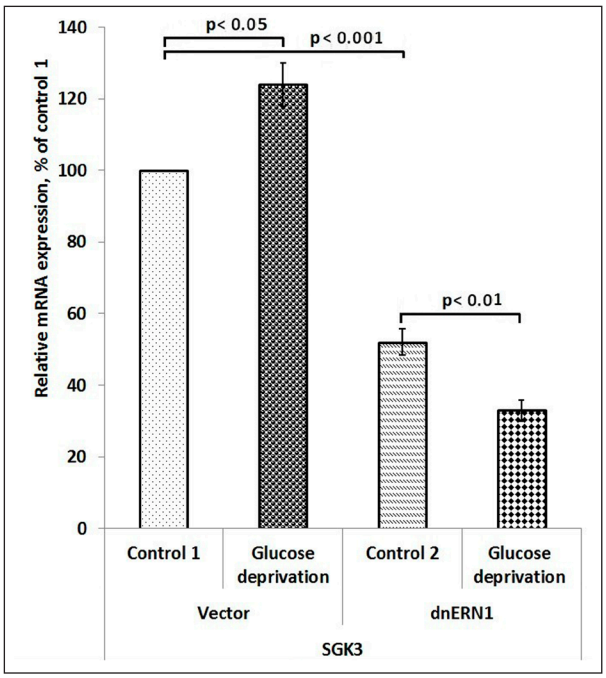

Figure 8. Effect of hypoxia on the expression level of serum/glucocorticoid regulated kinase family, member 3 (SGK3) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of SGK3 mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

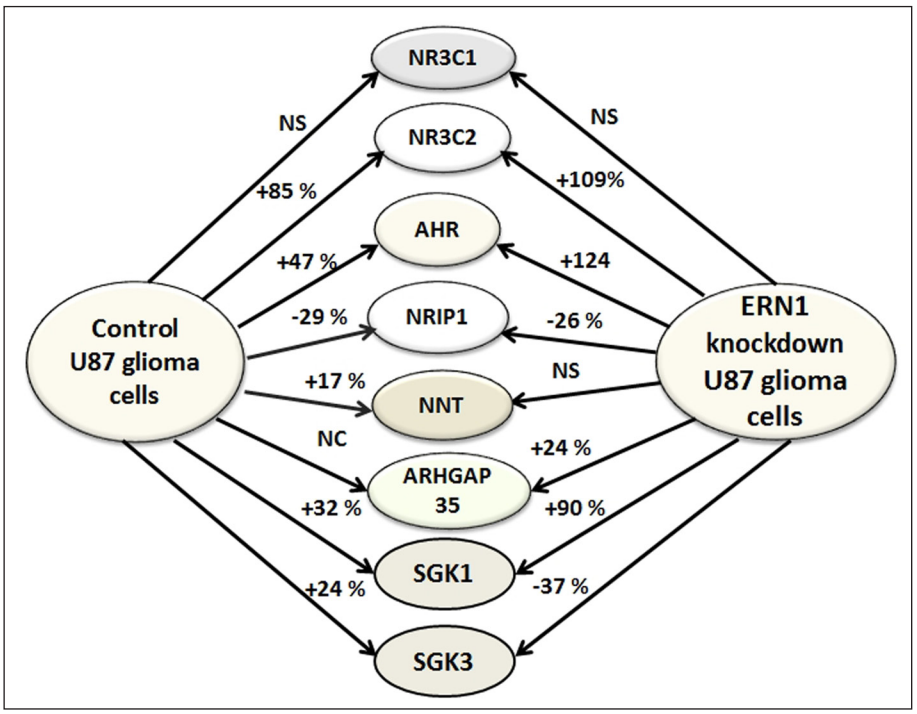

Figure 9. Schematic demonstration of *NR3C1*, *NR3C2*, *AHR*, *NRIP1*, *NNT*, *ARHGAP35*, *SGK1*, and *SGK3* genes expression profile in control and ERN1 knockdown glioma cells under glucose deprivation; NS – no significant changes.

cating their participation in ERN1 mediated network of unfolded protein response and possible contribution in the control of glioma cell proliferation. All proteins encoding by these genes are multifunctional and it is possible that different sensitivity of these gene expressions to glucose deprivation is associated with their specific functions in glioma cells. Thus, receptor for glucocorticoids (NR3C1) functions, as a transcription factor and also a modulator of other transcription factors, plays an important role in the regulation of numerous metabolic and proliferative processes, including tumorigenesis and metabolic diseases (de Guia and Herzig 2015; Pufall 2015; Huang et al. 2016; Liang et al. 2017). It is possible that resistance of NR3C1 to glucose deprivation in glioma cells is associated with an important multifunctional role of this receptor.

We showed that the mineralocorticoid receptor gene expression is very sensitive to glucose deprivation and this sensitivity is enhanced by ERN1 knockdown. This receptor has functional kinship with the glucocorticoid receptor and can stimulate the glucocorticoid-responsive promoter. Thus, interaction of both receptors in a combinatorial fashion allows achieving complex physiologic control (Arriza et al. 1987; Le Billan et al. 2018). At the same time, the mineralocorticoid receptor participates in tumor growth because targeting of NR3C2 by microRNA-766 promotes cancer progression in hepatocellular carcinoma (Mitsuishi et al. 2018; Yang et al. 2019). This up-regulation of *NR3C2* gene expression in glioma cells under glucose deprivation condition correlates well with anti-proliferative function of mineralocorticoid receptor as well as with anti-proliferative effect of ERN1 inhibition (Auf et al. 2010, 2013; Minchenko et al. 2015a,b,c; Yang et al. 2019). We also found that the expression of glucocorticoid receptor DNA binding factor 1 (ARHGAP35) is increased in ERN1 knockdown glioma cells. This data correlate well with its functional role as a tumor suppressor (Choi et al. 2018; Onodera et al. 2018).

It is possible that increased expression of *AHR* gene in glioma cells under glucose deprivation and more significant up-regulation of this gene expression after ERN1 knockdown contributes to the suppression of proliferation and glioma growth from cells with inhibited ERN1 function (Auf et al. 2010, 2013; Minchenko et al. 2015a,c). These results conform the data that *AHR* has mainly anti-proliferative functions through interaction with different proteins and EGFR pathway (Dvorak et al. 2018; Kubli et al. 2019).

It is interesting to note that glucose deprivation leads to a down-regulation of *NRIP1* gene independently from ERN1 inhibition. It is a nuclear protein that specifically interacts with the hormone-dependent activation domain of nuclear receptors NR3C1, NR3C2, and ESR1 and modulates their transcriptional activity. The NRIP1 protein is a positive regulator of the circadian clock genes and plays a critical role in promoting the progression and development of breast cancer because its expression is altered in breast cancers and suppressing of NRIP1 inhibits growth of breast cancer cells (Aziz et al. 2015; Jalaguier et al. 2017; Muller et al. 2019). Our data about down-regulation of *NRIP1* gene expression under glucose deprivation conform well the pro-proliferative role of NRIP1 protein. We also showed that the expression of NNT, which is an integral protein of the inner mitochondrial membrane, is up-regulated in glioma cells under glucose deprivation condition and that ERN1 knockdown eliminates this effect. It is possible that increased expression of *NNT* under glucose deprivation is connected with pro-oncogenic role of this protein and its important function in mitochondria (Li et al. 2018a).

We also observed up-regulation of serum/glucocorticoid regulated kinases 1 and 3 (SGK1 and SGK3), which also are induced by a very large spectrum of stimuli distinct from the glucocorticoids and serum (Liu et al. 2015; Xiaobo et al. 2016; Cao et al. 2019; Fan et al. 2019). Both kinases have pro-oncogenic properties: SGK1 expression is positively regulated in the triple negative breast cancer cells by SRC proto-oncogene, non-receptor tyrosine kinase and down-regulation of SGK3 through overexpression of miR-376a inhibits RCC cell proliferation, migration, and invasion (Fan et al. 2019; Ma et al. 2019). Thus, up-regulation of the expression of these multifunctional kinases (SGK1 and SGK3) in glioma cells under glucose deprivation conforms well to pro-proliferative role of these proteins. At the same time, inhibition of ERN1 signaling enzyme function has strong but different effect on their expression indicating ERN1 dependent character of these gene expressions and variable role in ERN1 signaling (Chen et al. 2018; Li et al. 2018b; Cao et al. 2019; Fan et al. 2019; Ma et al. 2019).

We showed that glucose deprivation condition up-regulates the expression of most genes studied and that inhibition of endoplasmic reticulum stress signaling mediated by ERN1 signaling enzyme modifies the effect of glucose deprivation on the expression of almost all the glucocorticoid receptor related factors in gene-specific manner, i.e. enhances the effect of glucose deprivation on *NR3C2*, *AHR*, and *SGK1* genes in U87 glioma cells, introduces

sensitivity of *ARHGAP35* gene, eliminates sensitivity of *NNT* gene, and suppresses the expression of *SGK3* gene.

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoded glucocorticoid receptor and some related proteins in glucose deprivation condition and their correlation with reduced cell proliferation in cells harboring dnERN1, attesting to the fact that endoplasmic reticulum stress is a necessary component of the malignant tumor growth and cell survival. Moreover, the expression of some genes studied under glucose deprivation are significantly depended on ERN1 signaling enzyme function. However, the detailed molecular mechanisms of this regulation are complex yet and warrants further study.

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