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Glucose deprivation affects the expression of genes encoding cAMP-activated protein kinase and related proteins in U87 glioma cells in ERN1 dependent manner

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Objective. The aim of this investigation was to study the expression of genes encoding cAMP-activated protein kinase catalytic and regulatory A subunits (PRKACA and PRKAR1A) and related proteins such as cAMP-dependent protein kinase inhibitors A and G (PKIA and PKIG), catalytic subunit A of protein phosphatase 3 (PPP3CA), A-kinase anchoring protein 12 (AKAP12), and praja ring finger ubiquitin ligase 2 (PJA2) in U87 glioma cells in response to glucose deprivation in both control U87 glioma cells and cells with ERN1 (endoplasmic reticulum to nucleus signaling 1) knockdown, the major pathway of the endoplasmic reticulum stress signaling, for evaluation of possible significance of glucose deprivation in ERN1 dependent regulation of glioma growth.

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

Results. It was shown that the expression level of *PRKACA* and *PKIA* genes was down-regulated in control glioma cells treated by glucose deprivation, but *PJA2* gene was up-regulated. At the same time, the expression of four other genes (*PRKAR1A*, *PKIG*, *AKAP12*, and *PPP3CA*) was resistant to this experimental condition. Furthermore, ERN1 knockdown of glioma cells significantly modified the effect glucose deprivation on the expression almost all studied genes. Thus, treatment of glioma cells with inhibited ERN1 enzymatic activity by glucose deprivation lead to a more significant down-regulation of the expression level of *PKIA* and to suppression *PRKAR1A* gene expressions. Moreover, the ERN1 knockdown introduced up-regulation of *PKIG* and *AKAP12* gene expressions in glioma cells treated by glucose deprivation and eliminated the sensitivity of *PJA2* gene to this experimental condition.

Conclusions. Results of this investigation demonstrated that ERN1 knockdown significantly modified the sensitivity of most studied PRKA related gene expressions to glucose deprivation and that these changes are a result of complex interactions of variable endoplasmic reticulum stress related and unrelated regulatory factors and contributed to the suppression of glioma cell proliferation and their possibly chemoresistance.

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

Protein kinase A (PKA, cAMP-activated protein kinase) is composed of two regulatory and two catalytic subunits and is involved in the regulation of a variety of cellular functions including glucose and lipid metabolism through phosphorylation of a large number of substrates in the cytoplasm and the

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nucleus and participate in the onset and progression of various tumors (Caretta and Mucignat-Caretta 2011; Beristain et al. 2015; Cohen et al. 2017; Singhi et al. 2020). Furthermore, cAMP-activated protein kinase is a component of the signal transduction mechanism of certain G protein-coupled receptors, which can increase cAMP to activate protein kinase A and inhibit mTORC1 through phosphorylation the mTORC1 component Raptor (Jewell et al. 2019). There are also data indicating that deletion of RAP1B reduces PKA-mediated thyroid cancer (Huk et al. 2018). It is interesting to note that regulatory subunit of PKA (PRKAR1A) is a functional tumor suppressor inhibiting ERK/Snail/E-cadherin pathway in lung adenocarcinoma and knockout of this gene leads to neuroendocrine tumorigenesis in the pancreas (Wang et al. 2016; Saloustros et al. 2017). The activity of cAMP-dependent protein kinase is controlled by specific protein kinase inhibitors including PKIA and PKIG as well as protein phosphatases (King et al. 1984; Knighton et al. 1991; Olsen and Uhler 1991; Saloustros et al. 2017; Rusmini et al. 2019). The A-kinase anchor protein 12 (AKAP12) binds to the regulatory subunit of the protein kinase A and mediates the subcellular compartmentalization of protein kinase A. It associates with protein kinases A and phosphatase and serves as a scaffold protein in signal transduction and as metastasis suppressor because AKAP12 inhibits cell migration in breast cancer (Lester and Scott 1997; Shih et al. 1999; Soh et al. 2018). In the control of PKA stability and signaling, RING ligase praja2 (PJA2, praja ring finger ubiquitin ligase 2) plays an important role, which also attenuates the tumor-suppressor Hippo signaling and supports glioblastoma growth via proteolysis of MOB1 and positively regulates the TLR2 signaling pathway that leads to the activation of the downstream JNK and p38 pathways (Lignitto et al. 2011, 2013; Zhong et al. 2017). PJA2 has E2-dependent E3 ubiquitin-protein ligase activity and responsible for ubiquitination of cAMP-dependent protein kinase regulatory subunits, thus controlling the strength and duration of PKA signaling in response to cAMP (Cantara et al. 2012; Hedrick et al. 2013). Furthermore, it is involved in several types of cancers and markedly is overexpressed in differentiated thyroid cancer. Its levels inversely correlate with the malignant phenotype of the tumor (Cantara et al. 2012; Gong et al. 2020).

Malignant gliomas are highly aggressive tumors with very poor prognosis and glucose as substrate for glycolysis is an important to glioma development as well as a more aggressive behavior through regulation of the cell cycle (Minchenko et al. 2002; Colombo et al. 2011; Yalcin et al. 2014; Zhao et al. 2017). Furthermore, glucose supply as well as endoplasmic reticulum stress is important and complementary factors for tumor growth (Huber et al. 2013; Minchenko et al. 2013; Iurlaro et al. 2017). It is interesting to note that ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1/inositol requiring enzyme 1) mediated stress signaling significantly modifies the effects of glucose deprivation on numerous gene expressions (Minchenko et al. 2015b; Tsymbal et al. 2016; Riabovol et al. 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). A glucose-related risk signature for the malignancy of glioma was identified by bioinformatic profiling (Zhao et al. 2017). However, the detailed molecular mechanisms of the interaction of glucose deprivation with ERN1 mediated stress signaling pathway are complex and prospective studies are needed for further clarifications. It is interesting to note that the glucose level in the cells is an important factor of cancer cells chemoresistance (Awale et al. 2006). There are data indicating that glucose deprivation possibly by blocking the unfolded protein response increases the sensitivity of various cancer cells to arctigenin, a natural lignan compound extracted from Arctium lappa, which inhibits the tumor growth and induces tumor cell death only under glucose deprivation (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 a suppression of chemoresistance through unknown mechanisms and further research is needed to confirm these findings.

Malignant tumors use endoplasmic reticulum stress response and its signaling pathways strongly enhances the tumor cells proliferation under stressful environmental conditions (Papaioannou and Chevet 2018; Hughes and Mallucci 2019). It is well known that activation of ERN1 branch of the endoplasmic reticulum stress response is tightly linked to apoptosis, and suppression of its functional activity has been demonstrated to result in significant anti-proliferative effect in malignant tumors including glioma (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. 2015d).

The aim of this study was to examine the expression of genes encoding cAMP-activated protein

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
PRKACA	protein kinase cAMP-activated catalytic subunit alpha	F: 5'- gcaagctgtcaactttccgt R: 5'- agattctccggcttcaggtc	503–522 733–714	NM_002730
PRKAR1A	protein kinase cAMP-dependent type I regulatory subunit alpha	F: 5'- gatgggcagaagattgtggt R: 5'- ggccaagaacacgttcaaat	1008–1027 1257–1238	NM_002734
PKIA	cAMP-dependent protein kinase inhibitor alpha	F: 5'- cagaacaaagtggggaagcc R: 5'- tgcagcacagccattttctt	706–725 867–848	NM_006823
PKIG	cAMP-dependent protein kinase inhibitor gamma	F: 5'- acttcatctcctgtgaccgg R: 5'- gtctctcgtccagccttctt	320–339 556–537	NM_007066
PPP3CA	protein phosphatase 3 catalytic subunit alpha	F: 5'- tgatcccaagttgtcgacga R: 5'- acactctcttccagccttcc	751–770 909–890	NM_000944
PJA2	praja ring finger ubiquitin ligase 2	F: 5'- gatgttgaggtggccaatcc R: 5'- ggcatgttcccgacttttgt	2018–2037 2234–2215	NM_014819
AKAP12	A-kinase anchoring protein 12	F: 5'- cccaagcacaggaggagtta R: 5'- tgcctgctctccaattctca	5554–5573 5751–5732	NM_005100
ACTB	beta-actin	F: 5'- ggacttcgagcaagagatgg R: 5'- agcactgtgttggcgtacag	747–766 980–961	NM_001101

kinase and related proteins such as PKIA, PKIG, PPP3CA, AKAP12, and PJA2 in U87 glioma cells in response to glucose deprivation in both control U87 glioma cells and cells with knockdown of ERN1 for evaluation of possible significance of glucose deprivation condition in ERN1 dependent regulation of glioma growth.

Materials and methods

Cell lines and culture conditions. We used two sublines of U87 glioma cells, which has been described previously (Auf et al. 2010; Minchenko et al. 2015d). Cells 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. One subline was obtained by selection of stable transfected clones with overexpression of vector pcDNA3.1, which was used for creation of dnERN1, and this untreated subline of glioma cells was used as control 1 (control glioma cells) 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) and suppression of both the protein kinase and endoribonuclease activities of this signaling enzyme (Auf et al. 2010, 2013). It has been shown that cells with dnERN1 have a lower proliferation rate, do not express 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. 2013). 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 sublines of glioma cells, used in this study, are grown in the presence of geneticin (G418), while these cells carrying empty vector pcDNA3.1 or dnERN1 construct. Glucose deprivation condition was 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) and RNA pellets were washed with 75% ethanol. RNA samples were dissolved in nuclease-free water and for additional purification were re-precipitated with 95% ethanol (Minchenko et al. 2018). RNA pellets re-dissolved again in nuclease-free water and concentration of RNAs as well as their spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. The expression levels of genes encoding cAMP-activated protein kinase and related proteins 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 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. 2020). Comparison of two means was performed by the use of two-tailed Student's t-test (Bochkov et al. 2006). The value $p<0.05$ 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 in the expression level of genes encoding cAMP-activated protein kinase and related proteins in relation to the suppression of endoplasmic reticulum stress signaling mediated by ERN1, we studied the effect of glucose deprivation on gene expressions in U87 glioma cells with and without ERN1 functional activity. As shown in Figure 1, the expression of catalytic alpha subunit of cAMP-activated protein kinase (PRKACA) mRNA in control glioma cells transfected by empty vector pcDNA3.1, is decreased (–19%) after exposure of these cells under glucose deprivation condition in comparison with the cells growing in

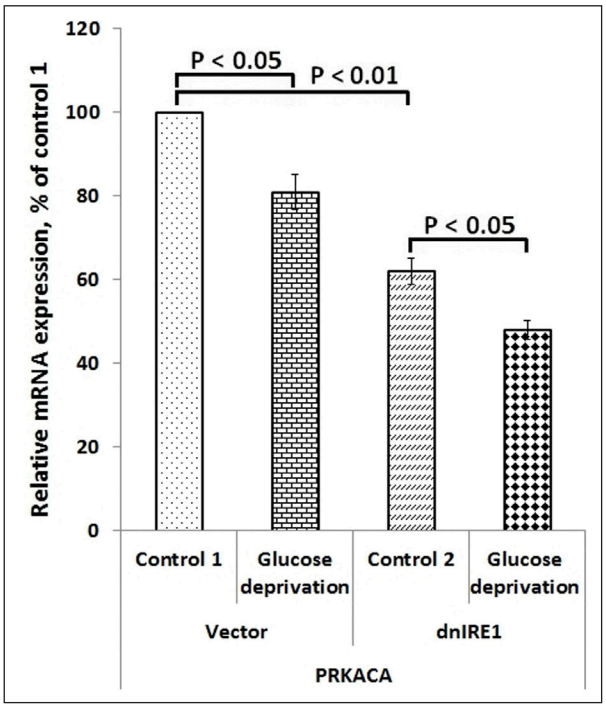

Figure 1. Effect of glucose deprivation on the expression level of PRKACA (protein kinase cAMP-activated catalytic subunit alpha) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

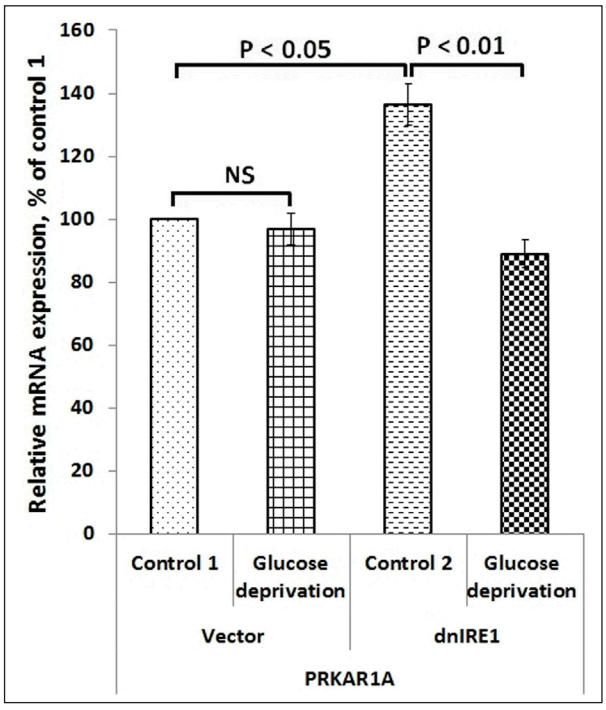

Figure 2. Effect of glucose deprivation on the expression level of PRKAR1A (protein kinase cAMP-dependent type I regulatory subunit alpha) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

complete DMEM medium. Furthermore, inhibition of ERN1 signaling enzyme function by dnERN1 did not significantly change the sensitivity of *PRKACA* gene expression to this experimental condition (Figure 1). Thus, the level of PRKACA mRNA expression is decreased on 23% in cells without ERN1 signaling enzyme function. Next, we investigated the effect of glucose deprivation on the expression of gene encoding regulatory alpha subunit of type I cAMP-dependent protein kinase (PRKAR1A) in relation to inhibition of ERN1 function. As shown in Figure 2, the expression of *PRKAR1A* gene in transfected by empty vector control glioma cells is resistant to glucose deprivation condition. At the same time, inhibition of ERN1 signaling protein function leads to significant down-regulation of the expression level of this gene (–35%; Figure 2).

We also studied the effect of glucose deprivation on the expression of gene encoding cAMP-dependent protein kinase inhibitor alpha (PKIA) in control U87 glioma cells and cells with ERN1 knockdown. As shown in Figure 3, the expression of PKIA mRNA in control glioma cells exposure under glucose deprivation condition is down-regulated (–39%) but inhibition of ERN1 signaling leads to more significant down-regulation of this mRNA expression (–65%) in comparison with dnERN1 cells growing with glucose.

As shown in Figure 4, the expression level of cAMP-dependent protein kinase inhibitor gamma (PKIG) mRNA is resistant to glucose deprivation condition in control glioma cells in comparison with cells growing in regular medium. Furthermore, inhibition of ERN1 signaling enzyme function in glioma cells significantly up-regulates this mRNA expression (+25%) as compared to corresponding control cells (transfected by dnERN1; Figure 4). At the same time, exposure of control glioma cells under glucose deprivation condition does not change the expression level of catalytic alpha subunit of protein phosphatase 3 (PPP3CA) mRNA and suppression of ERN1 signaling does not modify the sensitivity of this gene expression to glucose deprivation condition (Figure 5).

We also studied the effect of glucose deprivation on the expression of gene encoding praja ring finger

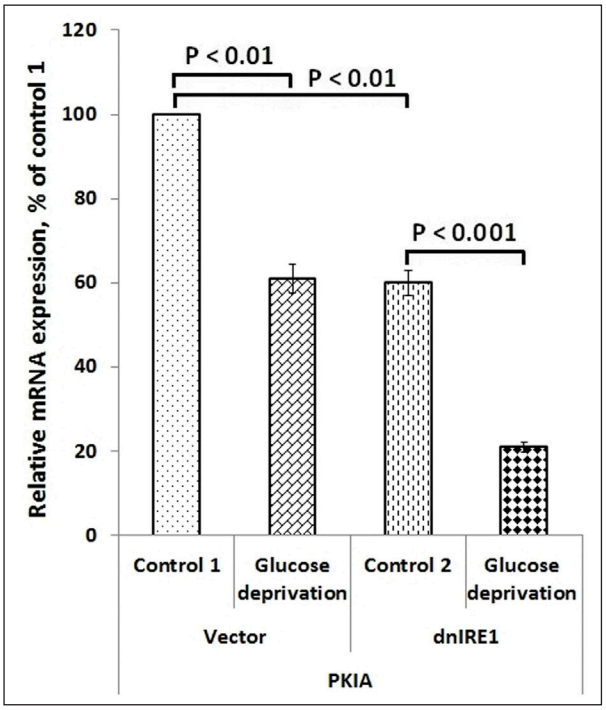

Figure 3. Effect of glucose deprivation on the expression level of cAMP-dependent protein kinase inhibitor alpha (PKIA) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PKIA mRNA expression were normalized to beta-actin mRNA level and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

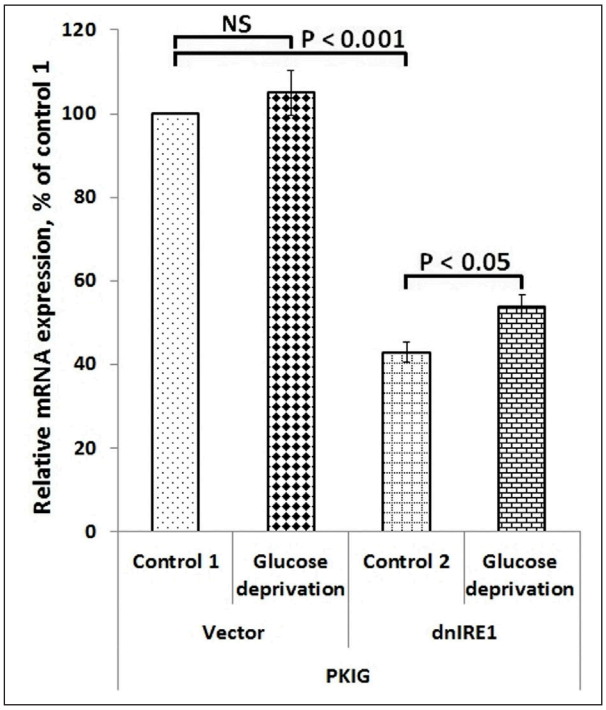

Figure 4. Effect of glucose deprivation on the expression level of cAMP-dependent protein kinase inhibitor gamma (PKIG) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PKIG mRNA expression were normalized to beta-actin mRNA level and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

ubiquitin ligase 2 (PJA2) in glioma cells in relation to ERN1 knockdown. As shown in Figure 6, exposure of glioma cells under glucose deprivation condition leads to significant up-regulation of mRNA expression (+25%) in comparison with control cells growing under condition with glucose in the medium. Furthermore, inhibition of both enzymatic activities of ERN1 completely eliminates the effect of glucose deprivation condition on the expression of this gene (Figure 6). At the same time, the expression of gene encoding A-kinase anchoring protein 12 (AKAP12) in control glioma cells is resistant to glucose deprivation condition, but ERN1 knockdown introduces the sensitivity of this gene to glucose deprivation (Figure 7). Thus, inhibition of ERN1 signaling enzyme function in glioma cells significantly up-regulates this mRNA expression (+42%) as compared to corresponding control cells growing under condition with glucose.

Thus, glucose deprivation condition affects the expression of genes encoding cAMP-activated protein kinase and related proteins in gene-specific manner and the effect of glucose deprivation on gene expressions preferentially depends on ERN1 signaling. Results of this investigation are summarized in Figure 8, which clearly demonstrated the ERN1-dependent character of changes in the expression profile of most studied genes encoding cAMP-activated protein kinase and related proteins in glioma cells under glucose deprivation.

Discussion

In this work, we studied the effect of glucose deprivation on the expression of genes encoding cAMP-activated protein kinase and related proteins, which are involved in the regulation of a variety of cellular functions including glucose and lipid metabolism through phosphorylation of a large number of substrates in the cytoplasm and the nucleus and participate in the onset and progression of various tumors (Caretta and Mucignat-Caretta 2011; Beristain et al. 2015; Cohen et al. 2017; Singhi et al. 2020). It is important to note that glucose supply as well as endoplasmic reticulum

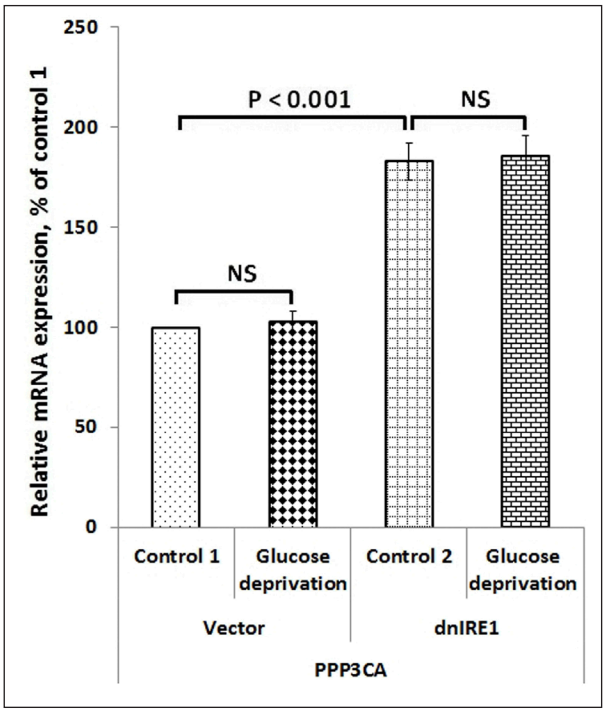

Figure 5. Effect of glucose deprivation on the expression level of protein phosphatase 3 catalytic subunit alpha (PPP3CA) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); NS – no significant changes; n=4.

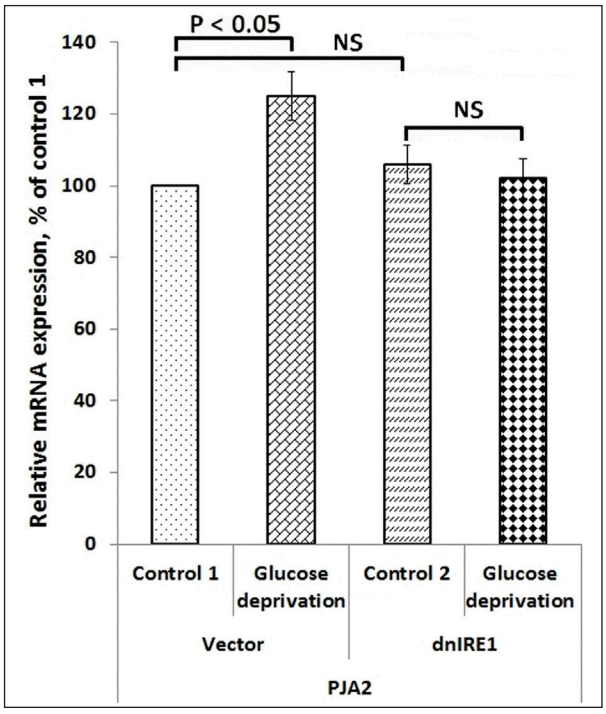

Figure 6. Effect of glucose deprivation on the expression level of PJA2 (praja ring finger ubiquitin ligase 2) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PJA2 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

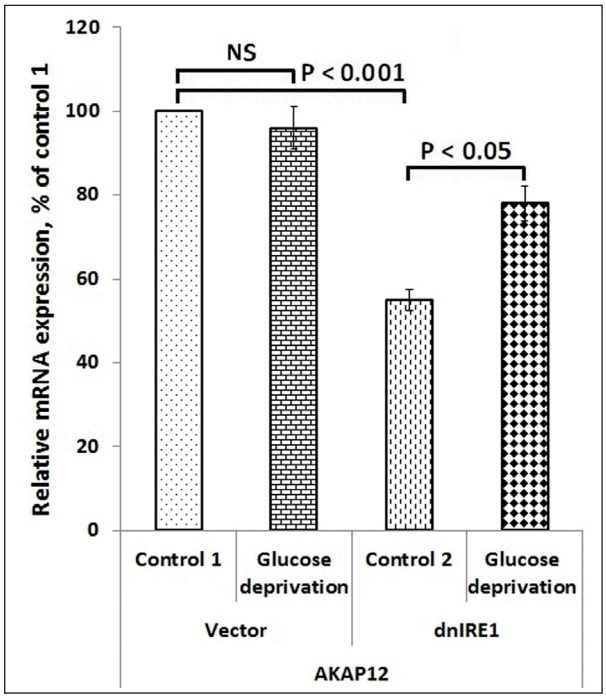

Figure 7. Effect of glucose deprivation on the expression level of A-kinase anchoring protein 12 (AKAP12) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of AKAP12 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

stress are important and complementary factors for tumor growth including glioma. Moreover, ERN1 signaling significantly modifies the effects of glucose deprivation on numerous gene expressions (Kubaichuk et al. 2013; Kryvdiuk et al. 2015; Minchenko et al. 2015b; Tsymbal et al. 2016; Riabovol et al. 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). Furthermore, a glucose-related risk signature for the malignancy of glioma was identified by bioinformatic profiling (Zhao et al. 2017). It is interesting to note that similar situation concerns the hypoxic regulation. Thus, there are data indicating that the endoplasmic reticulum stress plays an important role in the development of cancer cells resistance to toxic effects of hypoxia and that ERN1 inhibition modifies the effect of hypoxia in numerous gene expressions through genome reprogramming (Minchenko et al. 2015a, c, 2016a, b, c; Chevet et al. 2015; Tsymbal et al. 2017). It is possible that this mechanism may underline the resistance of cancer cells to toxic effects of hypoxia.

In this study, we obtained data indicating that glucose deprivation condition affects the expression of genes encoding cAMP-activated protein kinase and related proteins in gene-specific manner and that effect of glucose deprivation on the expression

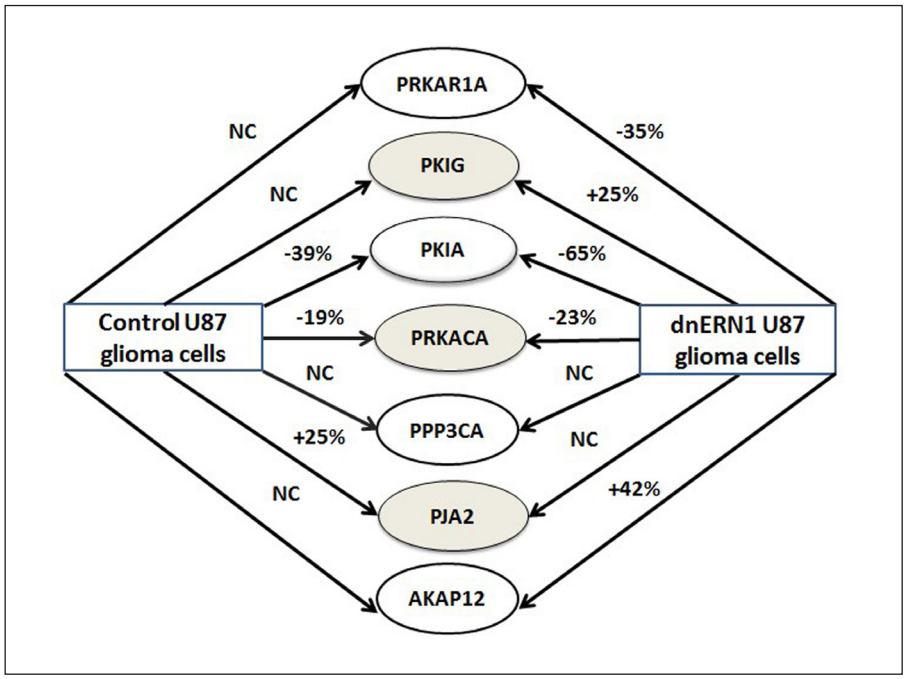

Figure 8. Schematic demonstration of changes in the expression profile of cAMP-activated protein kinase related genes in the control and ERN1 knockdown U87 glioma cells under glucose deprivation; NS – no significant changes.

of most studied genes preferentially depends on ERN1 signaling. This is important for evaluation of possible significance of 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; Minchenko et al. 2014; Tsymbal et al. 2017; Lhomond et al. 2018; Logue et al. 2018). Furthermore, there are data indicating 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 this experimental 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 cAMP-related proteins.

We have shown that exposure of control glioma cells under glucose deprivation condition leads to a down-regulation of the expression of *PRKACA* gene and up-regulation of *PJA2* gene. These data agree well with the functional significance of proteins encoding by these genes: PRKACA participates in the onset and progression of various tumors, but PJA2 has reverse effect because it is responsible for ubiquitination of cAMP-dependent protein kinase regulatory subunits, thus controlling the strength and duration of PKA signaling (Caretta and Mucignat-Caretta 2011; Cantara et al. 2012; Beristain et al. 2015). Furthermore, in ERN1 knockdown glioma cells glucose deprivation condition introduces similar down-regulation of *PRKACA* gene expression as well as suppression of *PRKAR1A* and up-regulation of *PKIG* gene expressions. These changes of *PRKAR1A* and *PRKACA* gene expressions under glucose deprivation condition in ERN1 knockdown glioma cells possibly contribute to decreased proliferation potential of these cells as it has been shown by Auf et al. (2010, 2013) and Minchenko et al. (2015d). We also showed that glucose deprivation down-regulates the expression of *PKIA* gene in both control and ERN1 knockdown glioma cells. It is possible that this decrease in *PKIA* gene expression has protective effect on the cells because there are data indicating that highly expressed protein kinase A inhibitor α and suppression of protein kinase A may potentiate acetaminophen-induced hepatotoxicity (Yun et al. 2014). It is possible that our results should contribute to decreased chemoresistance of ERN1 knockdown glioma cells upon both glucose deprivation and inhibition of endoplasmic reticulum stress, which agree with data reported by Kim et al. (2010), Gu et al. (2012), He et al. (2018), and Logue et al. (2018).

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding cAMP-activated protein kinase and related proteins in glioma cells in response to glucose deprivation and inhibition of ERN1 activity and their correlation with reduced cell proliferation in cells harboring dnERN1, attesting to the fact that endoplasmic reticulum stress is a necessary component of malignant tumor growth and cell survival, including glucose deprivation. It is important to note that our results validate a tight interaction of endoplasmic reticulum stress signaling pathways with glucose deprivation in the regulation of the expression of genes encoding cAMP-activated protein kinase and some related proteins and clarify some aspects of chemoresistance mechanism, but the detailed molecular mechanisms of this regulation have not been yet clearly defined and warrant further investigation.

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References

Auf G, Jabouille A, Guerit S, Pineau R, Delugin M, Bouchecareilh M, Favereaux A, Maitre M, Gaiser T, von Deimling A, Czabanka M, Vajkoczy P, Chevet E, Bikfalvi A, Moenner M. A shift from an angiogenic to invasive phenotype induced in malignant glioma by inhibition of the unfolded protein response sensor IRE1. Proc Natl Acad Sci U S A 107, 15553–15558, 2010.

Auf G, Jabouille A, Delugin M, Guerit S, Pineau R, North S, Platonova N, Maitre M, Favereaux A, Vajkoczy P, Seno M, Bikfalvi A, Minchenko D, Minchenko O, Moenner M. High epiregulin expression in human U87 glioma cells relies on IRE1alpha and promotes autocrine growth through EGF receptor. BMC Cancer 13, 597, 2013.

Awale S, Lu J, Kalauni SK, Kurashima Y, Tezuka Y, Kadota S, Esumi H. Identification of arctigenin as an antitumor agent having the ability to eliminate the tolerance of cancer cells to nutrient starvation. Cancer Res 66, 1751–1757, 2006.

Beristain AG, Molyneux, Joshi PA, Pomroy NC, Di Grappa MA, Chang MC, Kirschner LS, Prive GG, Pujana MA, Khokha R. PKA SD signaling drives mammary tumorigenesis through Src. Oncogene 34, 1160–1173, 2015.

Bochkov VN, Philippova M, Oskolkova O, Kadl A, Furnkranz A, Karabeg E, Breuss J, Minchenko OH, Mechtcheriakova D, Hohensinner P, Rychli K, Wojta J, Resink T, Binder BR, Leitinger N. Oxidized phospholipids stimulate angiogenesis via induction of VEGF, IL-8, COX-2 and ADAMTS-1 metalloprotease, implicating a novel role for lipid oxidation in progression and destabilization of atherosclerotic lesions. Circ Res 99, 900–908, 2006.

Cantara S, D'Angeli F, Toti P, Lignitto L, Castagna MG, Capuano S, Prabhakar BS, Feliciello A, Pacini F. Expression of the ring ligase PRAJA2 in thyroid cancer. J Clin Endocrinol Metab 97, 4253–4259, 2012.

Caretta A, Mucignat-Caretta C. Protein kinase A in cancer. Cancers (Basel) 3, 913–926, 2011.

Chevet E, Hetz C, Samali A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. Cancer Discov 5, 586–597, 2015.

Cohen JN, Joseph NM, North JP, Onodera C, Zembowicz A, LeBoit PE. Genomic analysis of pigmented epithelioid melanocytomas reveals recurrent alterations in PRKAR1A, and PRKCA genes. Am J Surg Pathol 41, 1333–1346, 2017.

Colombo SL, Palacios-Callender M, Frakich N, Carcamo S, Kovacs I, Tudzarova S, Moncada S. Molecular basis for the differential use of glucose and glutamine in cell proliferation as revealed by synchronized HeLa cells. Proc Natl Acad Sci USA 108, 21069–21074, 2011.

Gong M, Ye S, Li WX, Zhang J, Liu Y, Zhu J, Lv W, Zhang H, Wang J, Lu A, He K. Regulatory function of praja ring finger ubiquitin ligase 2 mediated by the *P2rx3/P2rx7* axis in mouse hippocampal neuronal cells. Am J Physiol Cell Physiol 318, C1123–C1135, 2020.

Gu Y, Qi C, Sun X, Ma X, Zhang H, Hu L, Yuan J, Yu Q. Arctigenin preferentially induces tumor cell death under glucose deprivation by inhibiting cellular energy metabolism. Biochem Pharmacol 84, 468–476, 2012.

Hassler JR, Scheuner DL, Wang S, Han J, Kodali VK, Li P, Nguyen J, George JS, Davis C, Wu SP, Bai Y, Sartor M, Cavalcoli J, Malhi H, Baudouin G, Zhang Y, Yates Iii JR, Itkin-Ansari P, Volkmann N, Kaufman RJ. The IRE1α/XBP1s pathway is essential for the glucose response and protection of β cells. PLoS Biol 13, e1002277, 2015.

He Y, Fan Q, Cai T, Huang W, Xie X, Wen Y, Shi Z. Molecular mechanisms of the action of Arctigenin in cancer. Biomed Pharmacother 108, 403–407, 2018.

Hedrick ED, Agarwal E, Leiphrakpam PD, Haferbier KL, Brattain MG, Chowdhury S. Differential PKA activation and AKAP association determines cell fate in cancer cells. J Mol Signal 8, 10, 2013.

Hetz C, Axten JM, Patterson JB. Pharmacological targeting of the unfolded protein response for disease intervention. Nat Chem Biol 15, 764–775, 2019.

Huber AL, Lebeau J, Guillaumot P, Petrilli V, Malek M, Chilloux J, Fauvet F, Payen L, Kfoury A, Renno T, Chevet E, Manie SN. p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows malignant progression under low glucose. Mol Cell 49, 1049–1059, 2013.

Hughes D, Mallucci GR. The unfolded protein response in neurodegenerative disorders – therapeutic modulation of the PERK pathway. FEBS J 286, 342–355, 2019.

Huk DJ, Ashtekar A, Magner A, La Perle K, Kirschner LS. Deletion of Rap1b, but not Rap1a or Epac1, reduces protein kinase A-mediated thyroid cancer. Thyroid 28, 1153–1161, 2018.

Iurlaro R, Puschel F, Leon-Annicchiarico CL, O'Connor H, Martin SJ, Palou-Gramon D, Lucendo E, Munoz-Pinedo C. Glucose deprivation induces ATF4-mediated apoptosis through TRAIL death receptors. Mol Cell Biol 37, e00479–e00516, 2017.

Jewell JL, Fu V, Hong AW, Yu FX, Meng D, Melick CH, Wang H, Lam WM, Yuan HX, Taylor SS, Guan KL. GPCR signaling inhibits mTORC1 via PKA phosphorylation of Raptor. Elife 8, e43038, 2019.

Kim JY, Hwang JH, Cha MR, Yoon MY, Son ES, Tomida A, Ko B, Song SW, Shin-ya K, Hwang YI, Park HR. Arctigenin blocks the unfolded protein response and shows therapeutic antitumor activity. J Cell Physiol 224, 33–40, 2010.

King MM, Huang CY, Chock PB, Nairn AC, Hemmings HC Jr, Chan KF, Greengard P. Mammalian brain phosphoproteins as substrates for calcineurin. J Biol Chem 259, 8080–8083, 1984.

Knighton DR, Zheng JH, Ten Eyck LF, Xuong NH, Taylor SS, Sowadski JM. Structure of a peptide inhibitor bound to the catalytic subunit of cyclic adenosine monophosphate-dependent protein kinase. Science 253, 414–420, 1991.

Kryvdiuk IV, Minchenko DO Hlushchak NA, Ratushna OO, Karbovskyi LL, Minchenko OH. Inhibition of IRE1 signaling modifies effect of glucose deprivation on the expression of TNFα-induced protein and TNF receptor superfamily genes in U87 glioma cells. Ukr Biochem J 87, 36–51, 2015.

Kubaichuk K, Minchenko D, Kryvdiuk I, Hubenia O, Minchenko O. The expression of CTGF, TEIBS1, PLAU and PLAUR genes in U87 glioma cells with signaling enzyme ERNI loss of function: effect of glucose and glutamine deprivation. Eur J Cancer 49, S128–S130, 2013.

Lester LB, Scott JD. Anchoring and scaffold proteins for kinases and phosphatases. Recent Prog Horm Res 52, 409–429, 1997.

Lhomond S, Avril T, Dejeans N, Voutetakis K, Doultsinos D, McMahon M, Pineau R, Obacz J, Papadodima O, Jouan F, Bourien H, Logotheti M, Jegou G, Pallares-Lupon N, Schmit K, Le Reste PJ, Etcheverry A, Mosser J, Barroso K, Vauleon E, Maurel M, Samali A, Patterson JB, Pluquet O, Hetz C, Quillien V, Chatziioannou A, Chevet E. Dual IRE1 RNase functions dictate glioblastoma development. EMBO Mol Med 10, e7929, 2018.

Lignitto L, Carlucci A, Sepe M, Stefan E, Cuomo O, Nistico R, Scorziello A, Savoia C, Garbi C, Annunziato L, Feliciello A. Control of PKA stability and signalling by the RING ligase praja2. Nat Cell Biol 13, 412–422, 2011.

Lignitto L, Arcella A, Sepe M, Rinaldi L, Delle Donne R, Gallo A, Stefan E, Bachmann VA, Oliva MA, Tiziana Storlazzi C, L'Abbate A, Brunetti A, Gargiulo S, Gramanzini M, Insabato L, Garbi C, Gottesman ME, Feliciello A. Proteolysis of MOB1 by the ubiquitin ligase praja2 attenuates Hippo signalling and supports glioblastoma growth. Nat Commun 4, 1822, 2013.

Logue SE, McGrath EP, Cleary P, Greene S, Mnich K, Almanza A, Chevet E, Dwyer RM, Oommen A, Legembre P, Godey F, Madden EC, Leuzzi B, Obacz J, Zeng Q, Patterson JB, Jager R, Gorman AM, Samali A. Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. Nat Commun 9, 3267, 2018.

Minchenko AG, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead VE, Caro J. Hypoxia-inducible factor-1-mediated expression of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) gene. Its possible role in the Warburg effect. J Biol Chem 277, 6183–6187, 2002.

Minchenko DO, Kharkova AP, Hubenia OV, Minchenko OH. Insulin receptor, IRS1, IRS2, INSIG1, INSIG2, RRAD, and BAIAP2 gene expressions in glioma U87 cells with ERN1 loss of function: effect of hypoxia and glutamine or glucose deprivation. Endocr Reg 47, 15–26, 2013.

Minchenko DO, Danilovskyi SV, Kryvdiuk IV, Bakalets TV, Lypova NM, Karbovskyi LL, Minchenko OH. Inhibition of ERN1 modifies the hypoxic regulation of the expression of TP53-related genes in U87 glioma cells. Endoplasm Reticul Stress Dis 1, 18–26, 2014.

Minchenko DO, Kharkova AP, Tsymbal DO, Karbovskyi LL, Minchenko OH. Expression of insulin-like growth factor binding protein genes and its hypoxic regulation in U87 glioma cells depends on ERN1 mediated signaling pathway of endoplasmic reticulum stress. Endocr Regul 49, 73–83, 2015a.

Minchenko DO, Kharkova AP, Tsymbal DO, Karbovskyi LL, Minchenko OH. IRE1 inhibition affects the expression of insulin-like growth factor binding protein genes and modifies its sensitivity to glucose deprivation in U87 glioma cells. Endocr Regul 49, 185–197, 2015b.

Minchenko DO, Riabovol OO, Halkin OV, Ratushna OO, Tsymbal DO, Minchenko OH. IRE-1α regulates expression of ubiquitin specific peptidases during hypoxic response in U87 glioma cells. Endoplasm Reticul Stress Dis 3, 50–62, 2016a.

Minchenko DO, Riabovol OO, Tsymbal DO, Ratushna OO, Minchenko OH. Inhibition of IRE1 affects the expression of genes encoded glucocorticoid receptor and some related factors and their hypoxic regulation in U87 glioma cells. Endocr Regul 50, 127–136, 2016b.

Minchenko DO, Tsymbal DO, Riabovol OO, Viletska YM, Lahanovska YO, Sliusar MY, Bezrodnyi BH, Minchenko OH. Hypoxic regulation of EDN1, EDNRA, EDNRB, and ECE1 gene expressions in IRE1 knockdown U87 glioma cells. Endocr Reg 53, 250–262, 2019.

Minchenko DO, Tsymbal DO, Luzina OY, Riabovol OO, Danilovskyi SV, Minchenko OH. Silencing of NAMPT leads to up-regulation of insulin receptor substrate 1 gene expression in U87 glioma cells. Endocr Reg 54, 31–42, 2020.

Minchenko OH, Tsymbal DO, Minchenko DO, Kovalevska OV, Karbovskyi LL, Bikfalvi A. Inhibition of ERN1 signaling enzyme affects hypoxic regulation of the expression of *E2F8*, *EPAS1*, *HOXC6*, *ATF3*, *TBX3* and *FOXF1* genes in U87 glioma cells. Ukr Biochem J 87, 76–87, 2015c.

Minchenko OH, Tsymbal DO, Minchenko DO, Moenner M, Kovalevska OV, Lypova NM. Inhibition of kinase and endoribonuclease activity of ERN1/IRE1α affects expression of proliferation-related genes in U87 glioma cells. Endoplasm Reticul Stress Dis 2, 18–29, 2015d.

Minchenko OH, Kryvdiuk IV, Minchenko DO, Riabovol OO, Halkin OV. Inhibition of IRE1 signaling affects expression of a subset genes encoding for TNF-related factors and receptors and modifies their hypoxic regulation in U87 glioma cells. Endoplasm Reticul Stress Dis 3, 1–15, 2016c.

Minchenko OH, Tsymbal DO, Minchenko DO, Hnatiuk OS, Prylutskyy YI, Prylutska SV, Ritter U. Suppression of the expression of genes associated with immune response in normal human astrocytes upon treatment by single-walled carbon nanotubes. Toxicology in Vitro 52, 122–130, 2018.

Olsen SR, Uhler MD. Inhibition of protein kinase-A by overexpression of the cloned human protein kinase inhibitor. Mol Endocrinol 5, 1246–1256, 1991.

Papaioannou A, Chevet E. Driving cancer tumorigenesis and metastasis through UPR signaling. Curr Top Microbiol Immunol 414, 159–192, 2018.

Riabovol OO, Tsymbal DO, Minchenko DO, Lebid-Biletska KM, Sliusar MY, Rudnytska OV, Minchenko OH. Effect of glucose deprivation on the expression of genes encoding glucocorticoid receptor and some related factors in ERN1-knockdown U87 glioma cells. Endocr Regul 53, 237–249, 2019.

Rusmini P, Cortese K, Crippa V, Cristofani R, Cicardi ME, Ferrari V, Vezzoli G, Tedesco B, Meroni M, Messi E, Piccolella M, Galbiati M, Garrè M, Morelli E, Vaccari T, Poletti A. Trehalose induces autophagy via lysosomal-mediated TFEB activation in models of motoneuron degeneration. Autophagy 15, 631–651, 2019.

Saloustros E, Salpea P, Starost M, Liu S, Faucz FR, London E, Szarek E, Song WJ, Hussain M, Stratakis CA. Prkar1a gene knockout in the pancreas leads to neuroendocrine tumorigenesis. Endocr Relat Cancer 24, 31–40, 2017.

Shih M, Lin F, Scott JD, Wang HY, Malbon CC. Dynamic complexes of beta2-adrenergic receptors with protein kinases and phosphatases and the role of gravin. J Biol Chem 274, 1588–1595, 1999.

Singhi AD, Wood LD, Parks E, Torbenson MS, Felsenstein M, Hruban RH, Nikiforova MN, Wald AI, Kaya C, Nikiforov YE, Favazza L, He J, McGrath K, Fasanella KE, Brand RE, Lennon AM, Furlan A, Dasyam AK, Zureikat AH, Zeh HJ, Lee K, Bartlett DL, Slivka A. Recurrent rearrangements in PRKACA and PRKACB in intraductal oncocytic papillary neoplasms of the pancreas and bile duct. Gastroenterology 158, 573–582, 2020.

Soh RYZ, Lim JP, Samy RP, Chua PJ, Bay BH. A-kinase anchor protein 12 (AKAP12) inhibits cell migration in breast cancer. Exp Mol Pathol 105, 364–370, 2018.

Tsymbal DO, Minchenko DO, Kryvdiuk IV, Riabovol OO, Halkin OV, Ratushna OO, Minchenko OH. Expression of proliferation related transcription factor genes in U87 glioma cells with IRE1 knockdown upon glucose and glutamine deprivation. Fiziol Zh 62, 3–15, 2016.

Tsymbal DO, Minchenko DO, Hnatiuk OS, Luzina OY, Minchenko OH. Effect of hypoxia on the expression of a subset of proliferation related genes in IRE1 knockdown U87 glioma cells. Adv Biol Chem 7, 195–210, 2017.

Wang S, Cheng Y, Zheng Y, He Z, Chen W, Zhou W, Duan C, Zhang C. PRKAR1A is a functional tumor suppressor inhibiting ERK/Snail/E-cadherin pathway in lung adenocarcinoma. Sci Rep 6, 39630, 2016.

Yalcin A, Clem BF, Imbert-Fernandez Y, Ozcan SC, Peker S, O'Neal J, Klarer AC, Clem AL, Telang S, Chesney J. 6-Phosphofructo-2-kinase (PFKFB3) promotes cell cycle progression and suppresses apoptosis via Cdk1-mediated phosphorylation of p27. Cell Death Dis 5, e1337, 2014.

Yun JW, Kim M, Cho SD, Lee JY, Bae ON, Lim KM. Highly expressed protein kinase A inhibitor α and suppression of protein kinase A may potentiate acetaminophen-induced hepatotoxicity. Toxicol Lett 229, 59–65, 2014.

Zhao S, Cai J, Li J, Bao G, Li D, Li Y, Zhai X, Jiang C, Fan L. Bioinformatic profiling identifies a glucose-related risk signature for the malignancy of glioma and the survival of patients. Mol Neurobiol 54, 8203–8210, 2017.

Zhong J, Wang H, Chen W, Sun Z, Chen J, Xu Y, Weng M, Shi Q, Ma D, Miao C. Ubiquitylation of MFHAS1 by the ubiquitin ligase praja2 promotes M1 macrophage polarization by activating JNK and p38 pathways. Cell Death Dis 8, e2763, 2017.