![sciendo]

Expression of *IDE* and *PITRM1* genes in ERN1 knockdown U87 glioma cells: effect of hypoxia and glucose deprivation

Dmytro O. Minchenko[1,2], Olena O. Khita[1], Dariia O. Tsymbal[1], Serhij V. Danilovskyi[1], Olha V. Rudnytska[1], Oleh V. Halkin[1], Iryna V. Kryvdiuk[1], Maria V. Smeshkova[3], Mykhailo M. Yakymchuk[3], Borys H. Bezrodnyi[2], Oleksandr H. Minchenko[1]

[1]*Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine;* [2]*National Bohomolets Medical University, Kyiv, Ukraine;* [3]*I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine*
E-mail: ominchenko@yahoo.com

Objective. The aim of the present investigation was to study the expression of genes encoding polyfunctional proteins insulinase (insulin degrading enzyme, IDE) and pitrilysin metallopeptidase 1 (PITRM1) in U87 glioma cells in response to inhibition of endoplasmic reticulum stress signaling mediated by ERN1/IRE1 (endoplasmic reticulum to nucleus signaling 1) for evaluation of their possible significance in the control of metabolism through ERN1 signaling as well as hypoxia, glucose and glutamine deprivations.

Methods. The expression level of *IDE* and *PITRM1* genes was studied in control and ERN1 knockdown U87 glioma cells under glucose and glutamine deprivations as well as hypoxia by quantitative polymerase chain reaction.

Results. It was found that the expression level of *IDE* and *PITRM1* genes was down-regulated in ERN1 knockdown (without ERN1 protein kinase and endoribonuclease activity) glioma cells in comparison with the control glioma cells, being more significant for *PITRM1* gene. We also found up-regulation of microRNA MIR7-2 and MIRLET7A2, which have specific binding sites in 3'-untranslated region of IDE and PITRM1 mRNAs, correspondingly, and can participate in posttranscriptional regulation of these mRNA expressions. Only inhibition of ERN1 endoribonuclease did not change significantly the expression of IDE and PITRM1 genes in glioma cells. The expression of *IDE* and *PITRM1* genes is preferentially regulated by ERN1 protein kinase. We also showed that hypoxia down-regulated the expression of *IDE* and *PITRM1* genes and that knockdown of ERN1 signaling enzyme function modified the response of these gene expressions to hypoxia. Glucose deprivation increased the expression level of *IDE* and *PITRM1* genes, but ERN1 knockdown enhanced only the effect of glucose deprivation on *PITRM1* gene expression. Glutamine deprivation did not affect the expression of *IDE* gene in both types of glioma cells, but up-regulated *PITRM1* gene and this up-regulation was stronger in ERN1 knockdown cells.

Conclusions. Results of this investigation demonstrate that ERN1 knockdown significantly decreases the expression of *IDE* and *PITRM1* genes by ERN1 protein kinase mediated mechanism. The expression of both studied genes was sensitive to hypoxia as well as glucose deprivation and dependent on ERN1 signaling in gene-specific manner. It is possible that the level of these genes expression under hypoxia and glucose deprivation is a result of complex interaction of variable endoplasmic reticulum stress related and unrelated regulatory factors and contributed to the control of the cell metabolism.

Key words: ERN1 knockdown, IDE, PITRM1, mRNA expression, microRNA, hypoxia, glucose deprivation, U87 glioma cells

Corresponding author: Oleksandr H. Minchenko, Prof., Department of Molecular Biology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovycha 9, Kyiv 01030, Ukraine; e-mail: ominchenko@yahoo.com.

The insulin degrading enzyme (IDE), which is also known as insulinase, insulin protease, insulysin, and Abeta-degrading protease, is a zinc metallopeptidase, which has been initially discovered as the enzyme responsible for insulin catabolism; therefore, its involvement in the onset of diabetes has been largely investigated (Pivovarova et al. 2016; Tundo et al. 2017; Zhang et al. 2018). IDE is a multi-functional protein and present in the cytoplasm and different subcellular structures, including mitochondrion, as well as in extracellular matrix. This enzyme degrades intracellular insulin, and thereby terminates its activity. However, IDE also degrades glucagon, which elevates glucose levels and opposes the effect of insulin. The insulin degrading enzyme has ability to degrade several other polypeptides, such as amylin, bradykinin, atrial natriuretic peptide, kallidin, and β-amyloid, has participating in intercellular peptide signaling (da Costa et al. 2017; Kurochkin et al. 2017). At the same time, the extracellular matrix enriched with insulin-degrading enzyme suppresses the deposition of amyloid-beta peptide in Alzheimer's disease cell models (Zhang et al. 2019). Structural and biochemical analyses revealed the molecular basis for IDE-mediated destruction of amyloidogenic peptides and this information has been exploited to develop promising inhibitors of IDE to improve glucose homeostasis; however, the inhibition of IDE can also lead to glucose intolerance (Tang 2016).

Deficiencies in this protein's function are associated with type 2 diabetes mellitus and Alzheimer's disease. Thus, Ohyagi et al. (2019) have revealed that apomorphine treatment improves neuronal insulin resistance and activates insulin-degrading enzyme, a major Aβ-degrading enzyme. There are data indicating that liver-specific ablation of insulin-degrading enzyme causes hepatic insulin resistance and glucose intolerance (Villa-Perez et al. 2018) and that pancreatic β-cell-specific deletion of insulin-degrading enzyme leads to dysregulated insulin secretion (Fernandez-Diaz et al. 2019). Furthermore, insulin deprivation in rats with streptozotocin-induced diabetes down-regulates insulin degrading enzyme level in the cerebral cortex (Kazkayasi et al. 2018). There are also data indicating that ablation of amyloid precursor protein (APP) increases insulin-degrading enzyme levels and activity in brain and other tissues, which clearly demonstrate a novel role for APP as an upstream regulator of IDE in vivo and represents a new molecular link connecting APP to metabolic homeostasis (Kulas et al. 2019). The level of IDE is also controlled by microRNA-7 (Fernandez-de Frutos et al. 2019).

Recently, it was shown that somatostatin receptor subtype-4 agonists, which have been proposed for AD treatment, increased in 15-fold expression of insulin degrading enzyme (Sandoval et al. 2019). Thus, insulin-degrading enzyme represents a pathophysiological link between type 2 diabetes and Alzheimer's disease because insulin regulates the degradation of Aβ by inducing expression of IDE in astrocytes (Pivovarova et al. 2016; Yamamoto et al. 2018). However, because of the pleiotropic IDE action, the use of IDE modulators for the treatment of certain pathologies should be carefully considered to protect from possible adverse effects associated with multiplicity of IDE targets.

It was also shown that insulin-degrading enzyme is applied to catalyze hydrolysis of Nociceptin/Orphanin 1-16 (OFQ/N) indicating the involvement of the enzyme in the degradation of neuropeptides engaged in pain transmission (Zingale et al. 2019). Moreover, IDE degradative action towards insulin was inhibited by the OFQ/N fragments, suggesting a possible regulatory mechanism in the central nervous system. It has been found that OFQ/N and insulin affect each other degradation by IDE, although in a different manner. IDE cleaves neuropeptides and their released fragments act as inhibitors of IDE activity toward insulin. It is possible that IDE enzyme indirectly participates in neural communication of pain signals and that neuropeptides involved in pain transmission may contribute to the regulation of IDE activity (Zingale et al. 2019).

Insulin-degrading enzyme plays a multi-functional role in the interconnecting several basic cellular processes. In particular, the latest advances indicate that IDE behaves as a heat shock protein and modulates the ubiquitin-proteasome system, suggesting a major implication in proteins turnover and cell homeostasis, which is thought to be tightly linked to the malfunction of the "quality control" machinery of the cell (Tundo et al. 2017).

At this time, mitochondrial dysfunction and altered proteostasis are central features of neurodegenerative diseases. The pitrilysin metallopeptidase 1 (PITRM1) is an ATP-dependent mitochondrial matrix enzyme, which functions in peptide cleavage and degradation rather than in protein processing, it binds zinc and can also degrade the mitochondrial fraction of amyloid beta A4 protein, suggesting a possible role in Alzheimer's disease (Falkevall et al. 2006; King et al. 2014; Pinho et al. 2014). This metalloendopeptidase specifically cleaves oligopeptides, including the mitochondrial targeting sequences that are cleaved from proteins imported across the inner mitochon-

drial membrane (Chow et al. 2009; King et al. 2014). Defective mitochondrial peptidase PITRM1 is associated with Aβ-positive amyloid deposits, because this enzyme is responsible for significant Aβ degradation and impairment of its activity results in Aβ accumulation and amyloidotic neurodegeneration (Brunetti et al. 2016; Smith-Carpenter and Alper 2018).

The both insulin-degrading enzyme and pitrilysin metallopeptidase 1 play an important role in the normal brain including glial cell function as well as many diseases, such as insulin resistance, type 2 diabetes, neurodegenerative disorders, and cancer. An important feature of all these diseases is endoplasmic reticulum stress, which is linked to the maintenance of cellular homeostasis and the fine balance between health and disease (Almanza et al. 2019; Marciniak et al. 2019). This stress is an integrator of the signal transduction pathway in both normal and pathological cells, including diabetic, Aβ-positive, and malignant cells because endoplasmic reticulum stress signaling pathways have connections with other plasma membrane receptor signaling networks and with numerous metabolic pathways (Moenner et al. 2007; Auf et al. 2010; Bravo et al. 2013; Hetz et al. 2013; Lee and Ozcan 2014; Manie et al. 2014; Chevet et al. 2015; Wang and Kaufman et al. 2016; Doultsinos et al. 2017; McMahon et al. 2017).

The endoplasmic reticulum stress plays a key role in the regulating of protein misfolding and neurodegeneration and targeting of the unfolded protein response in Alzheimer's disease is emerging as an interesting therapeutic approach (Garcia-Gonzalez et al. 2018; Gerakis and Hetz 2018; Mercado et al. 2018; Hughes and Mallucci 2019; Martinez et al. 2019). Furthermore, there are data (Hassler et al. 2015) that the IRE1/XBP1 pathway is essential for the glucose response and protection of β cells. Malignant tumors use endoplasmic reticulum stress response and its signaling pathways to adapt and to enhance tumor cells proliferation under stressful environmental conditions (Manie et al. 2014; Papaioannou et al. 2018; Almanza et al. 2019). It is well known that the 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 significant anti-proliferative effect in glioma growth (Auf et al. 2010; Minchenko et al. 2014, 2015a; 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).

It is interesting to note that endoplasmic reticulum stress and hypoxia as well as glucose deprivation are very important and complementary factors for tumor growth and that ERN1 mediated stress signaling can significantly modify the effects of hypoxia and glucose deprivation on gene expressions (Minchenko et al. 2014, 2015b, 2016, 2017; 2019; Zhao et al. 2017). However, the detailed molecular mechanisms of the interaction of hypoxia and glucose deprivation with ERN1 mediated stress signaling pathway are complex and requires further study.

The aim of this study was to examine the expression of genes encoding polyfunctional proteins insulin-degrading enzyme (IDE) and pitrilysin metallopeptidase 1 (PITRM1) in response to complete inhibition of ERN1 activity (protein kinase and endoribonuclease) or only its endoribonuclease for evaluation of their possible significance in the control of metabolic processes in U87 glioma cells through endoplasmic reticulum stress signaling mediated by IRE1 as well as through hypoxia, glucose and glutamine 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 three sublines of these cells, which were described previously (Auf et al. 2010, 2013; Minchenko et al. 2015c). One subline was obtained by selection of stable transfected clones with overexpression of vector pcDNA3.1, which was used for creation of dnERN1 and dnrERN1. This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of the effect of hypoxia, glucose and glutamine deprivation on the expression level of these genes. 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). The third sub-line was obtained by the selection of stable transfected clones with the overexpression of dominant-negative ERN1 endoribonuclease mutant (dnrERN1), which was obtained by truncation of the carboxy-terminal 78 amino acids of ERN1 (Auf et al. 2013; Minchenko

et al. 2015c). It has been shown that these cells have a low proliferation rate, do not express spliced XBP1, a key transcription factor in ERN1 signaling, and have not the phosphorylated isoform ERN1 after induction of endoplasmic reticulum stress by tunicamycin (Auf et al. 2013; Minchenko et al. 2015c). The expression of the studied genes in cells with a deficiency of ERN1, introduced by dnERN1 and dnrERN1, was compared with cells transfected with the previously mentioned, empty vector (control glioma cells, pcDNA3.1). All used in this study sublines of glioma cells are grown in the presence of geneticin (G418) while these cells carrying empty vector pcDNA3.1, dnERN1 or dnrERN1 constructs. Hypoxic condition was created in special incubator with 3% oxygen and 5% carbon dioxide levels; culture plates with complete DMEM were exposed to these conditions for 16 h. Glucose and glutamine deprivation conditions were created by changing the complete DMEM medium into culture plates on DMEM medium without glucose or glutamine and plates were exposed to this condition for 16 h. For induction of endoplasmic reticulum stress in glioma cells with dnrERN1 we used tunicamycin (0.01 mg/ml for 2 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 IDE and PITRM1 mRNAs as well as ACTB mRNA were measured in control U87 glioma cells and cells with a deficiency of ERN1, introduced by dnERN1 and dnrERN1, 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). For polyadenylation and reverse transcription of miRNAs we used NCode™ miRNA First-Strand cDNA Synthesis Kit MIRC-10 (Invitrogen) as described previously (Minchenko et al. 2018b). 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 *IDE* and *PITRM1* 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 endoplasmic reticulum stress signaling mediated by ERN1 bifunctional enzyme in the expression level of mRNA

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
IDE	insulin degrading enzyme	F: 5'-aggccttcatacctcagctc R: 5'-gctgacttggaaggagaggt	1220–1239 2361–2342	NM_004969.4
PITRM1	pitrilysin metallopeptidase 1	F: 5'-acccaccatacctgtcacag R: 5'-tgctcccggtagtcaagaag	1708–1727 1914–1895	NM_014889.4
ACTB	beta-actin	F: 5'-ggacttcgagcaagagatgg R: 5'-agcactgtgttggcgtacag	747–766 980–961	NM_001101
MIR7-2	hsa-miR-7-5p	F: 5'-tggaagactagtgattttgttgtt	32–55	NR_029606
MIRLET7A2	hsa-let-7a-5p	F: 5'-tgaggtagtaggttgtatagtt	5–26	NR_029477

for insulin degrading enzyme (IDE) and pitrilysin metallopeptidase 1 (PITRM1) in U87 glioma cells, we used cells with a full deficiency of ERN1, introduced by dnERN1 (cells without both protein kinase and endoribonuclease activities), and with deficiency in ERN1 endoribonuclease, introduced by dnrERN1 (cells without endoribonuclease activity only). As shown in Figure 1, the expression of IDE mRNA in U87 glioma cells, transfected by dnERN1, is down-regulated (–17%) in comparison with control glioma cells, transfected by empty vector. At the same time, inhibition of ERN1 endoribonuclease does not significantly change the expression level of this gene (Figure 1). We next investigated the effect of endoplasmic reticulum stress induced by tunicamycin on the expression of gene encoding insulin degrading enzyme in glioma cells with mutation in ERN1 endoribonuclease. As shown in Figure 1, the expression of IDE mRNA is significantly up-regulated (+43%) in U87 glioma cells without ERN1 endoribonuclease activity after treatment with tunicamycin in comparison with no treated cells.

Furthermore, the expression level of PITRM1 mRNA in U87 glioma cells without both protein kinase and endoribonuclease activities is also down-regulated in comparison with control glioma cells, transfected by empty vector, but these changes were more significant (–45%; Figure 2). We have also shown that the expression level of pitrilysin metallopeptidase 1 mRNA in glioma cells with inhibition of ERN1 endoribonuclease, introduced by dnrERN1, does not significantly change as compared to control glioma cells (Figure 2). Moreover, as shown in Figure 2, induction of endoplasmic reticulum stress in glioma cells with suppressed ERN1 endoribonuclease by tunicamycin up-regulates the expression level of pitrilysin metallopeptidase 1 mRNA (+15%).

We also studied the expression of microRNAs MIR7-2 (miR-7; has-miR-7-5p) and MIRLET7A2 (miR-7a; has-let-7a-5p) in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 in relation to suppression of functional activity of ERN1 signaling enzyme. Bioinformatics analysis of the 3'-untranslated region of IDE and PITRM1 mRNAs identified possible target sites for MIR7-2 and MIRLET7A2, correspondingly, which can participate in posttranscriptional regulation of these mRNA expressions. As shown in Figure 3,
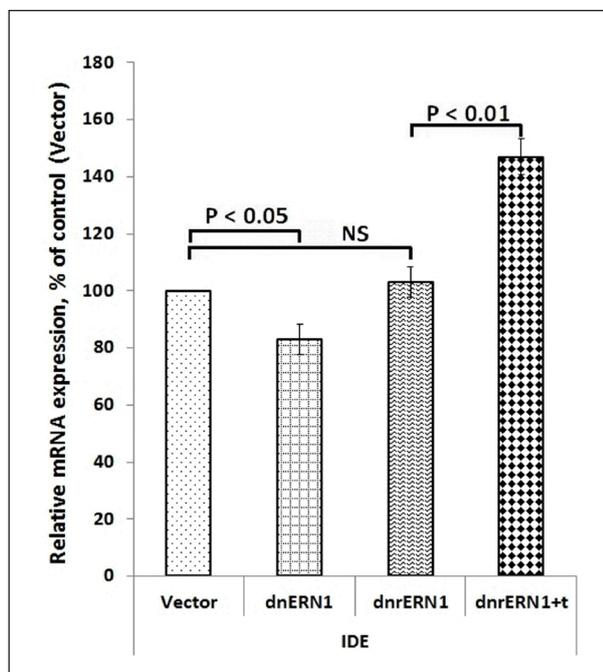
Figure 1. Insulin degrading enzyme (*IDE*) gene expression level in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 and dnrERN1 as well as in dnrERN1 cells treated by tunicamycin measured by qPCR. Values of IDE mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
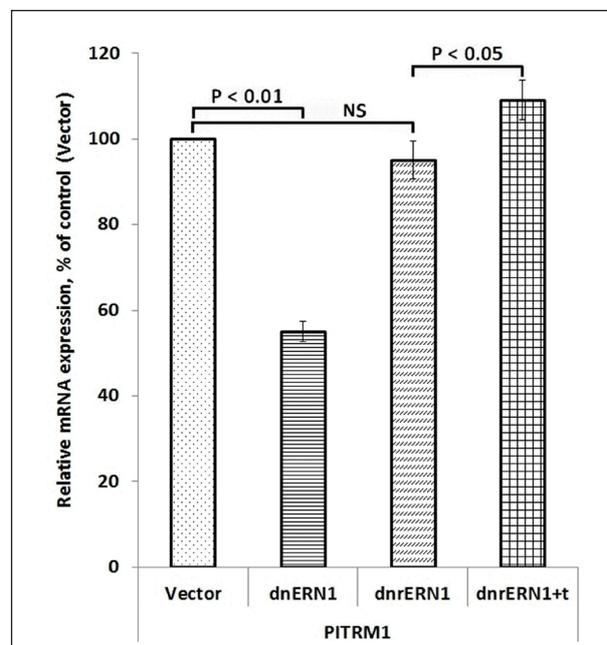
Figure 2. Pitrilysin metallopeptidase 1 (*PITRM1*) gene expression level in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 and dnrERN1 as well as in dnrERN1 cells treated by tunicamycin measured by qPCR. Values of PITRM1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
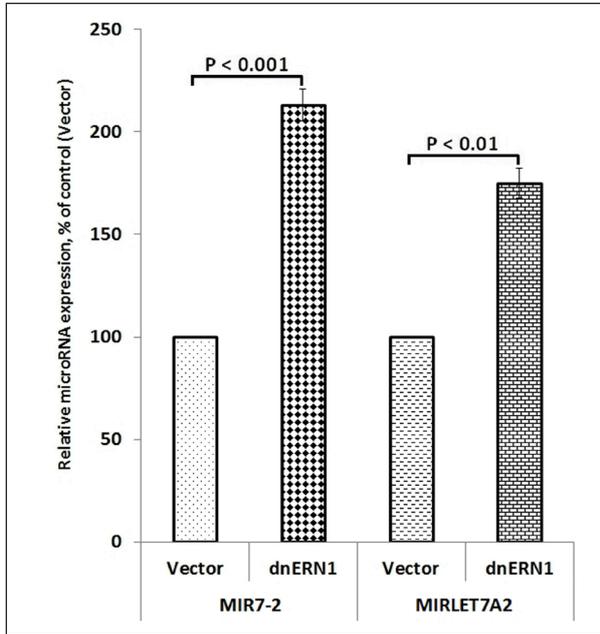
Figure 3. The expression level of microRNA MIR7-2 (miR-7; has-miR-7-5p) and MIRLET7A2 (miR-7a; has-let-7a-5p) in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of these mRNA expressions were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
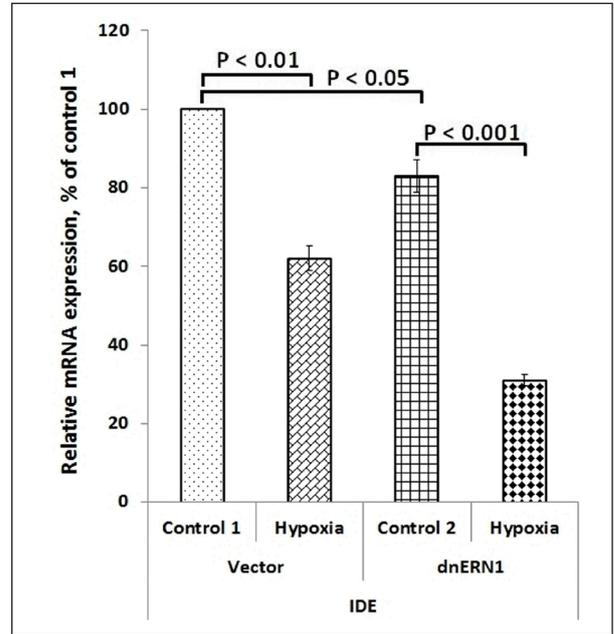
Figure 4. Effect of hypoxia on the expression level of insulin degrading enzyme (IDE) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of IDE mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
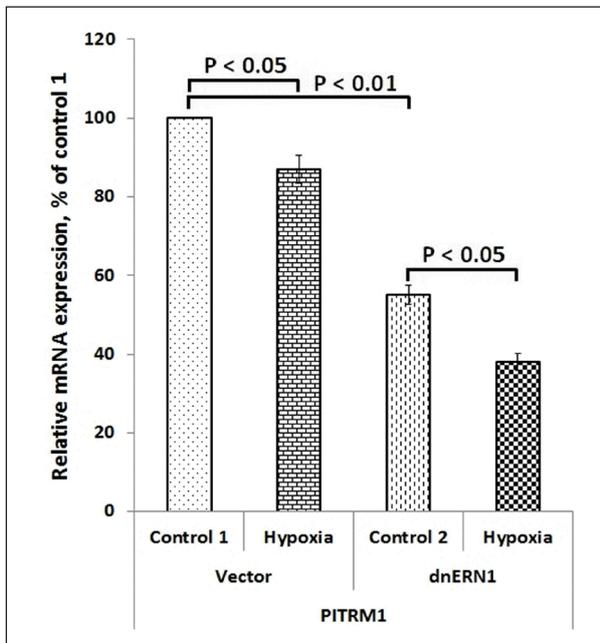
Figure 5. Effect of hypoxia on the expression level of pitrilysin metallopeptidase 1 (PITRM1) mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PITRM1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
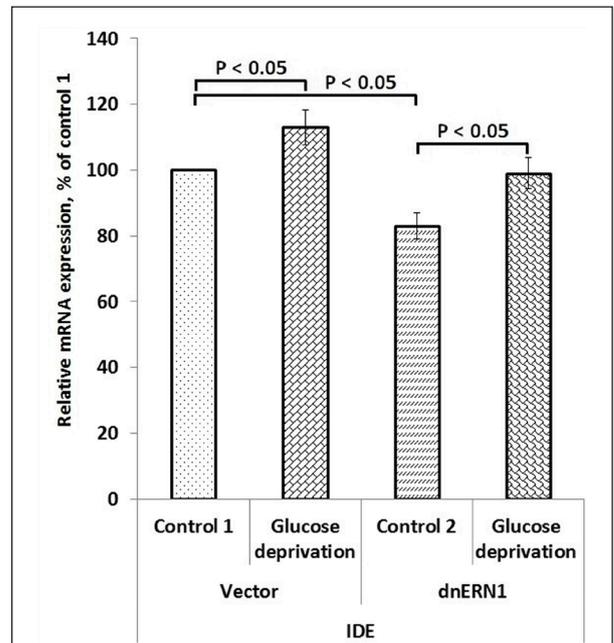
Figure 6. Effect of glucose deprivation on the expression level of insulin degrading enzyme mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of IDE mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

the expression level of microRNA MIR7-2 and MIRLET7A2 is up-regulated, being more significant for MIR7-2, in glioma cells without both ERN1 protein kinase and endoribonuclease activities in comparison with control cells (+113 and +75%, respectively). Therefore, the up-regulation of these microRNA levels correlates with down-regulation of corresponding mRNA (IDE and PITRM1) in ERN1 knockdown glioma cells.

We also investigated the effect of hypoxia on the expression of gene encoding insulin degrading enzyme in glioma cells in relation to complete inhibition of ERN1 signaling enzyme function (both protein kinase and endoribonuclease activities). It was shown that in control glioma cells (transfected by empty vector) the expression level of *IDE* gene is down-regulated (–38%) under hypoxic condition in comparison with cells growing in regular condition (Figure 4). At the same time, inhibition of ERN1 signaling enzyme function significantly enhances the effect of hypoxia on insulin degrading enzyme gene expression as compared to corresponding control cells, transfected by dnERN1 (–63%; Figure 4). Furthermore, effect of hypoxia on the expression of pitrilysin metallopeptidase 1 gene in control glioma cells was signifi-

cantly less than on *IDE* gene (Figure 5). Thus, exposure cells under hypoxia leads to down-regulation of *PITRM1* gene expression on 13%. It was also shown that complete inhibition of ERN1 signaling enzyme function (both protein kinase and endoribonuclease activities) also modifies the effect of hypoxia on this gene expression. Thus, the expression of pitrilysin metallopeptidase 1 gene is down-regulated (–31%) in comparison to control glioma cells, transfected with dnERN1 (Figure 5).

As shown in Figure 6 and Figure 7, exposure glioma cells under glucose deprivation condition leads to small but statistically significant up-regulation of IDE and PITRM1 mRNA expressions (+13 and +15%, respectively) in comparison with control cells growing under condition with glucose. Furthermore, inhibition of both enzymatic activities of ERN1 does not significantly modify the effect of glucose deprivation condition on the expression of insulin degrading enzyme, but increase the sensitivity of pitrilysin metallopeptidase 1 gene expression to this deprivation condition. Thus, exposure ERN1 knockdown glioma cells under glucose deprivation condition leads to up-regulation of IDE and PITRM1 mRNA expressions on 19% and 29%, respectively, in
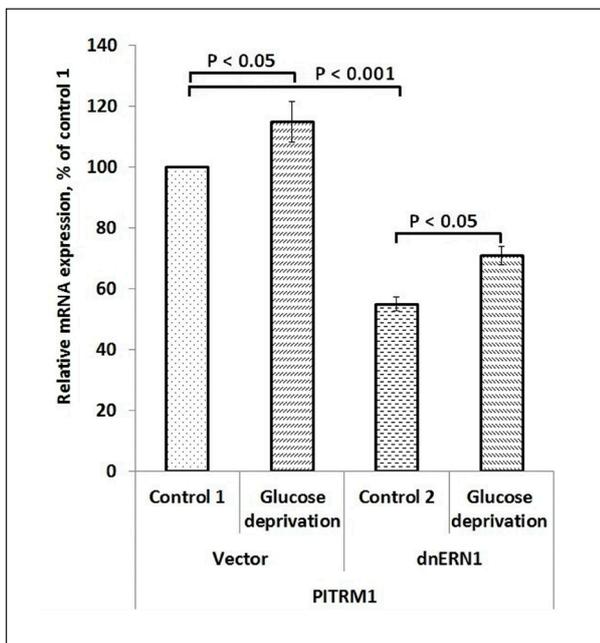
Figure 7. Effect of glucose deprivation on the expression level of PITRM1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PITRM1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
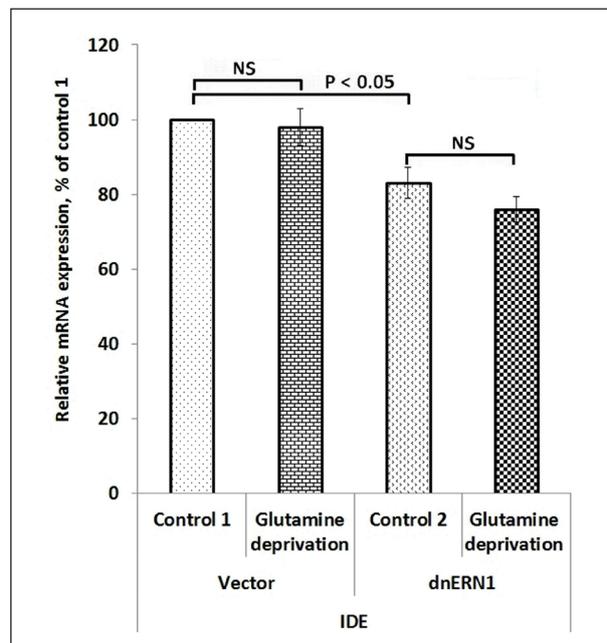
Figure 8. Effect of glutamine deprivation on the expression level of insulin degrading enzyme mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of IDE mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.
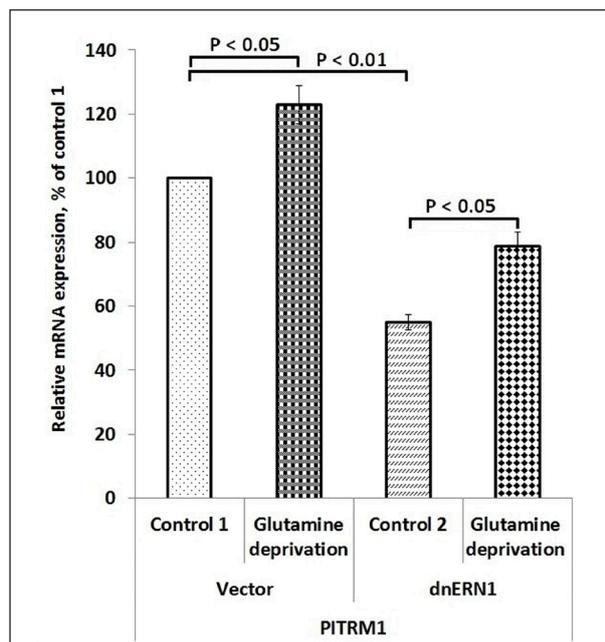
Figure 9. Effect of glutamine deprivation on the expression level of PITRM1 mRNA in control U87 glioma cells (Vector) and cells with a blockade of the ERN1 by dnERN1 measured by qPCR. Values of PITRM1 mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4.

comparison with control cells growing under condition with glucose (Figure 6 and Figure 7).

We also studied the expression of IDE and PITRM1 mRNAs in glioma cells exposure under glutamine deprivation condition in relation to inhibition of endoplasmic reticulum stress signaling mediated by ERN1. As shown in Figure 8 and Figure 9, exposure glioma cells under glutamine deprivation condition does not change significantly the expression of insulin degrading enzyme independently from ERN1 knockdown. At the same time, the expression of pitrilysin metallopeptidase 1 mRNA is up-regulated (+23%) in control glioma cells in comparison with control cells growing under condition with glutamine (Figure 8). Furthermore, inhibition of both enzymatic activities of ERN1 significantly enhances the effect of glutamine deprivation condition on pitrilysin metallopeptidase 1 mRNA expressions (+44%; Figure 9).

The inhibition of the endoplasmic reticulum stress signaling mediated by ERN1 affects the expression of insulin degrading enzyme and pitrilysin metallopeptidase 1 in U87 glioma cells through ERN1 protein kinase signaling pathway. Furthermore, hypoxia and glucose deprivation affected the expression of both

genes in gene-specific manner and these effects on gene expressions preferentially depended on ERN1 enzymatic activities.

Discussion

In this work, we studied the expression of genes encoding insulin degrading enzyme and pitrilysin metallopeptidase 1 in U87 glioma cells in relation to inhibition of ERN1, the major signaling pathway of the unfolded protein response. We used 2 variants of ERN1 deficiency: full ERN1 deficiency introduced by dnERN1 (cells without both protein kinase and endoribonuclease activities), and cells with deficiency in ERN1 endoribonuclease, introduced by dnrERN1 (cells without endoribonuclease activity only). This is important for evaluation of possible significance of *IDE* and *PITRM1* genes in the control of metabolism growth through endoplasmic reticulum stress signaling mediated by ERN1 because this stress signaling pathway is involved in numerous metabolic pathways and inhibition of ERN1 enzyme activity had 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).

We showed that the expression of studied genes *IDE* and *PITRM1* is responsible to endoplasmic reticulum stress signaling mediated by ERN1 and is down-regulated in glioma cells without both protein kinase and endoribonuclease activities. However, inhibition of ERN1 endoribonuclease activity does not affect the expression of both *IDE* and *PITRM1* genes. Thus, the expression of both these genes is regulated preferentially by ERN1 protein kinase signaling via mechanisms similar to that described by Auf et al. (2013) and Minchenko et al. (2015c). Functional significance of down-regulated expression of both *IDE* and *PITRM1* genes under inhibition of both ERN1 protein kinase and endoribonuclease activities is possibly connected with their important role in endoplasmic reticulum stress signaling and many other key processes because the expression of these genes is associated with type 2 diabetes, Alzheimer's disease, and cancer (Pivovarova et al. 2015; Brunetti et al. 2016; Liu et al. 2018; Fernandez-Diaz et al. 2019; Hetz et al. 2019; Kulas et al. 2019). There are data indicating that IDE knockdown affected the expression of genes involved in cell cycle and apoptosis pathways and that proliferation rate in IDE knockdown cells is lower than in controls (Pivovarova et al. 2015). Furthermore, we showed that the induction of endoplasmic reticulum stress by tunicamycin leads to up-regulation of the expression of both *IDE* and *PITRM1* genes in glioma cells with

suppressed ERN1 endoribonuclease. It is possible that the expression of these genes is also controlled by other signaling pathways of endoplasmic reticulum stress, not only ERN1 protein kinase. These agree well with data Mercado et al. (2018) that inhibition of PERK signaling has neuroprotective effect, which is accompanied by an increase in dopamine level and the expression of synaptic proteins.

We also observed up-regulation of microRNA MIR7-2 and MIRLET7A2, which have specific binding sites in 3'-untranslated region of IDE and PITRM1 mRNAs, correspondingly, in glioma cells with ERN1 knockdown. Furthermore, the level of these mRNA is down-regulated. Thus, our findings provide evidence that the decreased expression pattern of IDE and PITRM1 mRNAs is possibly regulated at both transcriptional and post-transcriptional levels. Thus, up-regulation of microRNA MIR7-2 agrees well with an important role of this microRNA in the control of insulin signaling and Aβ levels through posttranscriptional regulation of the insulin related proteins including insulin degrading enzyme (Fernandez-de Frutos et al. 2019). These results confirm to data that noncoding RNA functions in regulating adaptive pathways in both physiological and pathophysiological conditions, illustrating how they operate within the known UPR functions and contribute to diverse cellular outcomes (McMahon et al. 2017).

We also showed that hypoxia enhances the expression of *IDE* and *PITRM1* genes both in control and ERN1 knockdown glioma cells and that inhibition of ERN1 signaling enhances the effect of hypoxia on these genes' expression (Figure 10). Thus, blockade of ERN1 signaling modifies effect of hypoxia on the expression of *IDE* and *PITRM1* genes. It is possible that hypoxic stimulation of *IDE* and *PITRM1* gene expressions in glioma cells is realized by transcription factor HIF through specific hypoxia responsible elements, which we identified in promoter region of these genes by bioinformatics analysis. Moreover, in the promoter region of *IDE* gene we also identified three potential binding sites for XBP1, a major transcription factor of ERN1 signaling, and two of them are overlapping with HIF binding sites (CCACGC/TG). Similar results we also received for the promoter region of *PITRM1* gene: three potential binding sites for HIF and two of them are overlapping with XBP1 binding sites (CCACGCC). It is possible that hypoxic regulation of *IDE* and *PITRM1* genes is realized through specific interaction of HIF and XBP1 transcription factors on such binding sites, at least partially, and elimination of active XBP1 by ERN1 knockdown (Auf et al. 2010) modifies the effect of hypoxia on these gene expressions. At the same time, there are data indicating that many factors can facilitate interaction of HIF with endoplasmic reticulum
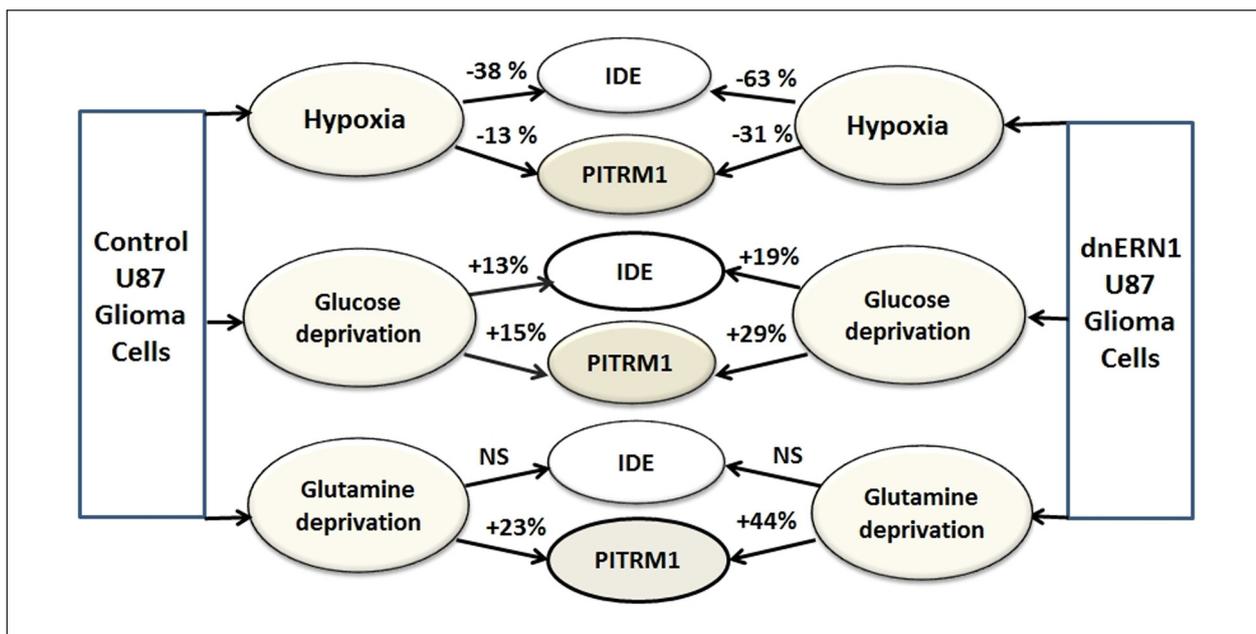
Figure 10. Schematic demonstration of *IDE* and *PITRM1* genes expression profile in control and ERN1 knockdown glioma cells under hypoxia, glucose and glutamine deprivations; NS – no significant changes.

stress response-signaling pathway mediated by ERN1 (Manie et al. 2014; Sun and Denko 2014; Chevet et al. 2015). Thus, there are data indicating that HIF-1α is associated with numerous upstream and downstream proteins and an examination of upstream hypoxic and nonhypoxic regulation of HIF1 as well as of downstream HIF1-regulated proteins may provide further insight into the role of this transcription factor in the pathophysiology of glioblastoma at the molecular level (Womeldorff et al. 2014; Semenza 2017). Our results validate tight interaction of endoplasmic reticulum stress signaling pathways with hypoxic regulation of the expression of genes encoding insulin-degrading enzyme and pitrilysin metallopeptidase 1, but precise molecular mechanisms are not clear yet and require further investigations.

We also demonstrated that exposure glioma cells under glucose deprivation condition leads to up-regulation of IDE and PITRM1 mRNA expressions and that inhibition of ERN1 signaling modify the effect of glucose deprivation condition on the expression of pitrilysin metallopeptidase 1 gene only (Figure 10). These results correlate with the data of Pivovarova et al. (2009) indicating that the insulin-induced activation of insulin-degrading enzyme in HepG2 cells is glucose dependent. Furthermore, our results demonstrate that the effect of glucose as well as glutamine deprivation condition on the expression of pitrilysin metallopeptidase 1 mRNA depends on ERN1 signaling and validates tight interaction of endoplasmic reticulum stress signaling pathways with glucose and glutamine levels. Previously it was shown that many gene expressions affected by glutamine deprivation condition in ERN1 dependent manner including ATF6 and PERK/EIF2AK3, key signaling pathways of endoplasmic reticulum stress (Minchenko et al. 2018a).

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding insulin-degrading enzyme and pitrilysin metallopeptidase 1 in glioma cells in response to complete inhibition of ERN1 activity (protein kinase and endoribonuclease) 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. Moreover, we identified microRNA, which increased expression can contribute to post-transcriptional up-regulation of IDE and PITRM1 mRNAs in ERN1 knockdown glioma cells. Thus, endoplasmic reticulum stress mediated changes in these gene expressions could possibly responsible for numerous pathological processes, in which insulin-degrading enzyme and pitrilysin metallopeptidase 1 play an important role as multifunctional proteins. Our results further demonstrate that hypoxia induces the expression of insulin-degrading enzyme and pitrilysin metallopeptidase 1 genes in control and ERN1 knockdown glioma cells and that inhibition of ERN1 signaling enhances the effect of hypoxia on these genes expression. It is interesting to note that our results validates tight interaction of endoplasmic reticulum stress signaling pathways with hypoxia and glucose deprivation in the regulation of the expression of genes encoding insulin-degrading enzyme and pitrilysin metallopeptidase 1, but the detailed molecular mechanisms of this regulation have not been yet clearly defined and require further investigation.

Acknowledgement

References

Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, Luis A, McCarthy N, Montibeller L, More S, Papaioannou A, Puschel F, Sassano ML, Skoko J, Agostinis P, de Belleroche J, Eriksson LA, Fulda S, Gorman AM, Healy S, Kozlov A, Munoz-Pinedo C, Rehm M, Chevet E, Samali A. Endoplasmic reticulum stress signalling - from basic mechanisms to clinical applications. FEBS J 286, 241–278, 2019.

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.

Bravo R, Parra V, Gatica D, Rodriguez AE, Torrealba N, Paredes F, Wang ZV, Zorzano A, Hill JA, Jaimovich E, Quest AF, Lavandero S. Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration. Int Rev Cell Mol Biol 301, 215–290, 2013.

Brunetti D, Torsvik J, Dallabona C, Teixeira P, Sztromwasser P, Fernandez-Vizarra E, Cerutti R, Reyes A, Preziuso C, D'Amati G, Baruffini E, Goffrini P, Viscomi C, Ferrero I, Boman H, Telstad W, Johansson S, Glaser E, Knappskog PM, Zeviani M, Bindoff LA. Defective PITRM1 mitochondrial peptidase is associated with Aβ amyloidotic neurodegeneration. EMBO Mol Med 8, 176–190, 2016.

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

Chow KM, Gakh O, Payne IC, Juliano MA, Juliano L, Isaya G, Hersh LB. Mammalian pitrilysin: substrate specificity and mitochondrial Targeting. Biochemistry 48, 2868–2877, 2009.

da Costa IB, de Labio RW, Rasmussen LT, Viani GA, Chen E, Villares J, Turecki G, Smith MAC, Payao SLM. Change in INSR, APBA2 and IDE gene expressions in brains of Alzheimer's disease patients. Curr Alzheimer Res 14, 760–765, 2017.

Doultsinos D, Avril T, Lhomond S, Dejeans N, Guedat P, Chevet E. Control of the unfolded protein response in health and disease. SLAS Discov 22, 787–800, 2017.

Falkevall A, Alikhani N, Bhushan S, Pavlov PF, Busch K, Johnson KA, Eneqvist T, Tjernberg L, Ankarcrona M, Glaser E. Degradation of the amyloid beta-protein by the novel mitochondrial peptidasome, PreP. J Biol Chem 281, 29096–29104, 2006.

Fernandez-de Frutos M, Galan-Chilet I, Goedeke L, Kim B, Pardo-Marques V, Perez-Garcia A, Herrero JI, Fernandez-Hernando C, Kim J, Ramirez CM. MicroRNA 7 impairs insulin signaling and regulates Aβ levels through posttranscriptional regulation of the insulin receptor substrate 2, insulin receptor, insulin-degrading enzyme, and liver X receptor pathway. Mol Cell Biol 39. pii: e00170–19, 2019.

Fernandez-Diaz CM, Merino B, Lopez-Acosta JF, Cidad P, de la Fuente MA, Lobaton CD, Moreno A, Leissring MA, Perdomo G, Cozar-Castellano I. Pancreatic β-cell-specific deletion of insulin-degrading enzyme leads to dysregulated insulin secretion and β-cell functional immaturity. Am J Physiol Endocrinol Metab 317, E805–E819, 2019.

Garcia-Gonzalez P, Cabral-Miranda F, Hetz C, Osorio F. Interplay between the unfolded protein response and immune function in the development of neurodegenerative diseases. Front Immunol 9, 2541, 2018.

Gerakis Y, Hetz C. Emerging roles of ER stress in the etiology and pathogenesis of Alzheimer's disease. FEBS J 285, 995–1011, 2018.

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.

Hetz C, Chevet E, Harding HP. Targeting the unfolded protein response in disease. Nat Rev Drug Discov 12, 703–719, 2013.

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

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

Kazkayasi I, Burul-Bozkurt N, Ismail MA, Merino-Serrais P, Pekiner C, Cedazo-Minguez A, Uma S. Insulin deprivation decreases insulin degrading enzyme levels in primary cultured cortical neurons and in the cerebral cortex of rats with streptozotocin-induced diabetes. Pharmacol Rep 70, 677–683, 2018.

King JV, Liang WG, Scherpelz KP, Schilling AB, Meredith SC, Tang WJ. Molecular basis of substrate recognition and degradation by human presequence protease. Structure 22, 996–1007, 2014.

Kulas JA, Franklin WF, Smith NA, Manocha GD, Puig KL, Nagamoto-Combs K, Hendrix RD, Taglialatela G, Barger SW, Combs CK. Ablation of amyloid precursor protein increases insulin-degrading enzyme levels and activity in brain and peripheral tissues. Am J Physiol Endocrinol Metab 316, E106–E120, 2019.

Kurochkin IV, Guarnera E, Wong JH, Eisenhaber F, Berezovsky IN. Toward allosterically increased catalytic activity of insulin-degrading enzyme against amyloid peptides. Biochemistry 56, 228–239, 2017.

Lee J, Ozcan U. Unfolded protein response signaling and metabolic diseases. J Biol Chem 289, 1203–1211, 2014.

Liu Z, Dai J, Shen H. Systematic analysis reveals long noncoding RNAs regulating neighboring transcription factors in human cancers. Biochim Biophys Acta Mol Basis Dis 1864, 2785–2792, 2018.

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.

Manie SN, Lebeau J, Chevet E. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 3. Orchestrating the unfolded protein response in oncogenesis: an update. Am J Physiol Cell Physiol 307, C901–C907, 2014.

Marciniak SJ. Endoplasmic reticulum stress: a key player in human disease. FEBS J 286, 228–231, 2019.

Martinez A, Lopez N, Gonzalez C, Hetz C. Targeting of the unfolded protein response (UPR) as therapy for Parkinson's disease. Biol Cell 111, 161–168, 2019.

McMahon M, Samali A, Chevet E. Regulation of the unfolded protein response by noncoding RNA. Am J Physiol Cell Physiol 313, C243–C254, 2017.

Mercado G, Castillo V, Soto P, Lopez N, Axten JM, Sardi SP, Hoozemans JJM, Hetz C. Targeting PERK signaling with the small molecule GSK2606414 prevents neurodegeneration in a model of Parkinson's disease. Neurobiol Dis 112, 136–148, 2018.

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, 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 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, 2015b.

Minchenko OH, Tsymbal DO, Minchenko DO, Moenner M, Kovalevska OV, Lypova NM. Inhibition of kinase and endoribonuclease activity of ERN1/IRE1 affects expression of proliferation-related genes in U87 glioma cells. Endoplasm Reticul Stress Dis 2, 18–29, 2015c.

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, 2016.

Minchenko OH, Luzina OY, Hnatiuk OS, Minchenko DO, Garmash YA, Ratushna OO. Expression of tumor growth related genes in IRE1 knockdown U87 glioma cells: effect of hypoxia. Ukr Biochem J 89, 40–51, 2017.

Minchenko OH, Kharkova AP, Hnatiuk OS, Luzina OY, Kryvdiuk IV, Kuznetsova AY. ERN1 modifies effect of glutamine deprivation on tumor growth related factors expression in U87 glioma cells. Ukr Biochem J 90, 49–61, 2018a.

Minchenko OH, Tsymbal DO, Minchenko DO, Hnatiuk OS, Prylutskyy YI, Prylutska SV, Ritter U. Single-walled carbon nanotubes affect the expression of genes associated with immune response in normal human astrocytes. Toxicol In Vitro 52, 122–130, 2018b.

Moenner M, Pluquet O, Bouchecareilh M, Chevet E. Integrated endoplasmic reticulum stress responses in cancer. Cancer Res 67, 10631–10634, 2007.

Ohyagi Y, Miyoshi K, Nakamura N. Therapeutic strategies for Alzheimer's disease in the view of diabetes mellitus. Adv Exp Med Biol 1128, 227–248, 2019.

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

Pinho CM, Teixeira PF, Glaser E. Mitochondrial import and degradation of amyloid-β peptide. Biochim Biophys Acta 1837, 1069–1074, 2014.

Pivovarova O, Gogebakan O, Pfeiffer AF, Rudovich N. Glucose inhibits the insulin-induced activation of the insulin-degrading enzyme in HepG2 cells. Diabetologia 52, 1656–1664, 2009.

Pivovarova O, von Loeffelholz C, Ilkavets I, Sticht C, Zhuk S, Murahovschi V, Lukowski S, Docke S, Kriebel J, de las Heras Gala T, Malashicheva A, Kostareva A, Lock JF, Stockmann M, Grallert H, Gretz N, Dooley S, Pfeiffer AF, Rudovich N. Modulation of insulin degrading enzyme activity and liver cell proliferation. Cell Cycle 14, 2293–2300, 2015.

Pivovarova O, Hohn A, Grune T, Pfeiffer AF, Rudovich N. Insulin-degrading enzyme: new therapeutic target for diabetes and Alzheimer's disease? Ann Med 48, 614–624, 2016.Sandoval K, Umbaugh D, House A, Crider A, Witt K. Somatostatin receptor subtype-4 regulates mRNA expression of amyloid-beta degrading enzymes and microglia mediators of phagocytosis in brains of 3xTg-AD mice. Neurochem Res 44, 2670–268, 2019.

Semenza GL. A compendium of proteins that interact with HIF-1α. Exp Cell Res 356, 128–135, 2017.

Smith-Carpenter JE, Alper BJ. Functional requirement for human pitrilysin metallopeptidase 1 arginine 183, mutated in amyloidogenic neuropathy. Protein Sci 27, 861–873, 2018.

Sun RC, Denko NC. Hypoxic regulation of glutamine metabolism through HIF1 and SIAH2 supports lipid synthesis that is necessary for tumor growth. Cell Metab 19, 285–292, 2014.

Tang WJ. Targeting insulin-degrading enzyme to treat Type 2 Diabetes Mellitus. Trends Endocrinol Metab 27, 24–34, 2016.

Tundo GR, Sbardella D, Ciaccio C, Grasso G, Gioia M, Coletta A, Polticelli F, Di Pierro D, Milardi D, Van Endert P, Marini S, Coletta M. Multiple functions of insulin-degrading enzyme: a metabolic crosslight? Crit Rev Biochem Mol Biol 52, 554–582, 2017.

Villa-Perez P, Merino B, Fernandez-Diaz CM, Cidad P, Lobaton CD, Moreno A, Muturi HT, Ghadieh HE, Najjar SM, Leissring MA, Cozar-Castellano I, Perdomo G. Liver-specific ablation of insulin-degrading enzyme causes hepatic insulin resistance and glucose intolerance, without affecting insulin clearance in mice. Metabolism 88, 1–11, 2018.

Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature 529, 326–335, 2016.

Womeldorff M, Gillespie D, Jensen RL. Hypoxia-inducible factor–1 and associated upstream and downstream proteins in the pathophysiology and management of glioblastoma. Neurosurg Focus 37, E8, 2014.

Yamamoto N, Ishikuro R, Tanida M, Suzuki K, Ikeda-Matsuo Y, Sobue K. Insulin-signaling pathway regulates the degradation of amyloid β-protein via astrocytes. Neuroscience 385, 227–236, 2018.

Zhang Z, Liang WG, Bailey LJ, Tan YZ, Wei H, Wang A, Farcasanu M, Woods VA, McCord LA, Lee D, Shang W, Deprez-Poulain R, Deprez B, Liu DR, Koide A, Koide S, Kossiakoff AA, Li S, Carragher B, Potter CS, Tang WJ. Ensemble cryoEM elucidates the mechanism of insulin capture and degradation by human insulin degrading enzyme. Elife 7, e33572, 2018.

Zhang S, Xiao T, Yu Y, Qiao Y, Xu Z, Geng J, Liang Y, Mei Y, Dong Q, Wang B, Wei J, Suo G. The extracellular matrix enriched with membrane metalloendopeptidase and insulin-degrading enzyme suppresses the deposition of amyloid-beta peptide in Alzheimer's disease cell models. J Tissue Eng Regen Med 13, 1759–1769, 2019.

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.

Zingale GA, Bellia F, Ahmed IMM, Mielczarek P, Silberring J, Grasso G. IDE degrades Nociceptin/Orphanin FQ through an insulin regulated mechanism. Int J Mol Sci 20, E4447, 2019.