UDC 577.112.7:616 doi: https://doi.org/10.15407/ubj97.05.116

SCIENTIFIC ACHIEVEMENTS OF THE DEPARTMENT OF MOLECULAR BIOLOGY IN UNDERSTANDING STRESS-DEPENDENT MECHANISMS OF GLIOMA GROWTH

O. H. MINCHENKO✉, Y. M. VILETSKA, M. Y. SLIUSAR, O. O. KHITA

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine,
Department of Molecular Biology, Kyiv;
✉e-mail: ominchenko@yahoo.com

Received: 09 July 2025; **Revised:** 25 July 2025; **Accepted:** 30 October 2025

Since 2005, the Department of Molecular Biology has initiated research aimed at solving key problems in biochemistry and molecular biology, with an emphasis on elucidating the molecular basis of malignant tumor growth and the mechanisms of hypoxic regulation, the role of alternative splicing in the mechanisms of gene expression regulation, as well as the fundamental importance of endoplasmic reticulum stress in maintaining homeostasis and the development of pathological conditions, in particular, the growth of glioblastomas, the most malignant brain tumors that are difficult to treat. It has been shown that the expression of different 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB), key glycolysis regulators, is exacerbated in various malignant tumors and that PFKFB4 is a marker of tumor growth. It has been established that the expression level of PFKFB4 is controlled under hypoxia by a HIF-dependent mechanism, and a HIF-specific sequence has been identified in the promoter, the mutation of which completely removes hypoxic regulation of the PFKFB4 gene. Numerous splice variants of different PFKFB and VEGFA genes have also been identified. It has been established that inhibition of endoplasmic reticulum stress, its ERN1 signaling pathway, reduces the proliferation of glioblastoma cells by changing the expression levels of oncogenes, tumor suppressors, mitochondrial enzymes, as well as insulin and glucocorticoid receptors and their dependent proteins. An important role of ERN1 protein kinase activity in regulating the expression of various genes has been revealed, and its inhibition has been shown to lead to increased invasiveness of glioblastoma cells upon ERN1 knockdown. Attention is focused on studying non-canonical mechanisms of hypoxic gene expression regulation and its dependence on endoplasmic reticulum stress.

K e y w o r d s: glioblastoma cells, hypoxia, endoplasmic reticulum stress, ERN1, gene expression.

It has previously been established that a key regulator of glucose is the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB), whose isoforms are encoded by four genes [1, 2]. This enzyme is responsible for maintaining cellular levels of fructose-2,6-bisphosphate, a potent allosteric activator of glycolysis. It has also been shown that the expression of these genes is controlled by hypoxia, but in different ways [1, 2]. Since glycolysis is a rather important factor in the growth of malignant tumors, studies have been conducted aimed at studying the expression level of PFKFB family genes in various malignant tumors, in particular adenocarcinomas of the lung, breast, stomach, and colon of humans, as well as at elucidating the molecular mechanisms of their hypoxic regulation.

PFKFB expression in malignant tumors, mechanisms of hypoxic regulation of gene expression

It has been established that the mRNA expression levels of *PFKFB1*, *PFKFB2*, *PFKFB3*, and *PFKFB4*, assessed by ribonuclease protection assay in human lung adenocarcinomas is increased compared to corresponding non-malignant tissue counterparts taken from the same patients, and this is associated with a sharp increase in the level of mRNA expression of the pro-tumorigenic genes glucose transporter 1 (*GLUT1*/*SLC2A1*) and vascular endothelial growth factor A (VEGFA) (Fig. 1) [3]. The intensity of RNase-protected bands from tumors and corresponding non-malignant tissue counterparts from 20 patients was quantified using a Fujix BAS

2000 Bio-Image Analyzer (Fuji Photo Film Co., Japan) and normalized to 18S rRNA [3].

The same figure also presents the results of a study of the expression level of the *PFKFB2, PFKFB3*, and *PFKFB4* genes in A_{549} human lung adenocarcinoma cells in culture. When comparing the expression levels of these genes in human lung tumors with expression in lung adenocarcinoma tumor cells, a large difference is revealed, especially in the expression of PFKFB4. The significantly lower level of expression of these genes in cultured tumor cells is due to hyperoxia (high oxygen levels in incubators), since keeping these cells in hypoxic conditions (3% oxygen for 16 hours) led to an increase in the expression level of the *PFKFB2*, *PFKFB3*, and *PFKFB4* genes in cultured lung adenocarcinoma cells, to the level found in human lung adenocarcinomas (Fig. 1) [3]. It is known that the oxygen level in malignant tumors is close to 0.5-1.5%; while in normal tissues it is much higher, from 3.4 to 8.4% (24-60 mm Hg) [4]. These results reveal the molecular mechanisms of increased glycolysis in malignant tumors and the role of hypoxia in this. It has also been demonstrated that increased expression of the *PFKFB3* and *PFKFB4* genes in malignant lung tumors is accompanied by a sharp increase in the level of proteins encoded by them [3].

The overexpression of *PFKFB3* and *PFKFB4* genes was also observed in the human breast and colon malignant tumors as compared to corresponding non-malignant tissue counterparts from same patients [5]. From the data presented in Fig. 2, it is clear that the expression of the genes *PFKFB3*, *PFKFB4*, and *HIF1A*, as well as GLUT1 and VEGFA, was dramatically increased in malignant breast tumors compared to corresponding non-malignant tissue counterparts from the same patient [5].

The results of *PFKFB3*, *PFKFB4*, *HIF1A, GLUT1*, and *VEGFA* gene expression obtained from 20 sites were quantified using a Fujix BAS 2000 Bio-Image Analyzer and statistically processed [5]. The results shown in this figure also show that PFKFB3 mRNA is present in relatively normal breast tissue, but PFKFB4 is not. At the same time, both mRNAs' levels are very high in tumour tissue [5]. This demonstrates that the so-called normoxia is hyperoxia, which is responsible for the sharp decrease in PFKFB3 and PFKFB4 in MCF7 breast adenocarcinoma cells during their culture. In human breast adenocarcinoma cells of the MCF7 line under normoxic conditions, mRNAs of both PFKFB3 and PFKFB4 were detected; however, in significantly lower quantities compared to tumor tissue. But hypoxia strongly increases the mRNA levels of both PFKFB3 and PFKFB4 in MCF7 breast adenocarcinoma cells.

However, PFKFB4 mRNA is still detected in MCF7 cells under normoxia, but is absent in condi-

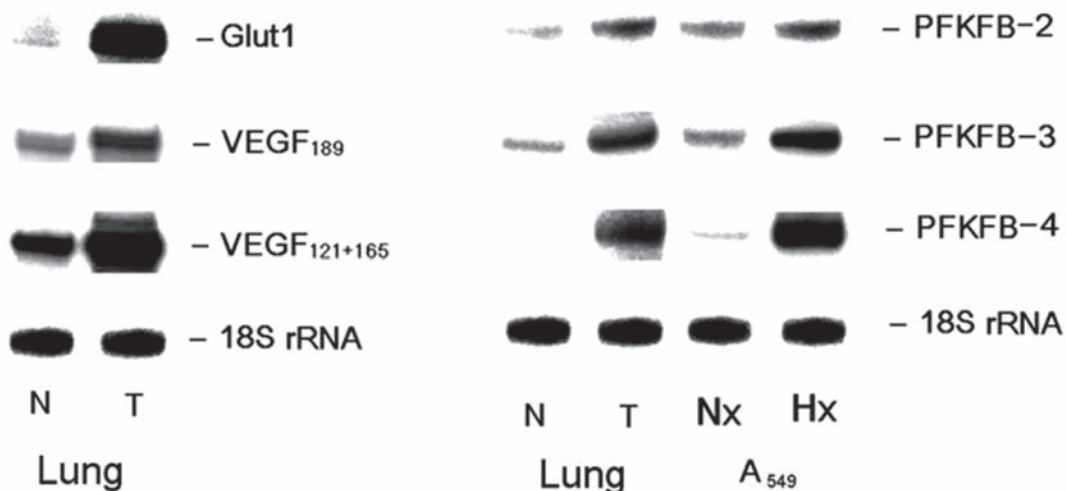

Fig. 1. Representative polyacrylamide gel autoradiograph employed in a typical ribonuclease protection assay of PFKFB-2, PFKFB-3, PFKFB-4, Glut1, and VEGF mRNAs from the human lung malignant tumors (T) and corresponding non-malignant tissue counterparts (N) from same patients as well as PFKFB2, PFKFB3, and PFKFB4 in A_{549} lung adenocarcinoma cells exposures under normoxic (Nx) and hypoxic (Hx) conditions, using 18 S rRNA as control of analyzed RNA quantity [3]

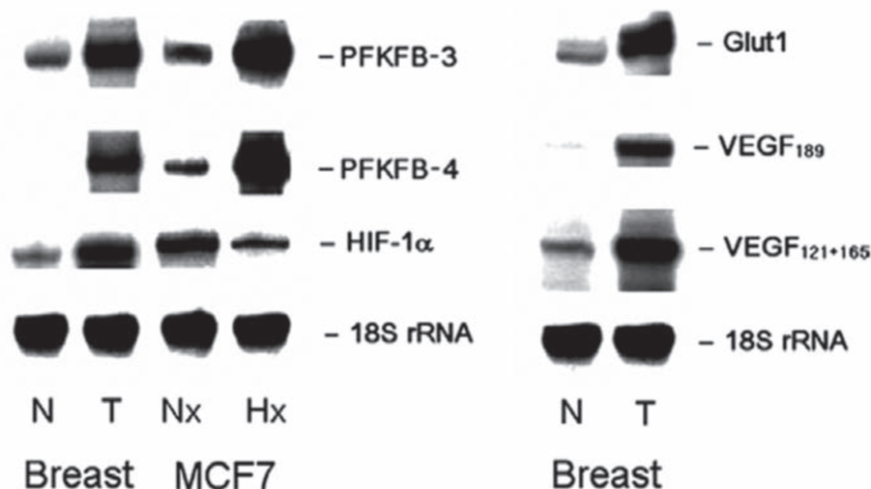

Fig. 2. Representative polyacrylamide gel autoradiograph employed in a typical ribonuclease protection assay of PFKFB-3, PFKFB-4, HIF1A, Glut1, and VEGF mRNAs from the human lung malignant tumors (T) and corresponding non-malignant tissue counterparts (N) from same patients as well as PFKFB3, PFKFB4, and HIF1A in MCF7 breast adenocarcinoma cells exposures under normoxic (Nx) and hypoxic (Hx) conditions, using 18 S rRNA as control of analyzed RNA quantity [5]

tionally normal (non-malignant) breast tissue. Similar results were obtained when studying the PFKFB4 protein (Fig. 3) [5].

At the same time, the level of HIF1A protein is detected in non-tumor breast tissue and increases during tumor growth; however, in MCF7 cells under normoxia, its level is significantly lower compared to tumor tissue and increases under hypoxia (Fig. 3) [5].

Thus, PFKFB4 expression in MCF7 adenocarcinoma cells is possibly controlled not only by hypoxia but also by oncogenes. It is important to note that the increased expression of PFKFB3 and PFKFB4 in MCF7 adenocarcinoma cells under hypoxia is accompanied by a decrease in HIF1A mRNA levels (Fig. 2). But the HIF1A protein level in these cells is increased, as is the PFKFB4 protein level (Fig. 3) [5]. An increase in HIF1A protein levels under hypoxia, accompanied by a decrease in HIF1A mRNA levels, has been shown for many other tumor and non-tumor cells [6-9].

Fig. 3 shows the results of a typical Western blot analysis of PFKFB3, PFKFB4, and HIF1A proteins in extracts from human breast and colon adenocarcinoma compared to corresponding conditionally normal tissue (N) from the same patients, as well as PFKFB4 and HIF1A from human breast adenocarcinoma cells of the MCF7 line under normoxia (Nx) and hypoxia (Hx) conditions. It has been shown that the levels of PFKFB3 and PFKFB4 proteins in extracts from conditionally normal breast tissue are very low, but increase intensely with tumor growth. At the same time, the HIF1A protein is present in conditionally normal tissue and increases in adenocarcinomas (Fig. 3). The level of PFKFB3 and PFKFB4 proteins in colon tumors also increased, but to a lesser extent, which is due to their high level in non-malignant tissue counterparts (Fig. 3) [5]. Thus, hypoxia inducible factor HIF1, together with PFKFB3 and PFKFB4, are important factors for solid tumor growth [1, 3, 5, 10-14].

In this regard, studies were conducted to investigate the impact of hypoxia on *PFKFB3* and *PFKFB4* gene expression in various tumor cell lines and elucidate the molecular basis of the hypoxic regulation of *PFKFB4* gene expression. Thus, it was found that in breast adenocarcinoma cells of the T47D, SKBR-3, MDA-MB-468, and BT549 lines, the level of *PFKFB4* gene expression increases under hypoxia, but with different magnitudes [16]. At the same time, the effect of hypoxia on *PFKFB3* gene expression was significantly smaller in most of these cell lines, but the basal level of its expression was much higher compared to PFKFB4 [16]. PFKFB4 protein levels also increased under hypoxia, although not as pronounced as the mRNA. Moreover, in adenocarcinoma cells of the MDA-MB-468 line, the level of PFKFB4 protein was practically unde-

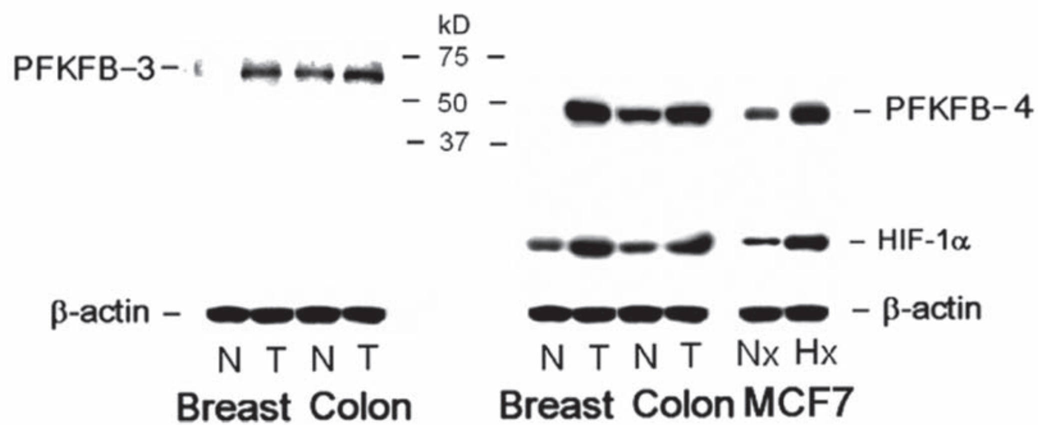

Fig. 3. Typical Western blot analysis of PFKFB3, PFKFB4, and HIF1A protein levels in extracts from human breast and colon malignant tumors (T) and corresponding non-malignant tissue counterparts (N) from the same patients, as well as PFKFB4 and HIF1A from human breast adenocarcinoma MCF7 cells under normoxia (Nx) and hypoxia (Hx) conditions; using β-actin to estimate the total amount of proteins taken for analysis [5]

tectable, despite the presence of mRNA [16]. The reason for this phenomenon is not entirely clear and warrants further study.

Interestingly, PFKFB4 mRNA expression was less sensitive to hypoxia in human gastric adenocarcinoma cells of the MKN45 and NUGC3 lines compared to cells of the Panc1 line (Fig. 4, *A*) [8]. From the data presented in this figure, it is also clear that PFKFB3 mRNA expression was, conversely, more sensitive to hypoxia in gastric adenocarcinoma cells of the MKN45 line and, especially, the NUGC3 line compared to Panc1 pancreatic adenocarcinoma cells [8]. This may indicate the cell-specific nature of hypoxic regulation of *PFKFB3* and *PFKFB*4 gene expression and their functional significance in different tumor cells. Moreover, in these cells, the level of PFKFB4 mRNA is relatively low under normoxia, as well as in gastric adenocarcinoma cells of both lines, which is even lower than in Panc1 pancreatic cells (Fig. 4, *A*). However, the protein level in Panc1 cells under these conditions is very low, and in gastric adenocarcinoma cells of both lines, it is very high, especially in NUGC3 cells (Fig. 4, *B*) [8]. Analysis of HIF1A protein levels in these three cell lines indicates the absence of clear relationships between different levels of PFKFB4 and HIF1A proteins under both normoxia and hypoxia (Fig. 4, *C*) [8]. Thus, the level of PFKFB4 protein is cell-specific and is determined not only by the mRNA level but also by several other mechanisms that affect its stability, in particular, post-translational modifications.

It was also demonstrated that PFKFB4 mRNA expression in PC-3, HeLa, Hep3B, and HepG2 tumor cell lines was highly sensitive to hypoxia, especially to dimethyloxalylglycine, an inhibitor of HIF1A prolyl hydroxylase. All this suggests that the response of this gene to hypoxia is regulated by the transcription factor HIF [17, 18]. To elucidate the molecular mechanisms of the dependence of PFKFB4 expression on hypoxia, the promoter region of the *PFKFB4* gene was isolated, reporter constructs with deletions and site-specific mutations were created, and the effect of hypoxia on the activity of these constructs was investigated in cells after transfection [18]. As a result of these studies, the hypoxia-responsive element (HRE) was identified in the promoter region of the *PFKFB4* gene, which mediates the hypoxic regulation of this gene. This was confirmed using two cell lines by site-specific mutagenesis of the promoter region of the *PFKFB4* gene [17, 18].

Investigations have also been conducted to study the impact of in vivo hypoxia on the expression of different *PFKFB* variants in various mouse and rat tissues. Moreover, the tissue-specific nature of various *PFKFB* gene expressions and their response to hypoxia was shown [2, 19, 20]. Many new alternative splice variants of various PFKFB and VEGFA mRNAs with unique characteristics have also been identified [18, 20-26]. Based on the identified alternative splice variants of PFKFB, as well as through the introduced mutations in the kinase domain, dominant-negative constructs were cre-

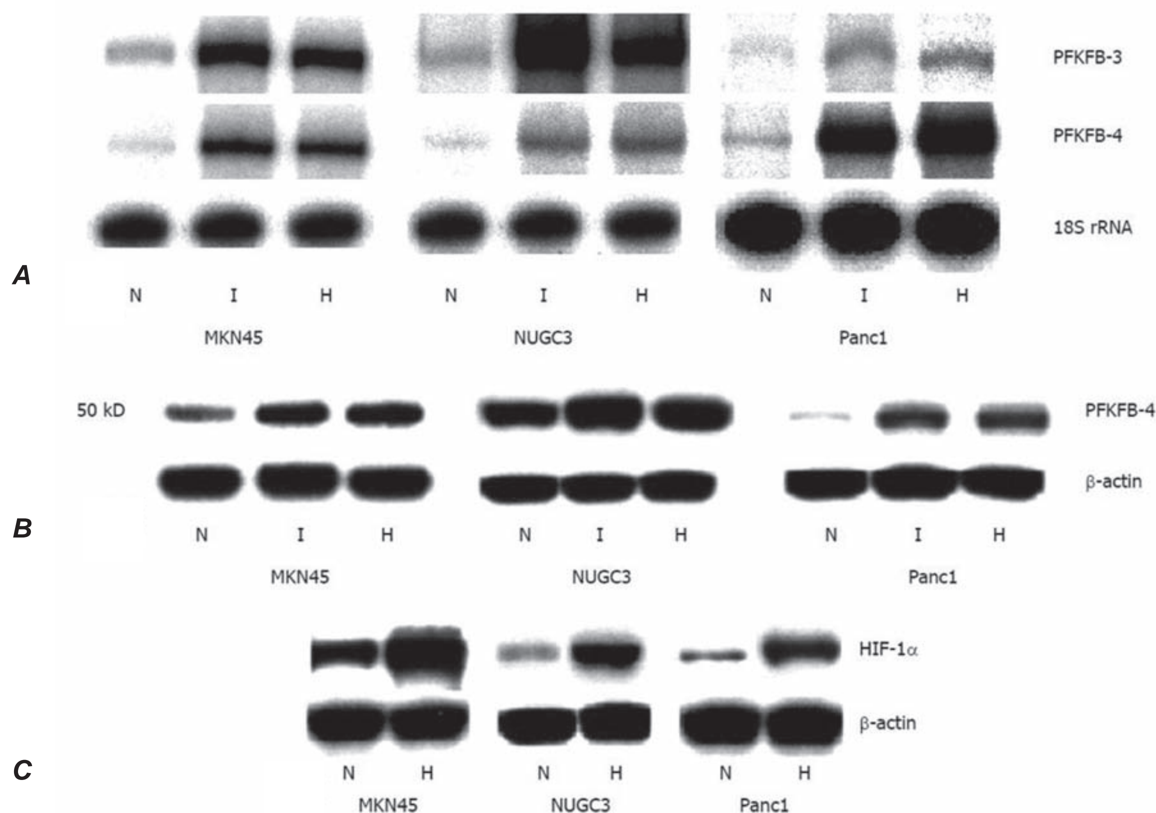

*Fig. 4. Effect of hypoxia (H) and hypoxia mimic prolyl hydroxylase inhibitor dimethyloxalylglycine (I) on the expression of PFKFB3 and PFKFB4 mRNA (**A**), protein level of PFKFB4 (**B**) and HIF1A (**C**) in human gastric cancer cell lines MKN45 and NUGC3 and pancreatic cancer cell line Panc1. The mRNA level was measured using a ribonuclease protection assay, and protein levels were measured using Western-blot analysis; N: normoxic (control) cells [8]*

ated to suppress tumor cells by reducing the level of PFKFB expression [27]. It is also important to note that PFKFB enzymes and hypoxia are not only key regulators of glycolysis; they also have other, equally important functions, such as transcriptional regulation and cell survival [4, 5].

The key role of endoplasmic reticulum stress and hypoxia in the control of gene expression during tumor growth

Endoplasmic reticulum stress and hypoxia are key regulators of malignant tumor development through changes in the expression of numerous genes that reprogram cellular metabolic processes for enhanced tumor growth and survival [28-32]. The rapid growth of malignant tumors generates microenvironmental changes in association with hypoxia, nutrient deficiency, and acidosis, which initiate angiogenesis and enhance cell proliferation [31, 33-35]. Malignant tumors utilize endoplasmic reticulum stress signaling systems to adapt to stressful environmental conditions [29, 31]. The response to endoplasmic reticulum stress is mediated by three interconnected sensory-signaling systems [30, 31]. The ERN1 (endoplasmic reticulum to nucleus signaling 1) sensory signaling pathway is the most evolutionarily conserved and responds to misfolded proteins in a program aimed at eliminating stress or apoptosis, making it a key regulator of cell life and death [29-31, 36].

The ERN1 protein contains two domains that exhibit protein kinase and endoribonuclease enzymatic activities, which control the expression of numerous stress-dependent genes [30, 31, 37]. Endoribonuclease activity of ERN1 initiates the cytosolic splicing of the pre-XBP1 (X-box binding protein 1) mRNA, whose mature transcript encodes for a transcription factor that controls the expression of

numerous stress-responsive genes, including regulatory and transcription factors [29, 30, 37, 38]. This ERN1 enzymatic activity is also involved in the degradation of a specific subset of mRNAs through the RIDD (Regulated ERN1-dependent decay of messenger RNAs) mechanism [39, 40]. Recently, the role of ERN1 endoribonuclease activity in CD95-mediated cell death has been demonstrated [36]. We have also shown that the ERN1 protein kinase activity is an important regulator of stress-dependent gene expression [41, 42]. For this purpose, a subline of glioblastoma cells with knockdown of ERN1 endoribonuclease activity was created [41]. This made it possible to establish that epiregulin (EREG), which is a ligand for the epidermal growth factor (EGF) receptor, is involved in glioblastoma growth through the signaling protein ERN1, namely through its protein kinase activity and further through JNK (c-Jun N-terminal kinase) [41] (Fig. 5).

It is important to note that ERN1-mediated EREG expression was not significantly dependent on the endoribonuclease activity of ERN1, as its blockade by a dominant-negative ERN1 construct with a mutation in the endoribonuclease domain or silencing of XBP1 did not alter EREG expression [41]. The leading role of ERN1 protein kinase activity in stress-dependent regulation of gene expression has also been demonstrated for the transcription factor ATF3 and endothelin 1 (EDN1), as well as for insulinase (IDE, insulin-degrading enzyme) and PITRM (pitrilysin metallopeptidase 1) [42-44]. The multifunctional enzymes insulin-degrading enzyme and pitrilysin metallopeptidase 1 are essential for normal brain function, including glial cells, as well as in diseases such as insulin resistance, type 2 diabetes, neurodegenerative disorders, and cancer [45-47]. The mechanism of stress-dependent regulation of gene expression mediated by ERN1 protein kinase activity has also been demonstrated for the transcription factors PBX3 and PRRX1, as well as for enzymes such as SHMT1 (serine hydroxymethyltransferase 1), SHMT2, PHGDH (phosphoglycerate dehydrogenase), CPE1 (carboxypeptidase 1), and EDEM1 (endoplasmic reticulum degradation enhancing alpha-mannosidase like protein 1) [48-52].

To study the role of ERN1 in regulating gene expression in glioblastoma cells and under hypoxia, cells with complete ERN1 knockdown were used [41, 53]. In these cells, both enzymatic activities of ERN1 (endoribonuclease and protein kinase) were inhibited using a dominant-negative construct of dnERN1. In glioblastoma cells with complete knockdown of ERN1, increased expression levels of the tumor suppressor TP53 and the ubiquitin-specific peptidase USP7, as well as TP53BP2 and SESN1, were observed [54, 55]. However, the expression level of the ubiquitin ligase MDM2 and the apoptosis effector TP53 (PERP), on the contrary, was reduced in glioblastoma cells with suppressed activity of the signaling protein ERN1 [54]. It was also found that changes in the expression of the *TP53*, *MDM2*, and *USP7* genes were mediated by the protein kinase ERN1, and the *PERP* gene was mediated by both enzymatic activities of the ERN1 protein [54]. In this regard, the expression level of the apoptosis effector TP53 in glioblastoma cells with only suppressed endoribonuclease activity sharply increased almost to the level of normal human astrocytes of the NHA/TS line [54]. The results of the study of the expression of the *TP53*, *MDM2*, *USP7*, and *PERP* genes in glioblastoma cells with suppressed activity of the ERN1 signaling protein indicate the antitumor effect of knockdown of this signaling protein by increasing the expression of the tumor suppressor TP53 and

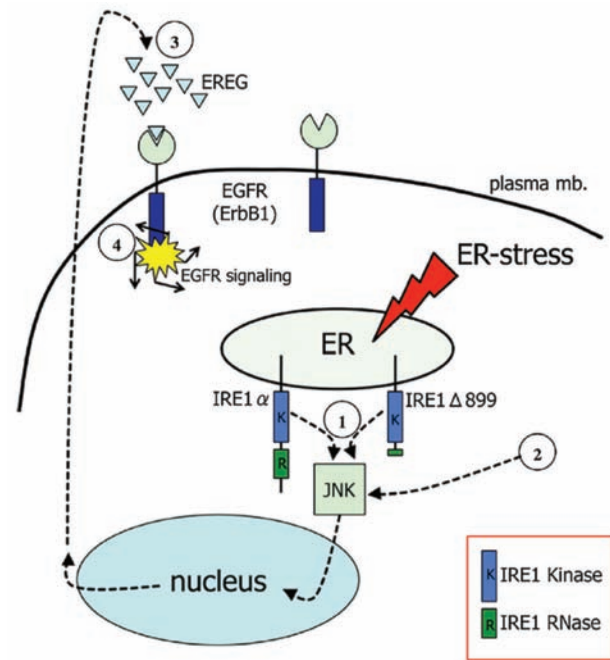

Fig. 5. Schematic representation of stress-dependent, ERN1/IRE1-mediated epiregulin (EREG) expression in glioblastoma cells through protein kinase activity of ERN1 with the participation of JNK (c-Jun N-terminal kinase) but not through ERN1 endoribonuclease activity [41]

the ubiquitin-specific peptidase USP7 and reducing the expression level of the ubiquitin ligase MDM2, which is a proto-oncogene responsible for TP53 ubiquitination and its proteosomal degradation.

When studying the effect of hypoxia on the expression of the *TP53*, *USP7*, and *PERP* genes, it was found that their expression level changes in glioblastoma cells under hypoxia and that ERN1 knockdown modifies the effect of hypoxia: it eliminates hypoxic regulation of *TP53* and *USP7* gene expressions, while increasing the sensitivity of the *PERP* gene expression to hypoxia [54]. And in the case of hypoxia, we also have a clear antitumor effect of ERN1 knockdown: elimination of the negative impact of hypoxia on the expression of the *TP53* and *USP7* genes, as well as an increase in the hypoxia impact on the level of *PERP* gene expression, which contributes to the suppression of proliferation and the enhancement of glioblastoma cell apoptosis.

Similar results were obtained when studying other tumor suppressor genes, in particular *RB1* (retinoblastoma 1) and *RBL1* (retinoblastoma-like 1), and the dependence of their expression on ERN1 knockdown in glioblastoma cells. Thus, it was found that knockdown of ERN1 in glioblastoma cells increases the expression levels of these tumor suppressors, and hypoxia sharply decreases the expression level of the *RBL1* gene, and inhibition of ERN1 activity reduces the effect of hypoxia on the expression of this gene [56]. Using the example of these genes, the anti-tumor effect is also observed in ERN1 knockdown glioblastoma cells both, at the level of gene expression and their sensitivity to hypoxia.

Molecular basis of inhibition of glioblastoma cell proliferation by knockdown of the signaling protein ERN1

Since endoplasmic reticulum stress is a key regulator of malignant tumor growth, mainly through the sensory-signaling protein ERN1, the main efforts of scientists are focused on finding effective ways to suppress its activity [29, 31, 41, 53, 57-61]. This is primarily the inhibition of the endoribonuclease activity of ERN1, but there are also inhibitors of its protein kinase activity.

It has been shown that inhibiting the function of the sensory signaling protein ERN1 by a dominant-negative construct of ERN1 (dnERN1) results in a significant antiproliferative effect on glioblastoma cells [50, 53, 54]. This is primarily due to a decrease in the expression of proangiogenic genes and an increase in the expression of antiangiogenic factors both *in vitro* and in the CAM (chorio-allantoic membrane) model, as well as in mice that were intracerebral transplanted with U87MG glioblastoma cells with dnERN1 [53] (Fig. 6).

The reduction in the proliferative potential of glioblastoma cells after inhibition of both enzymatic activities of the sensory-signaling protein ERN1 by the dominant-negative construct dnERN1, as well as after inhibition of only its endoribonuclease activity, was assessed by various methods [50, 54]. It was found that inhibition of only the endoribonuclease activity of ERN1 resulted in a significantly greater reduction in the proliferative potential of glioblastoma cells compared to inhibition of both of its enzymatic activities (Fig. 6) [50, 54].

The anti-proliferative effect of ERN1 knockdown may be mediated by VEGF (vascular endothelial growth factor). Its expression depends on hypoxia and endoplasmic reticulum stress signaling pathways, in particular ERN1 [62-64]. Thus, inhibition of the ERN1 signaling protein in glioblastoma cells reduces the level of VEGFA and VEGFB expression, and hypoxia increases their expression level [64]. However, ERN1 inhibition dramatically reduces the effect of hypoxia on the expression of both VEGFA and VEGFB, indicating that the hypoxic regulation of both *VEGF* gene expressions is ERN1-mediated [64]. In glioblastoma cells with ERN1 knockdown, a decrease in the protein of two VEGFA splice variants (VEGFA165 and VEGFA189) in the cytosolic fraction was also found [64]. Thus, the reduction in VEGFA levels in glioblastoma cells with ERN1 knockdown may be involved in the decrease in their proliferation and tumor growth in model experiments on the chorio-allantoic membrane and the mouse brain [41, 50, 53].

The uniqueness of this work lies in the detection of $VEGFA_{189}$ in nuclear extracts of glioblastoma cells, as well as in the significant increase of this VEGFA splice variant after ERN1 knockdown [64]. The functional significance of $VEGFA_{189}$ localization in the nuclei of glioblastoma cells and its increase after inhibition of the ERN1 has not yet been clarified and requires further study. However, it is worth noting that angiogenesis is controlled not only by VEGF, but also by many other pro-angiogenic and anti-angiogenic factors, and their role may differ significantly in different cells [65].

Insulin-like growth factors (IGFs) and proteins that bind to them and regulate their activity in dif-

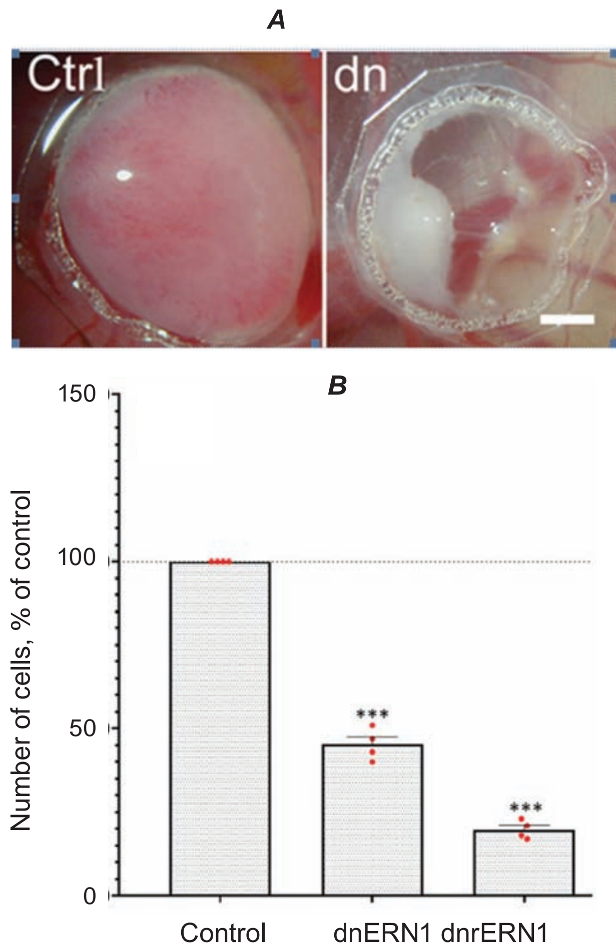

*Fig. 6. **A**: Growth of glioblastoma from cells with vector (control; Ctrl) and with dominant-negative construct dnERN1 (dn) in chorio-allantoic membrane (CAM) model [41]. **B**: Number of glioblastoma cells in culture after 72 h of cultivation according to flow cytometry data: U87, control – control glioblastoma cells transfected with an empty vector; dnERN1 – cells with both ERN1 enzymatic activities suppressed; dnrERN1 – cells with only ERN1 endoribonuclease activity suppressed; dnERN1 and dnrERN1 were compared with control; n = 4; ***P < 0.001 [50]*

ferent ways play an equally important role in tumor growth, although most of them are polyfunctional [66-69]. The IGF system is involved in the development of many malignant neoplasms. It has been found that ERN1 inhibition reduces the expression levels of IGF1, IGF2, IGFBP1, IGFBP2, IGF2BP3, IGFBP6, and NOV in glioblastoma cells [70-72], indicating a possible contribution of these factors to the antitumor effect of ERN1 knockdown. Moreover, the expression of most of these growth factors is regulated by hypoxia, and the effect of hypoxia was significantly reduced by inhibiting the enzymatic activity of the ERN1 protein, indicating that the detected changes in these gene expressions are mediated by ERN1 [70-72].

Serine synthesis enzymes and transcription factors, the expression levels of which are regulated by ERN1 and hypoxia, play an important role in glioblastoma growth [49, 50, 73, 74]. Therefore, understanding the molecular mechanisms of gene expression regulation on serine synthesis enzymes and key transcription factors in malignant tumor growth through ERN1 and hypoxia is important in the search for molecular targets to combat malignant growth. It has been established that inhibition of the endoribonuclease and protein kinase activities of ERN1 reduces the expression level of serine synthesis genes such as PHGDH (phosphoglycerate dehydrogenase), PSAT1 (phosphoserine aminotransferase 1), PSPH (phosphoserine phosphatase), SHMT2 (serine hydroxymethyltransferase 2) and the transcription factor ATF4, which controls the expression of the *PSAT1* gene but increases SHMT1 [50]. It has also been shown that the expression of PHGDH, SHMT1, and SHMT2 in glioblastoma cells is controlled by the protein kinase activity of ERN1, ATF4 by its endoribonuclease activity, and PSAT1 and PSPH by both enzymatic activities of this signaling protein [50]. Serine synthesis gene expression is sensitive to hypoxia in glioblastoma cells, but the effect of hypoxia is modified by ERN1 [73]. Moreover, it has been demonstrated that the regulation of *PSAT1* gene expression is controlled not only by endoplasmic reticulum stress but also by glucocorticoids in glioblastoma cells and normal human astrocytes differently [75]. These results indicate that decreased expression of serine synthesis genes in ERN1 knockdown glioblastoma cells is associated with their suppressed proliferation, as serine synthesis is essential for glioblastoma growth.

The expression of the pro-oncogenic transcription factors *TGIF1* and *ZEB2* genes in glioblastoma cells with ERN1 knockdown was also investigated. The reduction in the expression level of these genes in ERN1-knockdown glioblastoma cells was revealed and mediated by the endoribonuclease activity of ERN1, which is in good agreement with the antiproliferative effect of ERN1 knockdown [49]. However, the level of gene expression of pro-onco-

genic transcription factors PBX3, PRRX1, PBXIP1, and PAX6 in glioblastoma cells with suppressed protein kinase and endoribonuclease activities of ERN1, on the contrary, increases due to the suppression of the protein kinase activity of this signaling protein [49]. Since the transcription factors PBX3, PRRX1, PBXIP1, and PAX6, as well as the serine synthase enzyme SHMT1, are pro-invasive factors that are controlled by the protein kinase activity of ERN1. It has been suggested that the protein kinase activity of ERN1 controls not only proliferation but also invasion of glioblastoma cells. Since the transcription factors PBX3, PRRX1, PBXIP1, and PAX6, as well as the serine synthase enzyme SHMT1, are pro-invasive factors that are controlled by the protein kinase activity of ERN1, it has been suggested that ERN1 protein kinase activity regulates not only proliferation but also invasion of glioblastoma cells. This is consistent with data on enhanced invasiveness and significantly less reduction in proliferation of glioblastoma cells with suppressed protein kinase and endoribonuclease activities of ERN1 compared to suppression of its endoribonuclease activity alone [49, 50, 53].

Two other pro-invasive genes with oncogenic properties have also been investigated: CPE (carboxypeptidase E) and EDEM1 (endoplasmic reticulum degradation enhancing alpha-mannosidase like protein 1) [76-79]. Their expression is also controlled by the protein kinase activity of ERN1 [51, 52]. However, in glioblastoma cells with suppressed protein kinase and endoribonuclease activities of ERN1, the level of expression of the *CPE* gene is sharply increased, and that of the *EDEM1* gene, on the contrary, is reduced [51, 52]. These results indicate a multifaceted, orchestrating role of ERN1 protein kinase activity in stress-dependent regulation of gene expression in malignant tumor cells. Inhibition of ERN1 protein kinase activity contributes to the inhibition of tumor growth, particularly in glioblastoma [60]. Conversely, it enhances cell proliferation by increasing the expression of pro-oncogenic factors with pro-invasive properties [43, 49-51]. As a result, the proliferation rate of glioblastoma cells with suppressed endoribonuclease activity is significantly lower compared to cells with both ERN1 activities (protein kinase and endoribonuclease) suppressed (Fig. 6) [50].

Conclusions. Thus, the studies conducted in the Department of Molecular Biology contributed to the elucidation of the molecular basis of malignant tumor growth, the mechanisms of hypoxic regulation of gene expression through canonical and non-canonical pathways, and the role of alternative splicing in gene expression control. Methodological approaches were created for fundamental research into the role of endoplasmic reticulum stress in the development of pathological conditions, particularly the growth of glioblastomas, the most malignant brain tumors. The molecular mechanisms of glioblastoma cell proliferation inhibition through knockdown of the ERN1 signaling protein, as well as the molecular mechanisms of stress-dependent regulation of tumor suppressor and oncogene expressions, have been studied in detail. The role of ERN1 protein kinase activity in the regulation of gene expression, particularly genes responsive to cell invasion, has been identified and studied in detail in glioblastoma cells. This opens up a new and promising field for further scientific research.

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. Research reported in this publication was supported by funding from the National Academy of Sciences of Ukraine, projects N 0106U002051, 0111U002234, 0116U001027, and 0121U100662.

НАУКОВІ ДОСЯГНЕННЯ ВІДДІЛУ МОЛЕКУЛЯРНОЇ БІОЛОГІЇ У ПІЗНАННІ СТРЕС-ЗАЛЕЖНИХ МЕХАНІЗМІВ РОСТУ ГЛІОМ

О. Г. Мінченко✉*, Ю. М. Вілецька, М. Ю. Слюсар, О. О. Хіта*

Інститут біохімії ім. О.В. Палладіна Національної академії наук України, Київ;
✉e-mail: ominchenko@yahoo.com

З 2005 року у відділі молекулярної біології були розпочаті дослідження, направлені на вирішення ключових проблем біохімії та молекулярної біології з акцентом на виясненні молекулярних основ росту злоякісних пухлин, механізмів гіпоксичної регуляції, ролі альтернативного сплайсингу у механізмах регуляції експресії генів, а також фундаментального значення стресу ендоплазматичного ретикулума у підтриманні гомеостазу та розвитку патологічних станів, зокрема росту гліобластом, найбільш злоякісних пухлин головного мозку, що тяжко піддаються лікуванню. Показано,

що експресія різних 6-фосфофрукто-2-кіназ/фруктозо-2,6-біфосфатаз (PFKFB), ключових регуляторів гліколізу, посилюється у різних злоякісних пухлинах і що PFKFB4 є маркером пухлинного росту. Встановлено, що рівень експресії *PFKFB4* контролюється за гіпоксії HIF-залежним механізмом, а у промоторі ідентифікована специфічна до HIF послідовність, мутація якої повністю знімає гіпоксичну регуляцію гена PFKFB4. Ідентифіковані також численні сплайс-варіанти різних генів *PFKFB* та *VEGFA*. Встановлено, що пригнічення стресу ендоплазматичного ретикулума, зокрема його сигнального шляху ERN1, знижує проліферацію клітин гліобластоми змінюючи рівень експресії онкогенів, пухлинних супресорів, ензимів мітохондрій, а також рецепторів інсуліну і глюкокортикоїдів та залежних від них протеїнів. Виявлена важлива роль протеїнкіназної активності ERN1 у регуляції експресії різних генів і показано, що її пригнічення є причиною посиленої інвазивності клітин гліобластоми після нокдауну ERN1. Концентрується увага на ролі гормонів у контролі ефектів стресу ендоплазматичного ретикулума та гіпоксії, а також на неканонічних механізмах гіпоксичної регуляції експресії генів, її залежності від стресу.

Ключові слова: клітини гліобластоми, гіпоксія, стрес ендоплазматичного ретикулума, ERN1, експресія генів.

References

1. Minchenko A, Leshchinsky I, Opentanova I, Sang N, Srinivas V, Armstead V, 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.* 2002; 277(8): 6183-6187.
2. Minchenko O, Opentanova I, Caro J. Hypoxic regulation of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene family (PFKFB-1-4) expression *in vivo. FEBS Lett.* 2003; 554(3): 264-270.
3. Minchenko OH, Ogura T, Opentanova IL, Minchenko DO, Ochiai A, Caro J, Komisarenko SV, Esumi H. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene family overexpression in human lung tumor. *Ukr Biokhim Zhurn.* 2005; 77(6): 46-50.
4. Bertout JA, Patel SA, Simon MC. The impact of O_2 availability on human cancer. *Nat Rev Cancer.* 2008; 8(12): 967-975.
5. Minchenko OH, Ochiai A, Opentanova IL, Ogura T, Minchenko DO, Caro J, Komisarenko SV, Esumi H. Overexpression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 in the human breast and colon malignant tumors. *Biochimie.* 2005; 87(11): 1005-1010.
6. Minchenko A, Caro J. Regulation of endothelin-1 gene expression in human microvascular endothelial cells by hypoxia and cobalt: role of hypoxia responsive element. *Mol Cell Biochem.* 2000; 208(1-2): 53-62.
7. Bobarykina AY, Minchenko DO, Opentanova IL, Moenner M, Caro J, Esumi H, Minchenko OH. Hypoxic regulation of *PFKFB-3* and *PFKFB-4* gene expression in gastric and pancreatic cancer cell lines and expression of *PFKFB* genes in gastric cancers. *Acta Biochim Pol.* 2006; 53(4): 789-799.
8. Minchenko OH, Tsuchihara K, Minchenko DO, Bikfalvi A, Esumi H. Mechanisms of regulation of PFKFB expression in pancreatic and gastric cancer cells. *World J Gastroenterol.* 2014; 20(38): 13705-13717.
9. Bobarykina AIu, Minchenko DO, Opentanova IL, Kovtun OO, Komisarenko SV, Esumi H, Minchenko OH. HIF-1alpha, HIF-2alpha and VHL mRNA expression in different cell lines during hypoxia. *Ukr Biokhim Zhurn.* 2006; 78(2): 62-72. (In Ukrainian).
10. Ryan HE, Poloni M, McNulty W, Elson D, Gassmann M, Arbeit JM, Johnson RS. Hypoxia-inducible factor-1alpha is a positive factor in solid tumor growth. *Cancer Res.* 2000; 60(15): 4010-4015.
11. Yalcin A, Clem BF, Simmons A, Lane A, Nelson K, Clem AL, Brock E, Siow D, Wattenberg B, Telang S, Chesney J. Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. *J Biol Chem.* 2009; 284(36): 24223-24232.
12. Lypova N, Telang S, Chesney J, Imbert-Fernandez Y. Increased 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 activity in response to EGFR signaling contributes to non-small cell lung cancer cell survival. *J Biol Chem.* 2019; 294(27): 10530-10543.

13. Rider MH, Bertrand L, Vertommen D, Michels PA, Rousseau GG, Hue L. 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: head-to-head with a bifunctional enzyme that controls glycolysis. *Biochem J*. 2004; 381(Pt 3): 561-579.
14. Chesney J, Clark J, Klarer AC, Imbert-Fernandez Y, Lane AN, Telang S. Fructose-2,6-bisphosphate synthesis by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) is required for the glycolytic response to hypoxia and tumor growth. *Oncotarget*. 2014; 5(16): 6670-6686.
15. Brahimi-Horn MC, Chiche J, Pouysségur J. Hypoxia and cancer. *J Mol Med (Berl)*. 2007; 85(12): 1301-1307.
16. Minchenko OH, Opentanova IL, Ogura T, Minchenko DO, Komisarenko SV, Caro J, Esumi H. Expression and hypoxia-responsiveness of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 in mammary gland malignant cell lines. *Acta Biochim Pol*. 2005; 52(4): 881-888.
17. Minchenko O, Opentanova I, Minchenko D, Ogura T, Esumi H. Hypoxia induces transcription of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-4 gene via hypoxia-inducible factor-1alpha activation. *FEBS Lett*. 2004; 576(1-2): 14-20.
18. Minchenko OH, Ogura T, Opentanova IL, Minchenko DO, Esumi H. Splice isoform of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4: expression and hypoxic regulation. *Mol Cell Biochem*. 2005; 280(1-2): 227-234.
19. Mykhalchenko VG, Minchenko DO, Tsuchihara K, Moenner M, Komisarenko SV, Bikfalvi A, Esumi H, Minchenko OH. Expression of mouse 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 mRNA alternative splice variants in hypoxia. *Ukr Biokhim Zhurn*. 2008; 80(1): 19-25.
20. Minchenko DO, Mykhalchenko VG, Tsuchihara K, Kanehara S, Yavorovsky OP, Zavgorodny IV, Paustovsky YO, Komisarenko SV, Esumi H, Minchenko OH. Alternative splice variants of rat 6-phosphofructo-2-kinase/ fructose-2,6-bisphosphatase-4 mRNA. *Ukr Biokhim Zhurn*. 2008; 80(4): 66-73.
21. Minchenko DO, Tsuchihara K, Komisarenko SV, Moenner M, Bikfalvi A, Esumi H, Minchenko OH. Unique alternative splice variants of mouse PFKFB-3 mRNA: tissue specific expression. *Sci Bull Nat Bohomoletz Med Univ*. 2008; (1): 22-31.
22. Minchenko DO, Bozhko IV, Lypova NM, Mykhalchenko VG, Minchenko OH. Unique alternative splice variants of mouse and human 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-2 mRNA. *Studia Biologica*. 2010; 4(2): 13-24. (In Ukrainian).
23. Minchenko DO, Bobarykina AY, Kundieva AV, Lypova NM, Bozhko IV, Ratushna OO, Minchenko OH. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase genes: structural organization, expression and regulation of the expression. *Studia Biologica*. 2009; 3(3): 123-140. (In Ukrainian).
24. Lypova NM, Minchenko DO, Ratushna OO, Bozhko IV, Tsuchihara K, Esumi H, Minchenko OH. Expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-2 mRNA and its alternative splice variants in rats with experimental diabetes mellitus. *Ukr Biokhim Zhurn*. 2010; 82(1): 90-99. (In Ukrainian).
25. Mykhalchenko VG, Tsuchihara K, Minchenko DO, Esumi H, Prystupiuk OM, Minchenko OH. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase mRNA expression in streptozotocin-diabetic rats. *Biopolym Cell*. 2008; 24(3): 260-266.
26. Obrosova IG, Minchenko AG, Marinescu V, Fathallah L, Kennedy A, Stockert CM, Frank RN, Stevens MJ. Antioxidants attenuate early up regulation of retinal vascular endothelial growth factor in streptozotocin-diabetic rats. *Diabetologia*. 2001; 44(9): 1102-1110.
27. Minchenko DO, Bobarykina AY, Ratushna OO, Marunych RY, Tsuchihara K, Moenner M, Caro J, Esumi H, Minchenko OH. Dominant-negative constructs of human 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 and -4: effect on the expression of endogenous 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase mRNA. *Biotechnology*. 2008; (4): 49-56.
28. Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nat Rev Cancer*. 2008; 8(9): 705-713.
29. Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, Leuzzi B, Luís A, McCarthy N, Montibeller L, More S, Papaioannou A, Püschel F, Sassano ML, Skoko J, Agostinis P, de Belleroche J, Eriksson LA, Fulda S, Gorman AM, Healy S, Kozlov A, Muñoz-Pinedo C, Rehm M,

Chevet E, Samali A. Endoplasmic reticulum stress signalling - from basic mechanisms to clinical applications. *FEBS J.* 2019; 286(2): 241-278.

30. Minchenko OH, Kharkova AP, Bakalets TV, Kryvdiuk IV. Endoplasmic reticulum stress, its sensor and signalling systems and the role in regulation of gene expression at malignant tumor growth and hypoxia. *Ukr Biochem J.* 2013; 85(5): 5-16. (In Ukrainian).
31. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol.* 2020; 21(8): 421-438.
32. Doultsinos D, Avril T, Lhomond S, Dejeans N, Guédat P, Chevet E. Control of the Unfolded Protein Response in Health and Disease. *SLAS Discov.* 2017; 22(7): 787-800.
33. Warburg O. On respiratory impairment in cancer cells. *Science.* 1956; 124(3215): 269-270.
34. Batie M, Rocha S. Gene transcription and chromatin regulation in hypoxia. *Biochem Soc Trans.* 2020; 48(3): 1121-1128.
35. Minchenko OH, Kubaichuk KI, Minchenko DO, Kovalevska OV, Kulinich AO, Lypova N.M. Molecular mechanisms of ERN1-mediated angiogenesis. *Int J Physiol Pathophysiol.* 2014; 5(1): 1-22.
36. Pelizzari-Raymundo D, Maltret V, Nivet M, Pineau R, Papaioannou A, Zhou X, Caradec F, Martin S, Le Gallo M, Avril T, Chevet E, Lafont E. IRE1 RNase controls CD95-mediated cell death. *EMBO Rep.* 2024; 25(4): 1792-1813.
37. Acosta-Alvear D, Zhou Y, Blais A, Tsikitis M, Lents NH, Arias C, Lennon CJ, Kluger Y, Dynlacht BD. XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. Mol Cell. 2007; 27(1): 53-66.
38. Minchenko OH, Tsymbal DO, Khita OO, Minchenko DO. Inhibition of ERN1 signaling is important for the suppression of tumor growth. *Clin Cancer Drugs.* 2021; 8(1): 27-38.
39. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J Cell Biol.* 2009; 186(3): 323-331.
40. Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem Sci.* 2014; 39(5): 245-254.
41. Auf G, Jabouille A, Delugin M, Guérit 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 IRE1α and promotes autocrine growth through EGF receptor. *BMC Cancer.* 2013; 13: 597.
42. 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.* 2015; 2(1): 18-29.
43. 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 ERN1 knockdown U87 glioma cells. *Endocr Regul.* 2019; 53(4): 250-262.
44. Minchenko DO, Khita OO, Tsymbal DO, Danilovskyi SV, Rudnytska OV, Halkin OV, Kryvdiuk IV, Smeshkova MV, Yakymchuk MM, Bezrodnyi BH, Minchenko OH. Expression of IDE and PITRM1 genes in ERN1 knockdown U87 glioma cells: effect of hypoxia and glucose deprivation. *Endocr Regul.* 2020; 54(3): 183-195.
45. Tian Y, Jing G, Zhang M. Insulin-degrading enzyme: Roles and pathways in ameliorating cognitive impairment associated with Alzheimer's disease and diabetes. *Ageing Res Rev.* 2023; 90: 101999.
46. Feng YY, Hao JR, Zhang YJ, Qiu TT, Zhang ML, Qiao W, Wu JJ, Qiu P, Xu CF, Zhang YL, Du CY, Pan Z, Chang YS. Krüppel-like factor 9 alleviates Alzheimer's disease via IDE-mediated Aβ degradation. *Acta Pharmacol Sin.* 2025; 46(6): 1556-1566.
47. Brunetti D, Catania A, Viscomi C, Deleidi M, Bindoff LA, Ghezzi D, Zeviani M. Role of PITRM1 in Mitochondrial Dysfunction and Neurodegeneration. *Biomedicines.* 2021; 9(7): 833.
48. Minchenko OH, Sliusar MY, Khita OO, Viletska YM, Luzina OY, Danilovskyi SV, Minchenko DO. Endoplasmic reticulum stress-dependent regulation of the expression of serine hydroxymethyltransferase 2 in glioblastoma cells. *Endocr Regul.* 2024; 58(1): 144-152.
49. Minchenko OH, Khita OO, Krasnytska DA, Viletska YM, Rudnytska OV, Hnatiuk OS, Minchenko DO. Inhibition of ERN1 affects the

expression of TGIF1 and other homeobox gene expressions in U87MG glioblastoma cells. *Arch Biochem Biophys.* 2024; 758: 110073.
50. Minchenko OH, Sliusar MY, Khikhlo YP, Halkin OV, Viletska YM, Khita OO, Minchenko DO. Knockdown of ERN1 disturbs the expression of phosphoserine aminotransferase 1 and related genes in glioblastoma cells. *Arch Biochem Biophys.* 2024; 759: 110104.
51. Minchenko OH, Abramchuk AI, Khita OO, Sliusar MY, Viletska YM, Minchenko DO. Endoplasmic reticulum stress-dependent regulation of carboxypeptidase E expression in glioblastoma cells. *Endocr Regul.* 2024; 58(1): 206-214.
52. Minchenko OH, Hrebennykova VO, Viletska YM, Hnatiuk OS, Sliusar MY, Kozynkevych HE, Minchenko DO. The ERN1 signaling pathway of unfolded protein controls the expression of EDEM1 and its hypoxic regulation in glioblastoma cells. *Endocr Regul.* 2025; 59(1): 1-9.
53. Auf G, Jabouille A, Guérit S, Pineau R, Delugin M, Bouchecareilh M, Magnin N, Favereaux A, Maitre M, Gaiser T, von Deimling A, Czabanka M, Vajkoczy P, Chevet E, Bikfalvi A, Moenner M. Inositol-requiring enzyme 1alpha is a key regulator of angiogenesis and invasion in malignant glioma. *Proc Natl Acad Sci USA.* 2010; 107(35): 15553-15558.
54. Danilovskyi SV, Minchenko DO, Moliavko OS, Kovalevska OV, Karbovskyi LL, Minchenko OH. ERN1 knockdown modifies the hypoxic regulation of TP53, MDM2, USP7 and PERP gene expressions in U87 glioma cells. *Ukr Biochem J.* 2014; 86(4): 90-102.
55. 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.* 2014; 1(1): 18-26.
56. Minchenko DO, Karbovskyi LL, Danilovskyi SV, Moenner M, Minchenko OH. Effect of hypoxia and glutamine or glucose deprivation on the expression of retinoblastoma and retinoblastoma-related genes in ERN1 knockdown glioma U87 cell line. *Am J Mol Biol.* 2012; 2(1): 21-31.
57. Minchenko OH, Tsymbal DO, Minchenko DO. IRE-1alpha signaling as a key target for suppression of tumor growth. *Single Cell Biol.* 2015; 4(3): 118.
58. Le Reste PJ, Pineau R, Voutetakis K, Samal J, Jégou G, Lhomond S, Gorman AM, Samali A, Patterson JB, Zeng Q, Pandit A, Aubry M, Soriano N, Etcheverry A, Chatziioannou A, Mosser J, Avril T, Chevet E. Local intracerebral inhibition of IRE1 by MKC8866 sensitizes glioblastoma to irradiation/chemotherapy *in vivo. Cancer Lett.* 2020; 494: 73-83.
59. Raymundo DP, Doultsinos D, Guillory X, Carlesso A, Eriksson LA, Chevet E. Pharmacological Targeting of IRE1 in Cancer. *Trends Cancer.* 2020; 6(12): 1018-1030.
60. Pelizzari-Raymundo D, Doultsinos D, Pineau R, Sauzay C, Koutsandreas T, Langlais T, Carlesso A, Gkotsi E, Negroni L, Avril T, Chatziioannou A, Chevet E, Eriksson LA, Guillory X. A novel IRE1 kinase inhibitor for adjuvant glioblastoma treatment. *iScience.* 2023; 26(5): 106687.
61. 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, Jäger R, Gorman AM, Samali A. Inhibition of IRE1 RNase activity modulates the tumor cell secretome and enhances response to chemotherapy. *Nat Commun.* 2018; 9(1): 3267.
62. Minchenko A, Bauer T, Salceda S, Caro J. Hypoxic stimulation of vascular endothelial growth factor expression *in vitro* and *in vivo. Lab Invest.* 1994; 71(3): 374-379.
63. Drogat B, Auguste P, Nguyen DT, Bouchecareilh M, Pineau R, Nalbantoglu J, Kaufman RJ, Chevet E, Bikfalvi A, Moenner M. IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth *in vivo. Cancer Res.* 2007; 67(14): 6700-6707.
64. Minchenko DO, Kubaichuk KI, Ratushna OO, Komisarenko SV, Minchenko OH. The vascular endothelial growth factor genes expression in glioma U87 cells is dependent from ERN1 signaling enzyme function. *Adv Biol Chem.* 2012; 2(2): 198-206.
65. Hose D, Moreaux J, Meissner T, Seckinger A, Goldschmidt H, Benner A, Mahtouk K, Hillengass J, Rème T, De Vos J, Hundemer M, Condomines M, Bertsch U, Rossi JF, Jauch A,

Klein B, Möhler T. Induction of angiogenesis by normal and malignant plasma cells. *Blood.* 2009; 114(1): 128-143.

66. Cao J, Yee D. Disrupting Insulin and IGF Receptor Function in Cancer. *Int J Mol Sci.* 2021; 22(2): 555.
67. Wang P, Mak VC, Cheung LW. Drugging IGF-1R in cancer: New insights and emerging opportunities. Genes Dis. 2022;10(1):199-211.
68. Takahashi SI, Perks CM. Editorial: The Role of the IGF/Insulin-IGFBP Axis in Normal Physiology and Disease. *Front Endocrinol (Lausanne).* 2022; 13: 892140.
69. Liu Y, Shen S, Yan Z, Yan L, Ding H, Wang A, Xu Q, Sun L, Yuan Y. Expression characteristics and their functional role of IGFBP gene family in pan-cancer. *BMC Cancer.* 2023; 23(1): 371.
70. Minchenko DO, Kharkova AP, Halkin OV, Karbovskyi LL, Minchenko OH. Effect of hypoxia on the expression of genes encoding insulin-like growth factors and some related proteins in U87 glioma cells without IRE1 function. *Endocr Regul.* 2016; 50(2): 43-54.
71. Minchenko DO, Kharkova AP, 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.* 2015; 49(2): 73-83.
72. Minchenko OH, Kharkova AP, Minchenko DO, Karbovskyi LL. Effect of hypoxia on the expression of genes that encode some IGFBP and CCN proteins in U87 glioma cells depends on IRE1 signaling. *Ukr Biochem J.* 2015; 87(6): 52-63.
73. Sliusar MY, Minchenko DO, Khita OO, Tsymbal DO, Viletska YM, Luzina OY, Danilovskyi SV, Ratushna OO, Minchenko OH. Hypoxia controls the expression of genes responsible for serine synthesis in U87MG cells on ERN1-dependent manner. *Endocr Regul.* 2023; 57(1): 252-261.
74. 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.* 2015; 87(2): 76-87.
75. Minchenko OH, Abramchuk AI, Khikhlo YP, Sliusar MY, Halkin OV, Luzina OY, Danilovsryi SV, Viletska YM, Minchenko DO. Hydrocortisone interacts with endoplasmic reticulum stress in hypoxic regulation of phosphoserine aminotransferase 1 gene expression differently in normal human astrocytes and glioblastoma cells. *Endocr Regul.* 2025; 59(1): 48-56.
76. Miura K, Katsuki R, Yoshida S, Ohta R, Tamura T. Identification of EGF Receptor and Thrombospondin-1 as Endogenous Targets of ER-Associated Degradation Enhancer EDEM1 in HeLa Cells. *Int J Mol Sci.* 2023; 24(15): 12171.
77. Flintoaca Alexandru PR, Chiritoiu GN, Lixandru D, Zurac S, Ionescu-Targoviste C, Petrescu SM. EDEM1 regulates the insulin mRNA level by inhibiting the endoplasmic reticulum stress-induced IRE1/JNK/c-Jun pathway. *iScience.* 2023; 26(10): 107956.
78. Fan S, Gao X, Chen P, Li X. Carboxypeptidase E-ΔN promotes migration, invasiveness, and epithelial-mesenchymal transition of human osteosarcoma cells via the Wnt-β-catenin pathway. *Biochem Cell Biol.* 2019; 97(4): 446-453.
79. Kuo IY, Liu D, Lai WW, Wang YC, Loh YP. Carboxypeptidase E mRNA: Overexpression predicts recurrence and death in lung adenocarcinoma cancer patients. *Cancer Biomark.* 2022; 33(3): 369-377.