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Inhibition of ERN1 suppresses the expression of phosphoenolpyruvate carboxykinase 2 in U87MG glioblastoma cells and increases its sensitivity to glucose and glutamine deprivation

Yuliia M. Viletska [1], Oleksandr H. Minchenko [1], Olena O. Khita [1], Myroslava Y. Sliusar [1], Oleh V. Halkin [1], Halyna E. Kozynkevych [2], Dmytro O. Minchenko [2]

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

Objective. Phosphoenolpyruvate carboxykinase (PCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate and regulates pyruvate metabolism and gluconeogenesis in response to glucocorticoid and insulin stimuli. Mitochondrial isoform of this enzyme (PCK2) is overexpressed in glioblastoma cells and participates in metabolic reprogramming and cell proliferation. This study aims to examine the impact of ERN1 (endoplasmic reticulum to nucleus signaling 1) inhibition on PCK2 expression and sensitivity to glucose and glutamine deprivation to determine the role of ERN1 signaling in the regulating its expression in glioblastoma cells.

Methods. The glioblastoma cell line U87MG and two genetically modified variants of these cells were used. These were glioblastoma cell sublines with suppressed endoribonuclease and protein kinase activities of ERN1 (dnERN1) or only ERN1 endoribonuclease (dnrERN1), and control cells transfected with an empty vector. The suppression of ERN1 function by silencing of ERN1 and XBP1 mRNAs was also used. Hypoxia was generated using the HIF1A prolyl hydroxylase inhibitor dimethyloxalylglycine. For glucose and glutamine deprivation, DMEM medium without glucose or glutamine was used. The expression level of the PCK2 mRNA was analyzed by real-time qPCR and normalized to the beta-actin mRNA.

Results. It has been demonstrated that PCK2 mRNA expression is significantly decreased in dnERN1 glioblastoma cells. Similar suppression of this mRNA expression was also observed in cells with only the endoribonuclease activity of ERN1 inhibited, indicating that this enzymatic activity is involved in the regulation of PCK2 expression. The silencing of ERN1 and XBP1 mRNAs also induced similar changes in PCK2 mRNA expression, possibly mediated by XBP1s. The expression of PCK2 was enhanced under glutamine deprivation in control glioblastoma cells, but inhibition of ERN1 activity strongly increased this effect. Upregulated PCK2 expression was also observed in control glioblastoma cells under glucose deprivation. However, the inhibition of ERN1 activity strongly increased the sensitivity of this gene expression to glucose deprivation. Furthermore, PCK2 mRNA expression was resistant to hypoxic conditions in cells with native ERN1. At the same time, in glioblastoma cells with inhibited ERN1 activity, a strong induction of PCK2 expression was observed.

Conclusion. The results of this study demonstrated that ERN1 inhibition reduces PCK2 mRNA expression through the ERN1 endoribonuclease activity. This mRNA expression is upregulated under glutamine and glucose deprivation. Moreover, ERN1 inhibition strongly enhanced the sensitivity of PCK2 mRNA expression to glucose and glutamine deprivation as well as to hypoxia.

Corresponding author: Yuliia M. Viletska, PhD., and Oleksandr H. Minchenko, Prof., Department of Molecular Biology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovycha 9, Kyiv 01054, Ukraine; phone: +380442356151; e-mail: ominchenko@yahoo.com.

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Phosphoenolpyruvate carboxykinase 2 (PCK2), also known as PEPCK2, catalyzes the conversion of oxaloacetate to phosphoenolpyruvate derived from the citric acid cycle and plays a key role in regulating pyruvate metabolism and gluconeogenesis in response to glucocorticoid and insulin stimuli (Zhang et al. 2014; Hwang et al. 2018; Niu et al. 2018; He et al. 2020; Sharma et al. 2020; Chang et al. 2025). PCK2 catalyzes the rate-limiting step of gluconeogenesis, plays an important role in metabolic reprogramming, and is the dominant isoform in many cancers, including glioblastoma (Gunasekharan et al. 2024; Xue et al. 2024; Yi et al. 2025). Overexpression of PCK2 regulates cell proliferation, metastasis, cell survival, protein folding, and metabolic reprogramming in different cell types (Hyrossova et al. 2022; Ma et al. 2022; Li et al. 2024; Xue et al. 2024; Chang et al. 2025). Mitochondrial PCK2 (PEPCK-M) is upregulated by glucose deprivation, thereby enhancing cell survival (Hyrossova et al. 2022). The down-regulated PCK2 suppresses glioblastoma cell proliferation, inhibits epithelial-mesenchymal transition, and suppresses breast tumor growth (Lin et al. 2021; Ma et al. 2022).

Furthermore, PCK2 counteracts mitochondrial respiration and maintains redox balance in starved cancer cells, which sheds light on the adaptive responses of cancer cells to nutrient deprivation (Bluemel et al. 2021). PCK2 silencing activates the tricarboxylic acid cycle, augments mitochondrial respiration, and enhances glutathione oxidation under glucose starvation (Bluemel et al. 2021). Moreover, transcriptional upregulation of PCK2 by estrogen-related receptor alpha promotes glutamine metabolism utilization by the tricarboxylic acid cycle and cancer cell survival (Frodyma et al. 2022). Thus, regulation of PCK2 expression may have a significant impact on tumor development and progression, as well as its response to therapy; however, significance of endoplasmic reticulum (ER) stress signaling pathways in this regulation remains elusive.

Glioblastoma metabolic reprogramming and its growth need the unfolded protein response (UPR) induced by ER stress and hypoxia, which is realized through three tightly interconnected UPR signaling pathways (Denko 2008; Sun and Denko 2014; Almanza et al. 2019; Pelizzari-Raymundo et al. 2024; Acosta-Alvear et al. 2025). These signaling pathways are responsible for adapting glioblastoma cells, as well as other cancer cells to adverse environmental conditions (Hetz et al. 2020; Acosta-Alvear et al. 2025). ERN1 (ER to nucleus signaling 1) is a key signaling protein of the UPR. Its knockdown significantly suppresses glioblastoma cell proliferation and tumor growth *in vivo*; however, it causes an enhanced invasiveness of glioma cells (Auf et al. 2010; Pelizzari-Raymundo et al. 2023; Minchenko et al. 2024a,b). It is worth noting that metabolic reprogramming is a key characteristic of tumor cells that contributes to their rapid growth and resistance to treatment, mainly through UPR, including ERN1 endoribonuclease and protein kinase activities, and that PCK2 is implicated in this process (Chevet et al. 2015; Avril et al. 2017; Logue et al. 2018; Papaioannou and Chevet 2018; Le Reste et al. 2020).

ERN1 endoribonuclease activity is responsible for the formation of the transcription factor XBP1s, which regulates the expression of numerous chaperones and enzymes involved in the degradation of misfolded proteins and restoration of folding (Acosta-Alvear et al. 2007, 2025; Doultsinos et al. 2017). It is worth noting that XBP1s contributes in EDEM1-controlled insulin synthesis in pancreatic β-cells by inhibiting the IRE1/JNK/c-Jun pathway (Flintoaca Alexandru et al. 2023). Protein kinase activity of ERN1 also plays an important role in UPR signaling and the regulation of gene expression through JNK and maybe other pathways (Auf et al. 2013; Minchenko et al. 2019, 2024b,c). This enzymatic activity of ERN1 signaling protein plays an important role in regulating the expression of several homeobox genes associated with the proliferation and invasion of glioblastoma cells and that ERN1 knockdown significantly enhances their expression, despite inhibiting the proliferation rate of these cells (Minchenko et al. 2024a).

Previously we have demonstrated that inhibition of ERN1 modifies the sensitivity of key regulatory gene expression to glucose and glutamine deprivation as well as to hypoxia in glioblastoma cells (Minchenko et al. 2016, 2021, 2024d; 2025b,d; Riabovol et al. 2019; Krasnytska et al. 2023; Sliusar et al. 2023). Furthermore, both hypoxic and ER-dependent regulation of gene expression in glioblastoma cells significantly alters by glucocorticoids (Minchenko et al. 2025a,c). In this regard, it is important to study the role of ER stress, hypoxia, glucose and glutamine deficiency, as well as their interactions in the regulation of PCK2 expression.

Thus, ER stress, glucose and glutamine supply, and hypoxia are important factors of glioblastoma metabolic reprogramming, its progression, and therapeutic resistance. However, there is still no data on the molecular mechanisms by which these factors influence on PCK2 expression regulation in ERN1-knockdown glioblastoma cells. In this study, we demonstrate that inhibition of the endoribonuclease activity of ERN1 and both enzymatic activities of ERN1 have similar suppressive effect on the expression of PCK2 in glioblastoma cells, and that ERN1 inhibition significantly enhances the sensitivity of this gene expression to nutrient deprivation and hypoxia.

Materials and Methods

Cell lines and culture conditions. In this investigation, we used wild-type U87MG glioblastoma cells and three genetically modified sublines of these cells previously described (Auf et al. 2013). Cells were 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. Glioblastoma cells with overexpression of the empty vector pcDNA 3.1 were used as control (control glioblastoma cells). The cells with overexpression of ERN1 dominant/negative construct in pcDNA 3.1 (dnERN1) having suppression of both the ERN1 protein kinase and endoribonuclease activities, do not have the phosphorylated isoform of ERN1, and have a lower proliferation rate and do not express a XBP1s, a key transcription factor in the ERN1 signaling (Auf et al. 2013; Minchenko et al. 2024b). All sublines of glioblastoma cells used in this study were grown in the presence of geneticin (G418) as described previously (Auf et al. 2013). Hypoxia was created with 0.5 mM dimethyloxalylglycine (Sigma-Aldrich, St. Louis, MO, USA) as described previously (Minchenko et al. 2024b), and culture plates were exposed for 4 h. For glucose and glutamine deprivations, cells were cultured in DMEM without glucose or glutamine, respectively, for 16 h. We also exposed glioblastoma cells with inhibited ERN1 endoribonuclease activity to 500 ng/ml tunicamycin to clarify the possible participation of other ER stress signaling pathways in the regulation of PCK2 expression.

Small interfering RNA knockdown experiments. The silencing of ERN1 and XBP1 mRNAs was achieved using small interfering RNA (siRNA) in U87MG glioblastoma cells mainly as described previously (Auf et al. 2013; Minchenko et al. 2024a). Briefly, U87MG cells were seeded in 6-well plates and incubated until 50% confluency was reached. On the following day, the appropriate amount of siRNA against ERN1 and negative control siRNA were transfected into the cells using Lipofectamine RNAi/MAX reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Transfection was performed for 48 h.

RNA isolation. Total RNA from glioblastoma cells was extracted using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The RNA pellets were washed twice with 75% ethanol and dissolved in nuclease-free water. RNA concentration and spectral characteristic were measured using a NanoDrop spectrophotometer.

Reverse transcription and quantitative PCR analysis. The expression levels of PCK2 and ACTB mRNAs were measured in control U87MG cells and cells with a deficiency of ERN1 by quantitative PCR using Luna Universal qPCR Master Mix (New England Biolabs, Inc., Ipswich, MA, USA). Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described (Minchenko et al. 2024a). Polymerase chain reaction was performed in triplicate. The expression of ACTB mRNA was used as a control of analyzed mRNA quantity. The pair of primers specific for PCK2 was received from Sigma-Aldrich (St. Louis, MO, USA) and used for quantitative PCR: PCK2 forward 5'-atgtccccagctgatttcca and PCK2 reverse 5'-aaagtcaccatctcccaggg (NM_004563.4). Primers for ERN1, XBP1, and ACTB have been described previously (Minchenko et al. 2024a).

The results of quantitative PCR were analyzed using "Differential Expression Calculator". Statistical analysis of the obtained results was performed using GraphPad Prism8.0.1 program. The values of studied gene expression were normalized to the expression of beta-actin mRNA and expressed as a percentage of controls (100%). All values were expressed as mean±SEM from triplicate measurements performed in 4 independent experiments. In all cases, a value of $p<0.05$ was considered significant. All experimental qPCR data were analyzed for the normality of distribution using a graphical tool (normal probability plot) and a histogram. A normal distribution was shown for all analyzed data sets. The amplified DNA fragments were analyzed on a 2.5% agarose gel and then visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

Results

As shown in Figure 1, the expression level of PCK2 mRNA was decreased by 38% ($p<0.01$) in glioblastoma cells with inhibited ERN1 endoribonuclease and protein kinase activities (dnERN1) compared to control cells transfected by empty vector (vector). Furthermore, the similar downregulation of this mRNA expression (–37%; $p<0.01$) was also detected in glioblastoma cells without ERN1 endoribonuclease activities compared to control cells (Figure 1). This indicates that suppression of PCK2 mRNA expression is mediated by inhibition of ERN1 endoribonuclease activity, since additional inhibition of the protein kinase did not significantly alter the effect of ERN1 endoribonuclease inhibition on PCK2 mRNA expression.

Similar changes were observed in the expression of PCK2 mRNA in glioblastoma cells after ERN1 mRNA silencing. As shown in Figure 2, the expression level of PCK2 mRNA is decreased by 35% ($p<0.01$) in glioblastoma cells after 48 h of ERN1 mRNA silencing (ERN1 mRNA level was suppressed by 90%; $p<0.001$) compared with cells transfected by control siRNA. Furthermore, silencing of XBP1 mRNA resulted in a similar suppression of PCK2 mRNA expression in glioblastoma cells (–32%; $p<0.01$) compared to cells treated with control siRNA (Figure 3). The efficiency of silencing XBP1 mRNA was also high (–90%; $p<0.001$). The obtained results indicate that changes in PCK2 mRNA expression upon inhibition of ERN1 endoribonuclease activity are mediated by disruption of XBP1 mRNA alternative splicing.

Next, we investigated the impact of tunicamycin-induced ER stress on PCK2 mRNA expression in glioblastoma cells with inhibited ERN1 endoribonuclease activity to clarify the possible participation of other ER stress signaling pathways in regulating this gene expression. As shown in Figure 4, tunicamycin strongly increased the expression level of PCK2 mRNA (by 252%; $p<0.001$) in these glioblastoma cells, indicating the involvement of other ER stress signaling pathways in PCK2 expression.

We also studied the impact of glutamine and glucose deficiency on PCK2 mRNA expression in glioblastoma cells in relation to inhibition of ERN1 endoribonuclease and protein kinase activities. As shown in Figure 5, PCK2 mRNA expression is significantly upregulated in control glioblastoma cells treated with glutamine deprivation (by 136%; $p<0.001$); however, inhibition of ERN1 endoribonuclease and protein kinase activities strongly enhances the sensitivity of this gene expression to glutamine deprivation. Thus, the PCK2 mRNA expression is

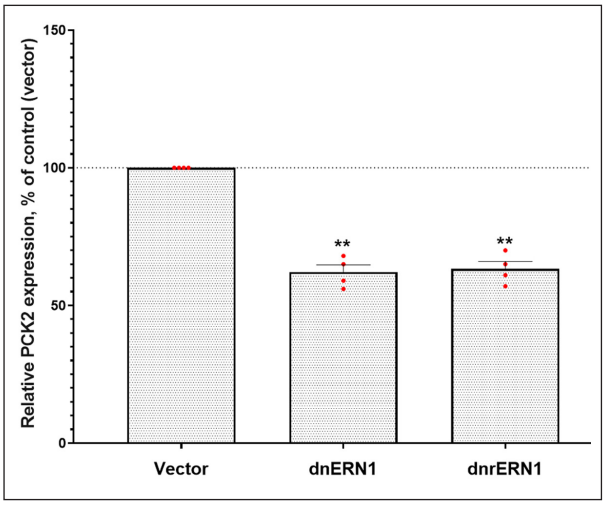

Figure 1. The expression level of phosphoenolpyruvate carboxykinase 2 (PCK2) in U87MG glioblastoma cells with suppressed protein kinase and endoribonuclease activities of ERN1 (dnERN1) and cells with inhibited only ERN1 endoribonuclease activity (dnrERN1) in comparison with control glioblastoma cells (transfected by an empty vector; control) measured by real-time qPCR. The values of this mRNA expression were normalized to ACTB mRNA and represented as a percentage of control (100%); mean±SEM; n=4; **p<0.01 vs control.

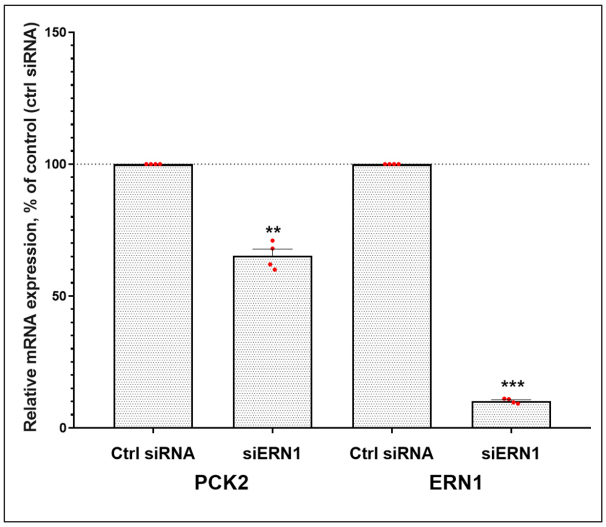

Figure 2. The effect of endoplasmic reticulum to nucleus signaling 1 (ERN1) mRNA silencing by siRNA specific to human ERN1 (48 h) on the expression of phosphoenolpyruvate carboxykinase 2 (PCK2) and ERN1 mRNAs in wild-type glioblastoma cells (real-time qPCR analysis). The values of these mRNA expressions were normalized to ACTB mRNA and represented as a percentage of control (Ctrl siRNA; 100%); mean±SEM; n=4; **p<0.01 and ***p<0.001 vs control.

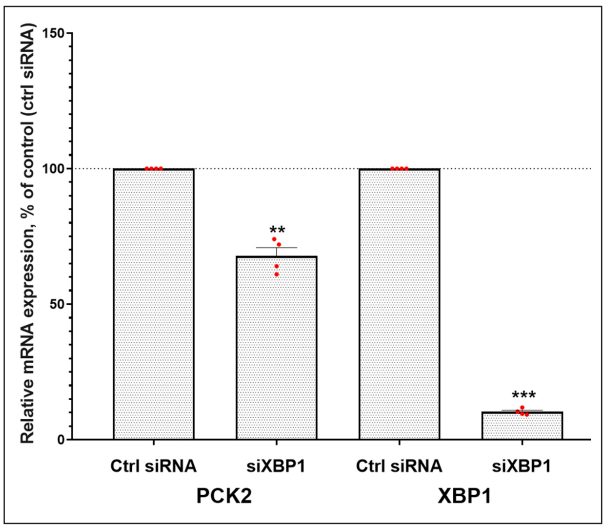

Figure 3. The effect of XBP1 (X-box binding protein 1) mRNA silencing by specific to human XBP1 siRNA (48 h) on the expression of phosphoenolpyruvate carboxykinase 2 (PCK2) and XBP1 mRNAs in wild-type glioblastoma cells (real-time qPCR analysis). The values of these mRNA expressions were normalized to ACTB mRNA and represented as a percentage of control (Ctrl siRNA; 100%); mean±SEM; n=4; ***p<0.001 vs control.

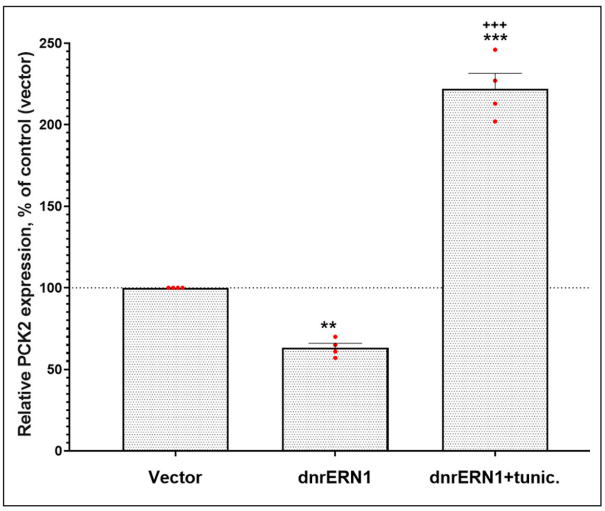

Figure 4. The effect of tunicamycin (tunic.; 500 ng/ml for 4 h), an inducer of endoplasmic reticulum (ER) stress, on the phosphoenolpyruvate carboxykinase 2 (PCK2) mRNA expression in glioblastoma cells without endoribonuclease activity of endoplasmic reticulum to nucleus signaling 1 (ERN1) (dnrERN1), measured by real-time qPCR. The values of PCK2 mRNA expression were normalized to ACTB mRNA and represented as a percentage of control (Control; 100%); mean±SEM; n=4; **p<0.01 and ***p<0.001 vs. control (vector); +++p<0.001 vs. dnrERN1.

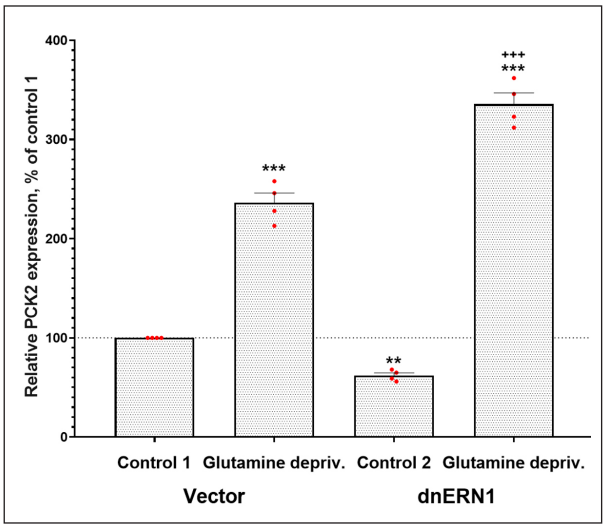

Figure 5. The effect of glutamine deprivation on the expression of phosphoenolpyruvate carboxykinase 2 (PCK2) mRNA in control glioblastoma cells (Vector) and cells with inhibited endoribonuclease and protein kinase activities of endoplasmic reticulum to nucleus signaling 1 (ERN1) (dnERN1), measured by real-time qPCR. The values of this mRNA expression were normalized to ACTB mRNA and represented as a percentage of control 1 (Vector; 100%). The impact of glutamine deprivation on PCK2 expression in cells with inhibited ERN1 was compared to that of control 2 (dnERN1); mean±SEM; n=4; **p<0.01 and ***p<0.001 vs. control 1; +++p<0.01 vs. control 2.

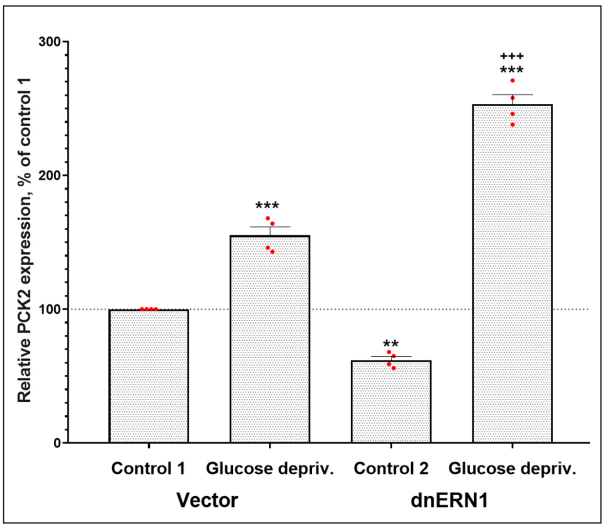

Figure 6. The effect of glucose deprivation on the expression of phosphoenolpyruvate carboxykinase 2 (PCK2) mRNA in control glioblastoma cells and cells with a deficiency of both enzymatic activities of ERN1 (dnERN1), measured by qPCR. The values of this mRNA expression were normalized to ACTB mRNA and represented as a percentage of control 1 (Vector; 100%). The impact of glucose deprivation on PCK2 expression in dnERN1 cells was compared to that of control 2 (dnERN1); mean±SEM; n=4; **p<0.01 and ***p<0.001 vs. control 1; +++p<0.001 vs. control 2.

increased by 442% in dnERN1 glioblastoma cells treated under glutamine deprivation compared with control 2 (Figure 5).

At the same time, glucose deficiency leads to less pronounced changes in PCK2 mRNA expression in glioblastoma cells with native ERN1 than in glutamine deficiency (Figure 6). Thus, in control glioblastoma cells treated with glucose deprivation, PCK2 mRNA expression upregulated by 55% ($p<0.001$) compared to the control 1 (Figure 6). However, the inhibition of ERN1 endoribonuclease and protein kinase activities significantly enhances the sensitivity of this gene expression to glucose deprivation (+308%; $p<0.001$), compared to the control 2. These results demonstrate the variable sensitivity of PCK2 mRNA expression to glutamine and glucose deprivation in glioblastoma cells, which depends on ERN1 activity.

Finally, we investigated the impact of hypoxia induced by dimethyloxalylglycine at normal oxygen tension on PCK2 RNA expression in control glioblastoma cells and cells with suppressed ERN1 protein kinase and endoribonuclease activities. It was found that PCK2 mRNA expression is resistant to hypoxia in control glioblastoma cells, but strongly upregulated (by 112%; $p<0.001$) in cells with suppressed ERN1 protein kinase and endoribonuclease activities treated by dimethyloxalylglycine (Figure 7). Thus, the sensitivity of PCK2 expression to hypoxia in glioblastoma cells is suppressed by ERN1 signaling protein.

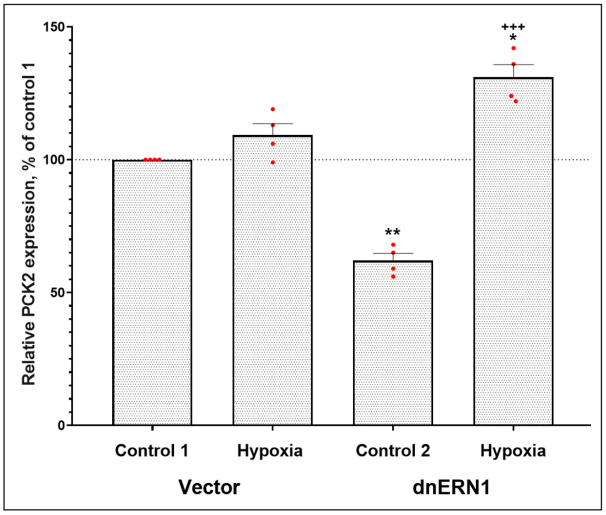

Figure 7. The effect of hypoxia induced by dimethyloxalylglycine on the expression of phosphoenolpyruvate carboxykinase 2 mRNA in control glioblastoma cells and cells with a deficiency of both enzymatic activities of ERN1 (dnERN1), measured by qPCR. The values of PCK2 mRNA expression were normalized to ACTB mRNA and represented as a percentage of control 1 (Vector; 100%). The impact of hypoxia on this mRNA expression in ERN1 knockdown cells was compared to that of control 2; mean±SEM; $n=4$; $^{*}p<0.05$ and $^{**}p<0.01$ vs. control 1; $^{+++}p<0.001$ vs. control 2.

Discussion

In this study, we demonstrated that the expression of PCK2 in U87MG glioblastoma cells is regulated by ERN1 endoribonuclease activity. This is shown both in cell sublines with suppressed ERN1 enzymatic activities and by silencing ERN1 and XBP1 (Figure 8). Changes in PCK2 expression upon silencing of ERN1 and XBP1 mRNA are similar to those observed in glioblastoma cells overexpressing dnERN1 and dnrERN1, confirming the important role of ERN1 in regulating PCK2 expression and consistent with previous studies (Auf et al. 2013; Minchenko et al. 2024a,b). ER stress-dependent regulation of gene expression through ERN1 endoribonuclease activity has been shown for many genes (Acosta-Alvear et al. 2007; Auf et al. 2013; Minchenko et al. 2019, 2024a,b, 2025b). Moreover, XBP1 silencing experiments indicate that the effect of inhibiting ERN1 endoribonuclease activity on PCK2 mRNA expression is mediated primarily by the transcription factor XBP1s, eliminating the possibility of posttranscriptional regulation of PCK2 mRNA expression through RIDD (regulated Ire1-dependent decay) (Acosta-Alvear et al. 2007; Auf et al. 2013; Hollien et al. 2009, Minchenko et al. 2024a,b). Our results demonstrate that ER stress-dependent regulation of PCK2 expression is mediated by ERN1 endoribonuclease activity and are consistent with previously published data on the involvement of this enzymatic activity in the regulation of gene expression (Auf et al. 2013; Minchenko et al. 2019, 2024a,b, 2025b).

It is interesting to note that PCK2 plays an important role in the metabolic reprogramming of many cancer cells, including glioblastoma cells (Gunasekharan et al. 2024; Xue et al. 2024; Yi et al. 2025). However, inhibition of the ERN1 protein kinase also suppresses glioblastoma growth by downregulating proliferation-related genes while increasing pro-invasive gene expression (Auf et al. 2013; Minchenko et al. 2019, 2024a,b, 2025b; Pelizzari-Raymundo et al. 2023). PCK2 is overexpressed in cancer cells and regulates cell survival, proliferation, metastasis, and protein folding in different cell types (Hyrossova et al. 2022; Ma et al. 2022; Li et al. 2024; Chang et al. 2025). Moreover, the regulation of PCK2 expression is multilevel, from epigenetic and transcriptional regulation to posttranscriptional (Yu et al. 2021).

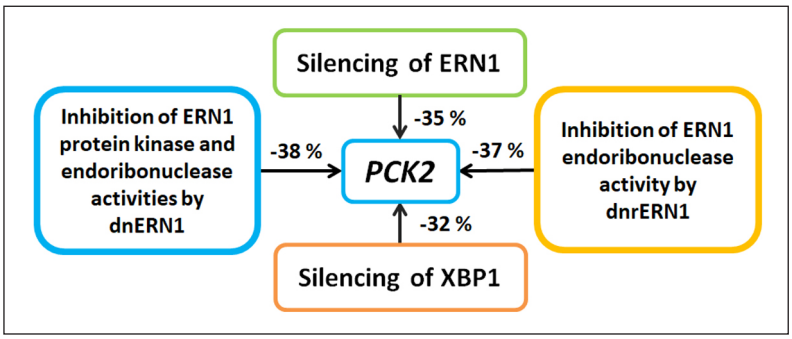

Figure 8. Schematic demonstration of the sensitivity of phosphoenolpyruvate carboxykinase 2 (PCK2) mRNA expression to inhibition of endoribonuclease and protein kinase activities of ERN1 by dnERN1 or only ERN1 endoribonuclease activity by dnrERN1 and by silencing of ERN1 and XBP1 mRNAs (48 h).

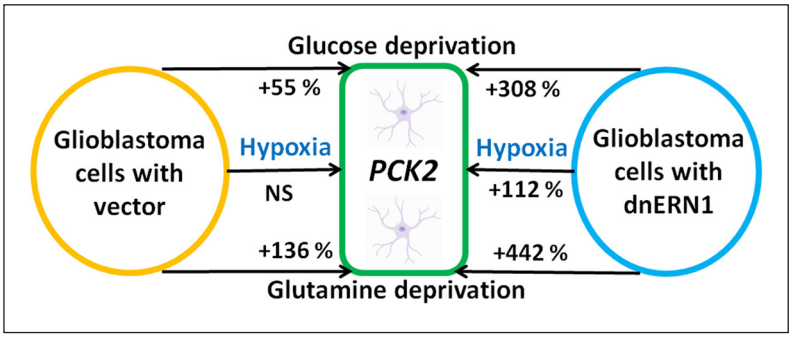

Figure 9. Schematic demonstration of the effect of glucose and glutamine deprivations as well as hypoxia on the expression of PCK2 mRNA in control U87MG glioblastoma cells (U87MG) and cells with a deficiency of both ERN1 protein kinase and endoribonuclease (dnERN1); NS – no significant changes.

In this work, we demonstrated that the expression of PCK2 is increased in glioblastoma cells under glutamine and glucose deprivation and that inhibition of ERN1 enzymatic activity strongly enhances the sensitivity of its expression to these nutrient deprivations. It can be assumed that in glioblastoma cells, under the influence of ERN1, PCK2 gene expression is resistant to glutamine and glucose deprivation, and upon ERN1 inhibition, this resistance is removed. These results are consistent with data that mitochondrial PCK is upregulated in glucose deficiency, thereby increasing cell survival (Hyrossova et al. 2022). Furthermore, our results are consistent with our previous data on ERN1-dependent expression of numerous genes under glutamine and glucose deprivation but in a gene-specific manner (Minchenko et al. 2019, 2021, 2024d, 2025d; Krasnytska et al. 2023). Changes in PCK2 gene expression under conditions of glucose and glutamine deficiency may represent ERN1-mediated stress adaptation. It is consistent with data that PCK2 silencing activates the tricarboxylic acid cycle, augments mitochondrial respiration, and enhances glutathione oxidation under glucose starvation, which sheds light on the adaptive responses of cancer cells to nutrient deprivation (Bluemel et al. 2021). It is interesting to note that ER stress plays an important role in these adaptive responses.

The dependence of PCK2 gene expression on glucose in glioblastoma cells and after inhibition of ERN1 that we have discovered indicates a close relationship between the implementation of genetic information from carbohydrates. This is consistent with the mechanism of ER stress induction, associated with disrupting post-translational protein modifications in the ER, including glycosylation, leading to genome reprogramming and changes in the expression of hundreds of genes (Almanza et al. 2019; Acosta-Alvear et al. 2025). This applies to genes responsible for glycosylation and other

post-translational modification of proteins, as well as post-transcriptional changes in RNA (Liu et al. 2023; Minchenko et al. 2025b).

Glutamine also plays an equally important role in controlling glioblastoma growth, providing carbon and nitrogen to support cancer cell proliferation. However, glutamine also modulates the tumor suppressor FBW7 through lysine glutaminylation by glutaminyl-tRNA synthetase (QARS) to promote cancer cell proliferation and survival (Wang et al. 2025). Moreover, this tRNA synthetase is a target of mascRNA, a small noncoding tRNA-like RNA deriving from the lncRNA MALAT1, and promotes global protein translation (Lu et al. 2020). All this emphasizes the very important and multivector regulatory role of both glucose and glutamine in the control of metabolic processes in normal and malignant tumors.

Hypoxia is a significant factor in glioblastoma growth because ER stress announces resistance to the toxic effects of hypoxia through genome reprogramming by HIF-dependent and HIF-independent mechanisms (Minchenko et al. 2004; Bobarykina et al. 2006; Denko 2008; Sun and Denko 2014; Chan et al. 2016; Semenza 2017; Sliusar et al. 2023). We showed that PCK2 expression in control glioblastoma cells is resistant to hypoxia. Inhibition of ERN1 renders glioblastoma cells sensitive to hypoxia. Thus, hypoxic regulation of this gene expression is controlled by ERN1. Data have shown that hypoxic regulation of numerous gene expressions is significantly modified by ERN1 knockdown and many other factors, by different mechanisms in a gene-specific manner (Minchenko and Caro 2000; Srinivas et al. 2001; Semenza 2017; Minchenko et al. 2014, 2019, 2020, 2021). The ERN1 signaling protein may modify the impact of hypoxia on gene expression via genome-specific changes in the additional regulatory factors that interact with HIF and modulate its activity (Minchenko and Caro 2000; Sun and Denko 2014; Semenza 2017; Batie and Rocha 2020). Many factors are known to influence the activity of HIF transcription factor. Thus, more than 150 proteins have been identified that can interact with HIF1A, altering its transcriptional activity and stability through different mechanisms, including phosphorylation (Minchenko and Caro 2000; Semenza 2017).

In conclusion, the data presented in this investigation identify the ERN1 endoribonuclease-mediated mechanism of the multifaceted regulation of PCK2 expression in glioblastoma cells, which is important for tumor growth. However, the detailed molecular mechanism of the interaction among hypoxia, glucose/glutamine deprivation, and the ERN1-mediated signaling pathway in regulating PCK2 expression as well as its significance for glioblastoma growth warrants further study.

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Conflicts of interest: *The authors declare no conflicts of interest.*

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