doi:10.2478/enr-2026-0005

ERN1-dependent regulation of BAG cochaperone 1 expression and the sensitivity to glutamine deprivation in U87MG glioblastoma cells

Yuliia M. Viletska [1], Oleksandr H. Minchenko [1], Olena O. Khita [1], Daria O. Tsymbal [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. The BAG cochaperone 1 (BAG1) binds to oncogene BCL2 and markedly enhances its anti-apoptotic effects. This cochaperone represents a link between growth factor receptors and anti-apoptotic mechanisms mediated by endoplasmic reticulum stress. BAG1 interacts with the glucocorticoid receptor and modulates its transcription activity. As a cochaperone for several HSP70 proteins, it participates in control of protein folding. The present study aims to investigate the regulation of the BAG1 mRNA expression in U87MG glioblastoma cells by hypoxia and glucose or glutamine deprivation, depending on the inhibition of ERN1 (endoplasmic reticulum to nucleus signaling 1) with the intent to reveal the role of ERN1 signaling in the regulation of this gene expression and function in oncogenesis.

Methods. The U87MG glioblastoma cells (transfected by an empty vector; control) and cells with inhibited ERN1 endoribonuclease and protein kinase (dnERN1) or only ERN1 endoribonuclease (dnrERN1) were used. Silencing of ERN1 and XBP1 mRNAs for suppression of ERN1 function was also used. A hypoxic condition was created by dimethyloxalylglycine (4 h). DMEM medium without glucose or glutamine was used for glucose and glutamine deprivation (16 h). The expression level of the BAG1 mRNA was studied by real-time qPCR and normalized to the beta-actin mRNA.

Results. Inhibition of the endoribonuclease activity of ERN1 significantly decreased BAG1 mRNA expression. However, a lesser suppression of this mRNA expression was observed in dnERN1 cells (with inhibited ERN1 endoribonuclease and protein kinase) indicating the involvement of protein kinase in controlling BAG1 expression. The silencing of ERN1 and XBP1 mRNAs also reduced the expression of BAG1 mRNA demonstrating the involvement of XBP1s in this regulation. The expression of the *BAG1* gene was resistant to glutamine deprivation and upregulated in response to glucose deprivation in control glioblastoma cells. However, the inhibition of ERN1 increased the sensitivity of *BAG1* gene expression to both glucose and glutamine deprivation. Furthermore, the expression of the *BAG1* gene was increased under hypoxia in control U87MG cells; however, a greater induction was observed in dnERN1 cells.

Conclusion. The results of this study demonstrated that ERN1 inhibition reduces BAG1 mRNA expression through the endoribonuclease activity of ERN1 and that protein kinase activity counteracts endoribonuclease in regulating the expression of BAG1 mRNA. Moreover, ERN1 inhibition also enhances the sensitivity of BAG1 mRNA expression to nutrient supply and hypoxia resulting in reduced resistance of glioblastoma cells.

Keywords: BAG1, gene expression, ERN1 inhibition, ERN1 endoribonuclease, ERN1 protein kinase, ERN1 and XBP1 silencing, hypoxia, glutamine and glucose deprivation, glioblastoma cells

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

The BAG cochaperone 1 (BAG1) is a multifunctional protein that exists as several functionally diverse variants and interacts with chaperones, glucocorticoid and other steroid hormone receptors, signaling molecules, the cellular stress response protein PPP1R15A/GADD34 (Protein Phosphatase 1 Regulatory Subunit 15A/Growth Arrest And DNA-Damage-Inducible 34), and the anti-apoptotic BCL2 protein (BCL2 apoptosis regulator) (Takayama et al. 1997; Witcher et al. 2001; Hung et al. 2003; Liu et al. 2009; Rauch et al. 2016; Pattingre and Turtoi 2022; Kizilboga et al. 2024). Overexpression of BAG1 regulates various cellular processes including cell proliferation, apoptosis, metastasis, cell motility, protein folding, and homeostasis in different cell types (Kilbas et al. 2022; Hou et al. 2024). The BAG1 is overexpressed in various malignant tumors and enhances chemoresistance (Yang et al. 1998; Zhao et al. 2024). It has a pro-survival role and suppression of BAG1 increases chemosensitivity of carcinoma cells (Townsend et al. 2005; Mariotto et al. 2021; Zhao et al. 2024). BAG1 participates in cancerogenesis by regulating both the cell survival and response to nuclear hormone receptors modifying gene expression at the transcriptional level (Sharp et al. 2004; Kilbas et al. 2022). This cochaperone protein negatively regulates glucocorticoid receptor action, however, it enhances the estrogen-dependent transcription (Cato and Mink 2001; Cutress et al. 2003). BAG1 also controls the activity of the androgen receptor (AR) and is upregulated in prostate cancer (Kuznik et al. 2022). Thus, regulation of nuclear hormone receptor function, apoptosis susceptibility, and endoplasmic reticulum (ER) stress signaling pathways by BAG1 may have a significant impact on development, progression, cell viability, and response to therapy.

Glioblastoma growth and its metabolic reprogramming require the unfolded protein response induced by ER stress and hypoxia (Denko 2008; Sun and Denko 2014; Almanza et al. 2019; Pelizzari-Raymundo et al. 2024; Acosta-Alvear et al. 2025). Three tightly interconnected unfolded protein response signaling pathways are responsible for adapting cancer cells including glioblastoma to adverse environmental conditions (Hetz et al. 2020; Acosta-Alvear et al. 2025). Knockdown of ERN1 (ER to nucleus signaling 1), a key signaling protein of the unfolded protein response, significantly inhibited glioblastoma cell proliferation and tumor growth *in vivo*; however, it resulted in an enhanced invasiveness of the glioma cells (Auf et al. 2010; Pelizzari-Raymundo et al. 2023; Minchenko et al. 2024a,b). Metabolic reprogramming is a basic characteristic of tumor cells that promotes their rapid growth and resistance to treatment, preferentially through ER stress (Chevet et al. 2015; Avril et al. 2017; Logue et al. 2018; Papaioannou and Chevet 2018; Le Reste et al. 2020).

The ERN1 is an ER transmembrane signaling protein with protein kinase and endoribonuclease activities in the cytoplasmic domain (Almanza et al. 2019; Hetz et al. 2020). The endoribonuclease activity of ERN1 is responsible for the alternative splicing of the XBP1 (X-box binding protein 1) pre-mRNA encoding the splice variant of XBP1 (XBP1s). This transcription factor regulates the expression of chaperones and enzymes involved in the degradation of misfolded proteins and restoration of folding (Acosta-Alvear et al. 2007; Doultsinos et al. 2017; Hetz et al. 2020). It is worth noting that XBP1s participates in EDEM1-controlled insulin synthesis in pancreatic β-cells by inhibiting the IRE1/JNK/c-Jun pathway (Alexandru et al. 2023). The protein kinase activity of ERN1 also plays a significant role in the ERN1 signaling and controlling the expression of numerous genes (Auf et al. 2013; Minchenko et al. 2019, 2024b,c). Recently, it has been demonstrated that the protein kinase activity of ERN1 plays a crucial role in regulating the expression of homeobox genes associated with glioblastoma cell proliferation and invasion. ERN1 knockdown significantly increases their expression, despite inhibiting the proliferation of these cells (Minchenko et al. 2024a).

There are also data indicating that inhibition of ERN1 modifies the hypoxic regulation of key regulatory gene expressions as well as their sensitivity to glucose and glutamine deprivations in glioblastoma cells (Minchenko et al. 2019, 2021, 2024d, 2025b,d; Riabovol et al. 2019; Krasnytska et al. 2023; Sliusar et al. 2023). This has also been shown for glucocorticoid and estrogen receptors (Minchenko et al. 2016, 2017). Furthermore, it has recently been demonstrated that hydrocortisone significantly alters both hypoxic and ER-dependent regulation of gene expression in glioblastoma cells (Minchenko et al. 2025a,c). It is known that hypoxic regulation of gene expression is mediated by transcription factor HIF in a gene-specific manner (Minchenko and Caro 2000; Srinivas et al. 2001; Minchenko et al. 2004, 2014; Bobarykina et al. 2006; Sun and Denko 2014; Semenza 2017; Batie and Rocha 2020). However, many factors are known to influence the activity of this 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 this regard, the question arises from the possible involvement of BAG1 in the mechanisms of interaction between ER stress, hypoxia, and glucocorticoids.

Thus, ER stress and hypoxia, as well as glucose and glutamine supply, are important factors of glioblastoma progression, metabolic reprogramming, and therapeutic resistance. However, there are still no available data concerning the molecular mechanisms of these factors' interaction in the regulation of *BAG1* gene expression in glioblastoma cells after ERN1 knockdown. In this study, we demonstrate that inhibition of both enzymatic activities of ERN1 reduces the expression level of BAG1 in glioblastoma cells; however, inhibition of only the endoribonuclease activity of ERN1 has a stronger effect. This indicates antagonism of these enzymatic activities of ERN1 in regulating BAG1 expression. Moreover, ERN1 knockdown enhances the sensitivity of this gene expression to nutrient deprivation and hypoxia.

Materials and Methods

Cell lines and culture conditions. The wild-type U87MG glioblastoma cells were used for RNA silencing. Three genetically modified sublines of glioblastoma cells were also used (Auf et al. 2013). Glioblastoma cells were grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (DMEM, 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. The wild-type U87MG glioblastoma cells and three sublines of these cells were described previously (Auf et al. 2013). The 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. A glioblastoma cell subline with dnrERN1-suppressed ERN1 endoribonuclease activity was also used (Auf et al. 2013). The cells with dnERN1 and dnrERN1 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). Moreover, the cells with dnERN1 do not have the phosphorylated isoform of ERN1 after induction of ER stress by tunicamycin (Auf et al. 2013). 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 by 0.5 mM dimethyloxalylglycine (Sigma-Aldrich, St. Louis, MO, USA) as described previously (Minchenko et al. 2024b) and cell cultures 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 the glioblastoma cells with inhibited endoribonuclease activity of ERN1 to tunicamycin (500 ng/ml) to clarify the role of other signaling pathways of ER stress all in the control of *BAG1* gene expression.

Small interfering RNA knockdown experiments. The ERN1 and XBP1 mRNAs were silenced in U87MG glioblastoma cells with small interfering RNA (siRNA) 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 was extracted from glioblastoma cells using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water. RNA concentration and spectral characteristic was measured using NanoDrop spectrophotometer.

Reverse transcription and quantitative PCR analysis. The expression levels of BAG1 and ACTB mRNA 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) 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. 2024a). Polymerase chain reaction was performed in triplicate. The expression of beta-actin mRNA was used as a control of analyzed mRNA quantity. The pair of primers specific for *BAG1* gene was received from Sigma-Aldrich (St. Louis, MO, USA) and used for quantitative PCR: BAG1 forward 5'- gagatgaatcggagccagga and BAG1 reverse 5'- ggacaactggttcactgctg (NM_004323.6). Primers for ERN1, XBP1, and ACTB have been described previously (Minchenko et al. 2024a).

Statistical analysis. 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 3% 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 BAG1 mRNA was decreased by 42% ($p<0.01$) in glioblastoma cells with inhibited endoribonuclease and protein kinase activities of ERN1 (dnERN1) in comparison to control cells transfected by empty vector. At the same time, the stronger downregulation of BAG1 mRNA expression (–62%; $p<0.001$) was observed in glioblastoma cells with inhibited only endoribonuclease activities of ERN1 (dnrERN1) as compared to control cells (Figure 1). This indicates that inhibition of ERN1 protein kinase activity leads to oppositely directed changes in BAG1 mRNA expression compared to inhibition of endoribonuclease activity, as a result of which the effect of endoribonuclease inhibition on the expression level of this mRNA is significantly reduced.

Similar changes were detected in the expression of the BAG1 mRNA in glioblastoma cells after silencing of ERN1 mRNA. As shown in Figure 2, the expression level of BAG1 mRNA was decreased by 38% ($p<0.01$) in glioblastoma cells after 48 h of ERN1 mRNA silencing. The level of ERN1 mRNA was suppressed by 89% ($p<0.001$) in comparison to cells transfected by control siRNA. Furthermore, silencing of XBP1 mRNA resulted in a more pronounced suppression of BAG1 mRNA expression in glioblastoma cells (–59%; $p<0.001$) 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 the level of BAG1 mRNA expression upon inhibition of ERN1

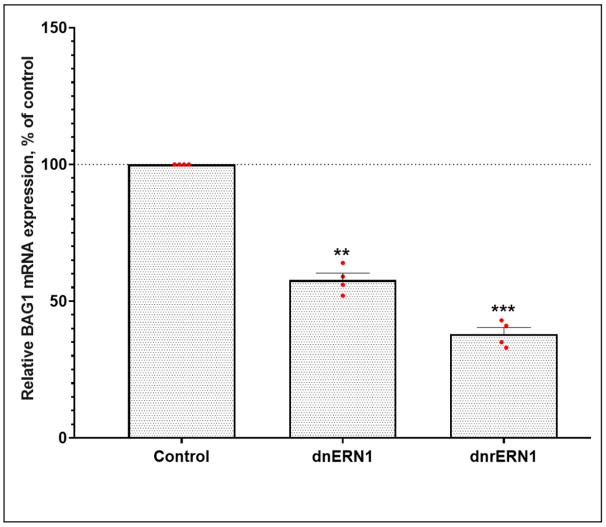

Figure 1. The expression level of BAG cochaperone 1 (BAG1) in control U87MG glioblastoma cells (transfected by an empty vector; control), cells with suppressed endoribonuclease and protein kinase activities of ERN1 (endoplasmic reticulum to nucleus signaling 1) (dnERN1), and cells with inhibited only endoribonuclease activity of ERN1 (dnrERN1) measured by real-time qPCR. The values of this mRNA expression were normalized to ACTB mRNA and presented as a percentage of control (100%). Mean±SEM; n=4; **p<0.01 and ***p<0.001 vs. control.

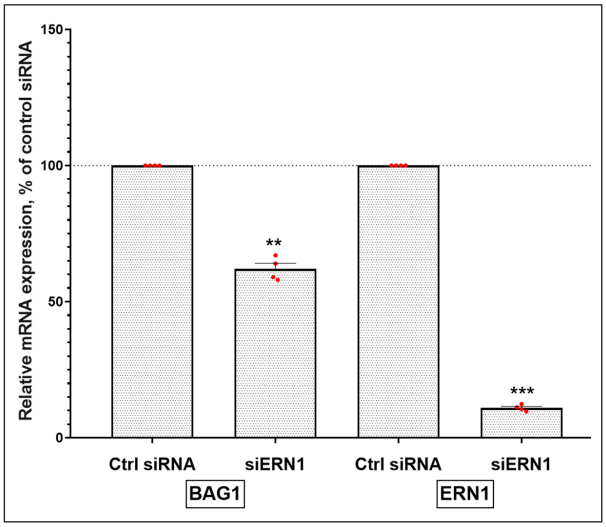

Figure 2. The impact of endoplasmic reticulum to nucleus signaling 1 (ERN1) mRNA silencing by specific ERN1 siRNA (48 h) on the expression of BAG cochaperone 1 (BAG1) and ERN1 mRNAs in wild-type glioblastoma cells (real-time qPCR analysis). The values of these mRNAs' expressions were normalized to beta-actin mRNA and presented as a percentage of control (Ctrl siRNA; 100%). Mean±SEM; n=4; **p<0.01 and ***p<0.001 vs. control.

endoribonuclease activity are mediated by disruption of alternative splicing of XBP1 mRNA.

We also investigated the impact of ER stress induced by tunicamycin on the expression of BAG1 mRNA in glioblastoma cells with inhibited endoribonuclease activity of ERN1 to clarify the role of other unfolded protein response signaling pathways in regulating this gene expression. As shown in Figure 4, tunicamycin increased the expression level of the BAG1 mRNA in these glioblastoma cells by 50% ($p<0.01$) indicating the involvement of other ER stress signaling pathways in the control of BAG1 mRNA expression.

Next, we investigated the impact of dimethyloxalylglycine, which introduces a hypoxic condition at normal oxygen tension on the expression of the BAG1 RNA in control glioblastoma cells and cells with suppressed ERN1 protein kinase and endoribonuclease activities compared to the corresponding control. It was found that under the influence of dimethyloxalylglycine, the level of BAG1 mRNA expression is upregulated by hypoxia (+16%; $p<0.05$) in control glioblastoma cells (Figure 5). However, the impact of dimethyloxalylglycine on BAG1 mRNA expression was significantly stronger in dnERN1 glioblastoma cells (+56%; $p<0.01$) compared to the control 2.

Finally, we studied the impact of glucose and glutamine deprivation on BAG1 mRNA expression in control glioblastoma cells and cells with inhibited ERN1 endoribonuclease and protein kinase activities. As shown in Figure 6, the level of BAG1 mRNA expression was increased in control glioblastoma cells exposed upon glucose deprivation condition by 49% ($p<0.01$) in comparison to the control 1. However, the inhibition of ERN1 endoribonuclease and protein kinase activities significantly enhances the sensitivity of this gene expression to glucose deprivation (+84%; $p<0.001$) compared to the corresponding control. At the same time, BAG1 mRNA expression was resistant to glutamine deprivation in control glioblastoma cells, however, it increased by 46% ($p<0.01$) in cells deprived of ERN1 endoribonuclease and protein kinase activities (Figure 7). Thus, the expression of BAG1 mRNA demonstrates different sensitivity to glucose and glutamine deprivation in glioblastoma cells and depends on the activity of ERN1.

Discussion

In this investigation, we demonstrated that the expression of polyfunctional protein BAG1 in U87MG glioblastoma cells is differently regulated by ERN1 endoribonuclease and protein kinase activities

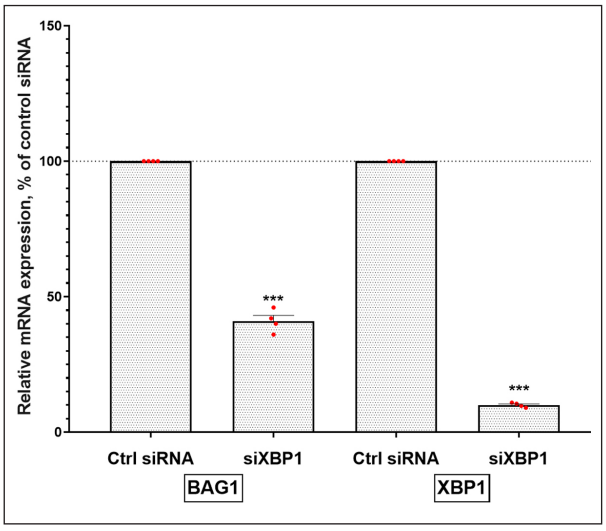

Figure 3. The impact of XBP1 (X-box binding protein 1) mRNA silencing by specific XBP1 siRNA (48 h) on the expression of BAG cochaperone 1 (BAG1) and XBP1 mRNAs in wild-type glioblastoma cells (real-time qPCR analysis). The values of these mRNAs' expressions were normalized to beta-actin mRNA and represented as a percentage of control (Ctrl siRNA; 100%). Mean±SEM; n=4; ***$p<0.001$ vs. control.

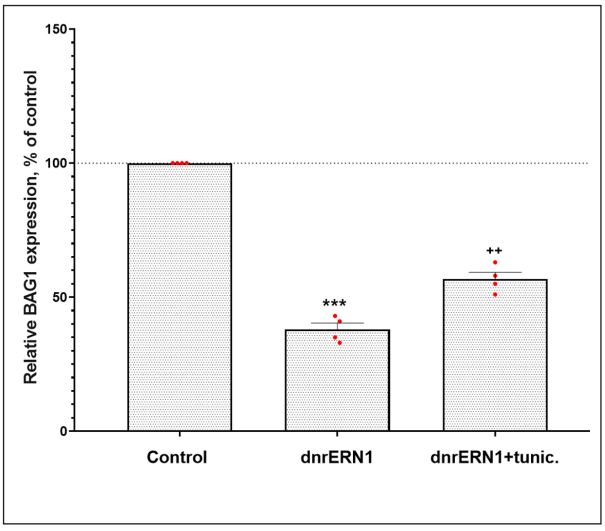

Figure 4. The impact of tunicamycin (tunic., 500 ng/ml for 4 h) on the BAG cochaperone 1 (BAG1) mRNA expression in glioblastoma cells without endoribonuclease activity of ERN1 (endoplasmic reticulum to nucleus signaling 1) (dnrERN1), measured by real-time qPCR. The values of BAG1 mRNA expression were normalized to beta-actin mRNA and represented as a percentage of control (control; 100%). Mean±SEM; n=4; ***$p<0.001$ vs. control (vector); $^{++}p<0.001$ vs. dnrERN1.

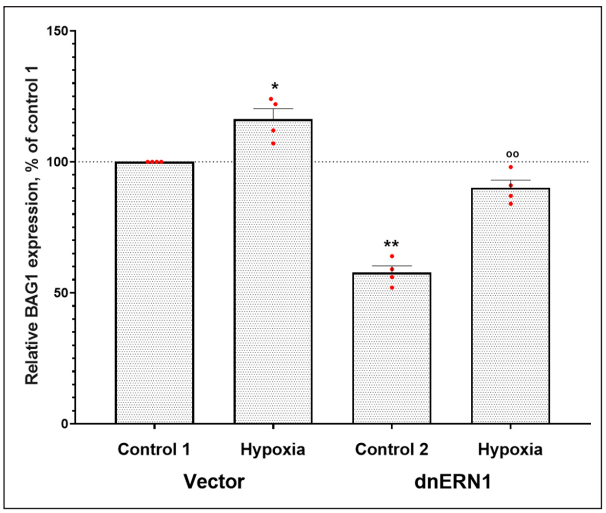

Figure 5. The impact of hypoxia induced by dimethyloxalylglycine on the expression of BAG cochaperone 1 (BAG1) mRNA in control glioblastoma cells (Vector) and cells with inhibited endoribonuclease and protein kinase activities of ERN1 (endoplasmic reticulum to nucleus signaling 1) (dnERN1) measured by real-time qPCR. The values of BAG1 mRNA expression were normalized to beta-actin mRNA and presented as a percentage of control 1 (Vector; 100%). The impact of hypoxia in cells with inhibited ERN1 was compared to that of control 2 (dnERN1). Mean±SEM; n=4; *p<0.05 and **p<0.01 vs. control 1; ^{oo}p<0.01 vs. control 2.

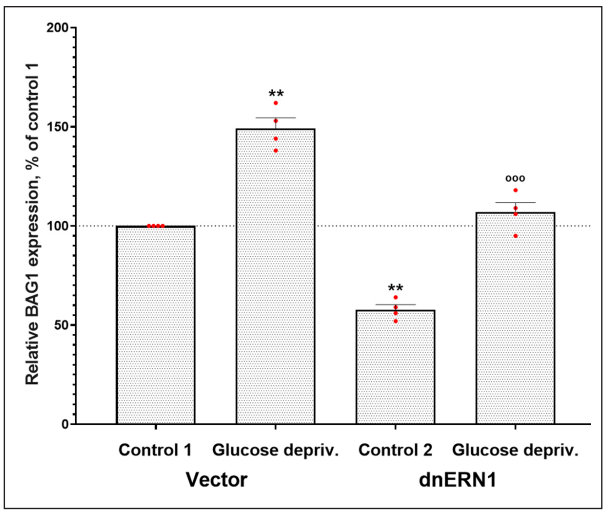

Figure 6. The impact of glucose deprivation on the expression of BAG cochaperone 1 (BAG1) mRNA in control glioblastoma cells and cells with a deficiency of both enzymatic activities of ERN1 (endoplasmic reticulum to nucleus signaling 1) (dnERN1) measured by qPCR. The values of BAG1 mRNA expression were normalized to beta-actin mRNA and presented as a percentage of control 1 (Vector; 100%). The impact of glucose deprivation in dnERN1 cells was compared to that of control 2 (dnERN1). Mean±SEM; n=4; **p<0.01 vs. control 1; ^{ooo}p<0.001 vs. control 2.

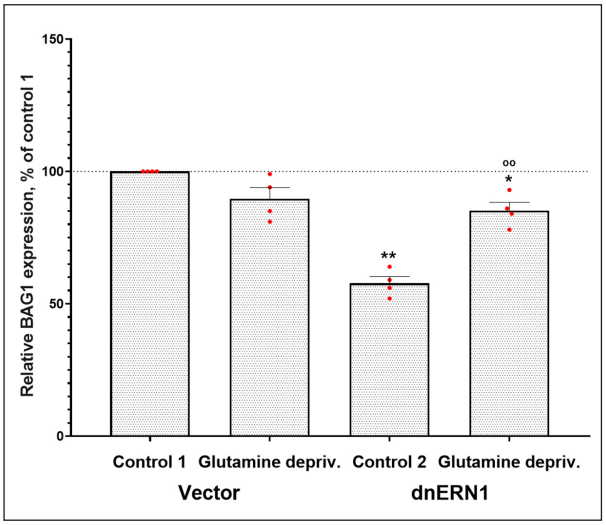

Figure 7. The impact of glutamine deprivation on the expression of BAG cochaperone 1 (BAG1) mRNA in control glioblastoma cells and cells with a deficiency of both enzymatic activities of ERN1 (endoplasmic reticulum to nucleus signaling 1) (dnERN1) measured by qPCR. The values of BAG1 mRNA expression were normalized to beta-actin mRNA and presented as a percentage of control 1 (Vector; 100%). The impact of glutamine deprivation 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; ^{oo}p<0.01 vs. control 2.

(Figure 8). Thus, inhibition of ERN1 endoribonuclease activity more significantly reduced BAG1 expression levels compared to inhibition of both enzymatic activities of this signaling protein. This suggests that the protein kinase activity of ERN1 exerts an opposite effect on the expression of BAG1 compared to its endoribonuclease activity. Silencing of ERN1 and XBP1 mRNAs has a similar effect to that of dnERN1 and dnrERN1 confirming the important role of ERN1 in regulating BAG1 expression and is consistent with previous studies (Auf et al. 2013; Minchenko et al. 2024a,b). This suggests that the effect of inhibiting ERN1 endoribonuclease activity on this mRNA expression is primarily mediated through transcription factor XBP1s, which practically eliminates posttranscriptional regulation of BAG1 mRNA expression through RIDD (regulated Ire1-dependent decay) (Acosta-Alvear et al. 2007; Auf et al. 2013; Minchenko et al. 2024a,b). These results clearly demonstrate that ER stress-dependent regulation of BAG1 expression is regulated by the interaction of endoribonuclease and protein kinase activities of ERN1, and agree well with previously reported data concerning the involvement of ERN1 protein kinase and endoribonuclease activities in regulating gene

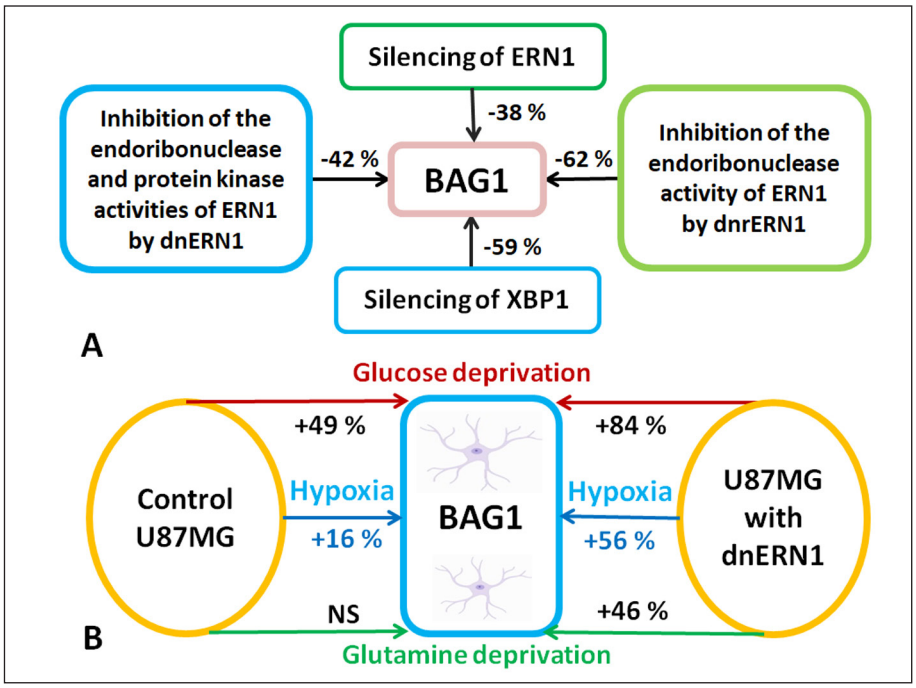

Figure 8. (A) Schematic demonstration of the sensitivity of BAG cochaperone 1 (BAG1) mRNA expression to inhibition of endoribonuclease and protein kinase activities of endoplasmic reticulum to nucleus signaling 1 (ERN1) by dnERN1 or only ERN1 endoribonuclease activity by dnrERN1 and by silencing of ERN1 and X-box binding protein 1 (XBP1) mRNAs (48 h). **(B)** Schematic demonstration of the impact of hypoxia, glucose, and glutamine deprivations on the expression of BAG1 mRNA in control U87MG glioblastoma cells (U87MG) and cells with a deficiency of both ERN1 protein kinase and endoribonuclease (dnERN1). NS – not-significant.

expression (Auf et al. 2013; Minchenko et al. 2019, 2024a,b, 2025b). It is interesting to note that BAG1 is a phosphorylation-dependent coactivator of c-JUN, through which the effects of the protein kinase ERN1 on EREG expression are mediated (Da Costa et al. 2010; Auf et al. 2013).

It has also been demonstrated that the inhibition of protein kinase activity of ERN1 is responsible for upregulation of EDN1, PBX3, PRRX1, PAX6, PBXIP1, and SHMT1 genes, which play a role in controlling cell proliferation and invasion (Minchenko et al. 2019, 2024a,b). It is worth to note that inhibition of both enzymatic activities of ERN1 inhibits the proliferation of glioblastoma cells to a lesser extent than inhibition of one endoribonuclease (Minchenko et al. 2024b). This is in good agreement with the reduction in BAG1 expression and the data that BAG1 is implicated in multiple cellular processes, including apoptosis, proliferation, transcription, metastasis, protein folding and homeostasis (Hung et al. 2003; Townsend et al. 2005; Pattingre and Turtoi 2022; Hou et al. 2024). This could be explained by the induction of some pro-oncogenic genes under the inhibition of ERN1 protein kinase (Minchenko et al. 2024a). However, inhibiting the protein kinase of ERN1 with a specific inhibitor also suppresses the glioblastoma growth (Pelizzari-Raymundo et al. 2023). Thus, inhibition of ERN1 protein kinase may suppress the proliferation-related EREG, PHGDH, and EDEM1 genes, and increase some invasion-responsible pro-proliferative genes.

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; Denko 2008; Sun and Denko 2014; Semenza 2017; Sliusar et al. 2023). We showed that hypoxia increases *BAG1* gene expression only in control glioblastoma cells. However, inhibition of ERN1 enhances the sensitivity of this gene expression to hypoxia resulting from the suppression of ERN1-mediated polyresistance of glioblastoma cells including resistance 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; Semenza 2017; Minchenko et al. 2004, 2019, 2020, 2021). The ERN1 may modify the impact of hypoxia on the expression of genes 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).

In this work, we demonstrated that the expression of BAG1 is increased in glioblastoma cells under glucose and glutamine deficiency in ERN1-dependent manner. These results are in good agreement with our previous data on ERN1-dependent expression of numerous genes under glucose and glutamine deprivation conditions in a gene-specific manner (Minchenko et al. 2019, 2021, 2024d, 2025d; Krasnytska et al. 2023).

Interestingly, the suppression of BAG1 expression in ERN1 knockout glioblastoma cells is associated with increased expression of the glucocorticoid receptor and decreased expression of estrogen receptor (Minchenko et al. 2016, 2017). This agrees with data that BAG1 interacts with glucocorticoid and estrogen receptors, modifying their functional activity (Cato and Mink 2001; Mariotto et al. 2021). Thus, interaction of this cochaperone with hormone receptors leads to inhibition of glucocorticoid receptor nuclear action at the level of regulation of the gene transcription and potentiates the role of estrogen in modulating chemotherapeutic-induced apoptosis as well as enhances estrogen-dependent transcription (Cato and Mink 2001; Cutress et al. 2003; Liu et al. 2009; Mariotto et al. 2021).

In conclusion, the data presented in this study identify the ERN1 endoribonuclease- and protein kinase-dependent mechanism in the regulation of BAG1 expression in U87MG glioblastoma cells, which is a multifunctional protein that controls tumor growth in different ways, interacts with ER stress and steroid receptors. However, the detailed molecular mechanism of the interaction between BAG1 and ERN1-mediated signaling pathways and glucocorticoid receptor function warrants further study.

Acknowledgement

This work was funded by the State Budget Program "Support for the Development of Priority Areas of Scientific Research" (Code: 6541030).

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

References

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 conditions-specific transcriptional regulatory networks. Molecular Cell 27, 53–66, 2007.

Acosta-Alvear D, Harnoss JM, Walter P, Ashkenazi A. Homeostasis control in health and disease by the unfolded protein response. Nat Rev Mol Cell Biol 26, 193–212, 2025.

Alexandru PRF, 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 26, 107956, 2023.

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 USA 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.

Avril T, Vauleon E, Chevet E. Endoplasmic reticulum stress signaling and chemotherapy resistance in solid cancers. Oncogenesis 6, e373, 2017.

Batie M, Rocha S. Gene transcription and chromatin regulation in hypoxia. Biochem Soc Trans 48, 1121–1128, 2020.
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 53, 789–799, 2006.
Cato AC, Mink S. BAG-1 family of cochaperones in the modulation of nuclear receptor action. J Steroid Biochem Mol Biol 78, 379–388, 2001.
Chevet E, Hetz C, Samali A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. Cancer Discov 5, 586–597, 2015.
Cutress RI, Townsend PA, Sharp A, Maison A, Wood L, Lee R, Brimmell M, Mullee MA, Johnson PW, Royle GT, Bateman AC, Packham G. The nuclear BAG-1 isoform, BAG-1L, enhances oestrogen-dependent transcription. Oncogene 22, 4973–4982, 2003.
Da Costa CR, Villadiego J, Sancho R, Fontana X, Packham G, Nateri AS, Behrens A. Bag1-L is a phosphorylation-dependent coactivator of c-Jun during neuronal apoptosis. Mol Cell Biol 30, 3842–3852, 2010.
Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumor. Nat Rev Cancer 8, 705–713, 2008.
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.
Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol 21, 421–438, 2020.
Hou M, Yue M, Han X, Sun T, Zhu Y, Li Z, Han J, Zhao B, Tu M, An Y. Comparative analysis of BAG1 and BAG2: Insights into their structures, functions and implications in disease pathogenesis. Int Immunopharmacol 143, 113369, 2024.
Hung WJ, Roberson RS, Taft J, Wu DY. Human BAG-1 proteins bind to the cellular stress response protein GADD34 and interfere with GADD34 functions. Mol Cell Biol 23, 3477–3486, 2003.
Kilbas PO, Can ND, Kizilboga T, Ezberci F, Doganay HL, Arisan ED, Dinler Doganay G. CRISPR/Cas9-mediated Bag-1 knockout increased mesenchymal characteristics of MCF-7 cells via Akt hyperactivation-mediated actin cytoskeleton remodeling. PLoS One 17, e0261062, 2022.
Kizilboga T, Ozden C, Can ND, Onay Ucar E, Dinler Doganay G. Bag-1-mediated HSF1 phosphorylation regulates expression of heat shock proteins in breast cancer cells. FEBS Open Bio 14, 1559–1569, 2024.
Krasnytska DA, Viletska YM, Minchenko DO, Khita OO, Tsymbal DO, Cherednychenko AA, Kozynkevych HE, Oksiom NS, Minchenko OH. ERN1 dependent impact of glucose and glutamine deprivations on *PBX3*, *PBXIP1*, *PAX6*, *MEIS1*, and *MEIS2* gene expressions in U87 glioma cells. Endocr Regul 57, 37–47, 2023.
Kuznik NC, Solozobova V, Lee II, Jung N, Yang L, Nienhaus K, Ntim EA, Rottenberg JT, Muhle-Goll C, Kumar AR, Peravali R, Graßle S, Gourain V, Deville C, Cato L, Neeb A, Dilger M, Cramer von Clausbruch CA, Weiss C, Kieffer B, Nienhaus GU, Brown M, Brase S, Cato ACB. A chemical probe for BAG1 targets androgen receptor-positive prostate cancer through oxidative stress signaling pathway. iScience 25, 104175, 2022.
Le Reste PJ, Pineau R, Voutetakis K, Samal J, Jegou 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 494, 73–83, 2020.
Liu HY, Wang ZM, Bai Y, Wang M, Li Y, Wei S, Zhou QH, Chen J. Different BAG-1 isoforms have distinct functions in modulating chemotherapeutic-induced apoptosis in breast cancer cells. Acta Pharmacol Sin 30, 235–241, 2009.
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.
Mariotto E, Corallo D, Pantile M, Giarin E, Pigazzi M, Basso G, Viola G, Aveic S. BAG1 down-regulation increases chemo-sensitivity of acute lymphoblastic leukaemia cells. J Cell Mol Med 25, 9060–9065, 2021.
Minchenko A, Caro J. Regulation of endothelin-1 gene expression in human microvascular endothelial cells by hypoxia and cobalt: role of hypoxia responsible element. Mol Cell Biochem 208, 53–62, 2000.
Minchenko O, Opentanova I, Minchenko D, Ogura T, Esumi H. Hypoxia induces transcription of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 gene via hypoxia-inducible factor-1alpha activation. FEBS Lett 576, 14–20, 2004.
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 20, 13705–13717, 2014.

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

Minchenko DO, Riabovol OO, Ratushna OO, Minchenko OH. Hypoxic regulation of the expression of genes encoded estrogen related proteins in U87 glioma cells: effect of IRE1 inhibition. Endocr Regul 51, 8–19, 2017.

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 Regul 53, 250–262, 2019.

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 54, 183–195, 2020.

Minchenko DO, Khita OO, Tsymbal DO, Viletska YM, Sliusar MY, Yefimova YV, Levadna LO, Krasnytska DA, Minchenko OH. ERN1 knockdown modifies the impact of glucose and glutamine deprivations on the expression of EDN1 and its receptors in glioma cells. Endocr Regul 55, 72–82, 2021.

Minchenko OH, Khita OO, Krasnytska DA, Viletska YM, Rudnytska OV, Hnatiuk OS, Minchenko DO. ERN1 knockdown affects the expression of TGIF1 and other homeobox genes in U87MG glioblastoma cells. Arch Biochem Biophys 758, 110073, 2024a.

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 759, 110104, 2024b.

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 58, 144–152, 2024c.

Minchenko OH, Sliusar MY, Khita OO, Minchenko DO, Viletska YM, Halkin OV, Ledvadna LO, Cherednychenko AA, Khikhlo YP. Inhibition of signaling protein ERN1 increases the sensitivity of serine synthesis gene expressions to glucose and glutamine deprivations in U87MG glioblastoma cells. Endocr Regul 58, 91–100, 2024d.

Minchenko OH, Abramchuk AI, Khikhlo YP, Sliusar MY, Halkin OV, Luzina OY, Danilovskyi 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 59, 48–56, 2025a.

Minchenko OH, Hrebennykova VO, Viletska YM, Hnatiuk OS, Sliusar MY, Kozynkevych HE, Minchenko DO. The ERN1 signaling pathway of unfolded protein response controls the expression of EDEM1 and their hypoxic regulation in glioblastoma cells. Endocr Regul 59,1–9, 2025b.

Minchenko OH, Viletska YM, Abramchuk AI, Halkin OV, Khikhlo YP, Sliusar MY, Danilovskyi SV, Luzina OY, Minchenko DO. Hypoxic regulation of insulin receptor substrate 2 gene expression is differently regulated by endoplasmic reticulum stress and hydrocortisone in normal human astrocytes and glioblastoma cells. Endocr Regul 59, 171–180, 2025c.

Minchenko OH, Viletska YM, Sliusar MY, Hnatiuk OS, Ratushna OO, Feldman TV, Kozynkevych HE, Bezrodnyi BH, Minchenko DO. Glucose-dependent control of insulin like growth factor 2 mRNA binding protein 2 and other gene expressions in ERN1 knockdown glioblastoma cells. Endocr Regul 59, 120–129, 2025d.

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

Pattingre S, Turtoi A. BAG family members as mitophagy regulators in mammals. Cells 11, 681, 2022.

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 26, 106687, 2023.

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 25, 1792–1813, 2024.

Rauch JN, Zuiderweg ER, Gestwicki JE. Non-canonical interactions between heat shock cognate protein 70 (Hsc70) and Bcl2-associated anthanogene (BAG) co-chaperones are important for client release. J Biol Chem 291, 19848–1985, 2016.

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

Semenza GL. A compendium of proteins that interact with HIF-1α. Exp Cell Res 356, 128–135, 2017.
Sharp A, Crabb SJ, Townsend PA, Cutress RI, Brimmell M, Wang XH, Packham G. BAG-1 in cancerogenesis. Expert Rev Mol Med 6, 1–15, 2004.
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 57, 252–261, 2023.
Srinivas V, Leshchinsky I, Sang N, King MP, Minchenko A, Caro J. Oxygen sensing and HIF-1 activation does not require an active mitochondrial respiratory chain electron-transfer pathway. J Biol Chem 276, 21995–21998, 2001.
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.
Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, Morimoto RI, Reed JC. BAG-1 modulates the chaperone activity of Hsp70/Hsc70. EMBO J 16, 4887–4896, 1997.
Townsend PA, Stephanou A, Packham G, Latchman DS. BAG-1: a multi-functional pro-survival molecule. Int J Biochem Cell Biol 37, 251–259, 2005.
Witcher M, Yang X, Pater A, Tang SC. BAG-1 p50 isoform interacts with the vitamin D receptor and its cellular overexpression inhibits the vitamin D pathway. Exp Cell Res 265, 167–173, 2001.
Yang X, Chernenko G, Hao Y, Ding Z, Pater MM, Pater A, Tang SC. Human BAG-1/RAP46 protein is generated as four isoforms by alternative translation initiation and overexpressed in cancer cells. Oncogene 17, 981–989, 1998.
Zhao B, Qin X, Fu R, Yang M, Hu X, Zhao S, Cui Y, Guo Q, Zhou W. Supramolecular nanodrug targeting CDK4/6 overcomes BAG1 mediated cisplatin resistance in oral squamous cell carcinoma. J Control Release 368, 623–636, 2024.