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The impact of ERN1 endoribonuclease activity inhibition on *TOB1*, *HBEGF*, and *TWIST1* genes expression in U87MG glioblastoma cells

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Objective. It is known that inhibition of the endoplasmic reticulum transmembrane signaling protein (ERN1) suppresses the glioblastoma cells proliferation. The present study aims to investigate the impact of inhibition of ERN1 endoribonuclease and protein kinase activities on the *TOB1*, *HBEGF*, and *TWIST1* gene expression in U87MG glioblastoma cells with an intent to reveal the role of ERN1 signaling in the regulation of expression of these genes.

Methods. The U87MG glioblastoma cells with inhibited ERN1 endoribonuclease (dnrERN1) or both enzymatic activities of ERN1 (endoribonuclease and protein kinase; dnERN1) were used. Cells transfected with empty vector served as controls. Wild-type glioblastoma cells were used for mRNA silencing. The expression level of the *TOB1*, *HBEGF*, and *TWIST1* genes and microRNA were studied by quantitative RT-PCR.

Results. We found that inhibition of ERN1 endoribonuclease activity led to a strong down-regulation of *HBEGF* gene expression in glioblastoma cells and did not significantly change the expression of *TOB1* and *TWIST1* genes. At the same time, inhibition of both enzymatic activities of ERN1 strongly increased the expression of the *TOB1* gene and down-regulated *HBEGF* and *TWIST1* genes in glioblastoma cells. The expression of *TWIST1* gene increased, but *HBEGF* and *TOB1* genes significantly decreased in cells with silencing of ERN1 mRNA by specific siRNA. At the same time, silencing of XBP1 mRNA reduced the expression of *HBEGF* gene only. In addition, in glioblastoma cells with ERN1 knockdown, the level of miR-96-5p was suppressed, but miR-182-5p was increased and could promote post-transcriptional expression of *TWIST1*, *HBEGF*, and *TOB1* mRNAs.

Conclusion. The results of the present study demonstrate that inhibition of ERN1 strongly up-regulated the expression of the anti-proliferative *TWIST1* gene through protein kinase activity of ERN1 and that decreased *HBEGF* and *TOB1* genes expression was also controlled preferentially by ERN1 protein kinase activity. These changes in the expression level of *TWIST1*, *HBEGF*, and *TOB1* genes may also contribute to ERN1 knockdown-mediated suppression of glioblastoma cells proliferation.

Keywords: TOB1, HBEGF, TWIST1, gene expression, microRNA, ERN1 protein kinase, glioblastoma cells

TWIST family bHLH transcription factor 1 (*TWIST1*) gene is hypermethylated and overexpressed in multiple human cancers. It plays an essential role in tumor initiation, growth, angiogenesis, invasion, metastasis, and chemo-resistance in a variety of carcinomas, sarcomas, and hematological malignancies (Zhao et al. 2017; Pires et al. 2021; Peng et al. 2024). Recently, it has been shown that TWIST1

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interacts with TMEM158, which functions as an oncogene and promotes lung adenocarcinoma progression through the PI3K/AKT pathway (Xu et al. 2024). High levels of TMEM158 promote cell proliferation, progression through the cell cycle, migration, and invasion, while suppressing apoptosis. Moreover, elevated expression of TMEM158 and TWIST1 in lung adenocarcinoma is associated with increased cancer aggressiveness and a poor prognosis (Xu et al. 2024). Heparin binding epidermal growth factor like growth factor (HBEGF), also known as diphtheria toxin receptor, is a multifunctional protein that mediates its effects through epidermal growth factor receptor (EGFR) and erb-b2 receptor tyrosine kinase 2 (ERBB2), promotes cell proliferation, and breast cancer intravasation, metastasis, and macrophage-independent invasion *in vivo* (Bos et al. 2009; Zhou et al. 2014; Xiao et al. 2020; Zhang et al. 2024). Park et al. (2022) have shown that secreted HBEGF activates EGFR on the cancer cell surface to induce epithelial-to-mesenchymal transition resulting in an increased migration and invasion *in vitro* and increased metastasis *in vivo*. Moreover, the impact of HBEGF on cell proliferation and apoptosis is mediated by microRNA miR-194 (Wu et al. 2021). Transducer of ERBB2, 1 (TOB1) operates as a tumor suppressor and exhibits anti-proliferative properties that have the potential to regulate cell growth via decreasing the activation of AKT/mTOR signaling pathway (Wang et al. 2022). Guan et al. (2017) and Wang et al. (2019) have shown that the decreased TOB1 expression and increased phosphorylation of nuclear TOB1 promotes gastric cancer and is associated with aggressive tumor behavior and poor prognosis. Moreover, TOB1 modulates the radiosensitivity of lung cancer cells through the MAPK/ERK signaling pathway (Sun et al. 2013). It has also been identified as a potential target gene regulated by miR-25-3p and miR-32-5p (Wang et al. 2020; Zhao et al. 2021). It has been suggested that miR-25-3p and miR-32-5p regulate cellular functions via TOB1 also in breast cancer (Wang et al. 2020; Zhao et al. 2021).

The endoplasmic reticulum (ER) stress, as in many malignant tumors, is an important factor in glioblastoma growth and its metabolic reprogramming (Chevet et al. 2015; Almanza et al. 2019; Minchenko et al. 2002, 2021). Previous studies have shown that the knockdown of ERN1 (endoplasmic reticulum to nucleus signaling 1) significantly suppressed the glioblastoma cells proliferation, tumor growth *in vivo,* and response to chemotherapy through genome reprogramming, but it increased the invasiveness (Auf et al. 2010, 2013; Avril et al. 2017; Logue et al. 2018; Minchenko et al. 2021). 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; Ediriweera and Jayasena 2023).

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; Minchenko et al. 2021). The endoribonuclease activity of ERN1 is responsible for alternative splicing of the XBP1 (X-box binding protein 1) pre-mRNA encoding splice variant of XBP1 (XBP1s), which regulates the expression of chaperons and enzymes for degradation of unfolded proteins and restoration of folding as well as many other proteins (Hetz et al. 2020; Minchenko et al. 2021; Pelizzari-Raymundo et al. 2024). The protein kinase activity of ERN1 also plays an important role in ERN1 signaling and controls the expression of many genes (Auf et al. 2013; Minchenko et al. 2019, 2020, 2024a, b, c; Pelizzari-Raymundo et al. 2023). Recently, it has been shown that the protein kinase activity of ERN1 plays an important role in controlling the expression of homeobox genes associated with glioblastoma cells proliferation and invasion and that ERN1 knockdown increases their expression although the proliferation of these cells is inhibited (Minchenko et al. 2024b).

Thus, the ER is a significant factor in malignant tumor progression, metabolic reprogramming, and therapeutic resistance. However, there is still not enough data available concerning the regulation of the expression of *TOB1*, *TWIST1*, and *HBEGF* genes, especially after suppression of glioblastoma cells proliferation by different ways of the ERN1 knockdown.

The aim of the present study was to investigate the impact of inhibition of ERN1 endoribonuclease activity or both enzymatic activities (endoribonuclease and protein kinase) on the *TOB1*, *HBEGF*, and *TWIST1* gene expression in glioblastoma cells with the intent to reveal the role of ERN1 signaling in the regulation of expression of these genes as well as molecular mechanism of gene-specific regulation.

Materials and Methods

Cell lines and culture conditions. The U87MG glioblastoma 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. In this study, we used wild-type U87MG glioblastoma cells and three sublines of these cells described previously (Minchenko et al. 2024b). One was obtained by selection of stably transfected clones with overexpression of vector pcDNA 3.1 and used as control (control glioblastoma cells). The second subline was obtained by selection of stably transfected clones with overexpression of ERN1 dominant/negative construct in pcDNA 3.1 (dnERN1) having suppression of both the ERN1 protein kinase and endoribonuclease activities. The third subline has inhibited ERN1 endoribonuclease only by dnrERN1 constructs (Auf et al. 2013). The cells with dnERN1 and dnrERN1 have a lower proliferation rate and do not express a spliced variant of XBP1, a key transcription factor in ERN1 signaling (Auf et al. 2010, 2013; Minchenko et al. 2024c). 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 high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium as described previously (Minchenko et al. 2024b). All three sublines of U87MG glioblastoma cells were grown in the presence of geneticin (G418), while these cells carried empty vector pcDNA3.1 or dominant/negative ERN1 constructs. We also exposed the glioblastoma cells with inhibited ERN1 endoribonuclease activity with tunicamycin (500 ng/ml) to clarify the role of other signaling pathways of ER stress in the control of *TOB1*, *HBEGF*, and *TWIST1* genes expression.

Small interfering RNA knockdown experiments. The ERN1 and XBP1 mRNAs in U87MG glioblastoma cells were silenced with specific small interfering RNA (siRNA) mainly as described previously (Auf et al. 2013; Minchenko et al. 2024b). The ON-TARGETplus SMARTpool siRNA J-004951-(19-22) against human ERN1 (catalog: L-0049251-02-0005), the ON-TARGETplus siRNA against human XBP1 (5'-GCUCUUUCCCUCAUGUAUAC), and control siRNA (ON-TARGETplus Control Pool, Non-Targeting pool; catalog: D-001810-10-05) was received from Dharmacon, a Horizon Discovery Group Company. 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, against XBP1 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 were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. The expression levels of TOB1, HBEGF, TWIST1, ERN1, XBP1, and ACTB mRNA were measured in control U87MG cells and cells with a deficiency of ERN1 by quantitative PCR using SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described (Rudnytska et al. 2021). PCR 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 TOB1, HBEGF, and TWIST1 mRNAs was received from Sigma-Aldrich (St. Louis, MO, USA) and used for quantitative PCR: HBEGF forward 5'- tggtgctgtcatctgtctgt and HBEGF reverse 5'-gtctttcccctctgcagtct (NM_001945.3); TOB1 forward 5'-agcccgaacaagatcactca and TOB1 reverse 5'-cacgtctcctgggaagctta (NM_005749.4); TWIST1 forward 5'-aagcacaagacacactgcag and TWIST1 reverse 5'-ccacgccctgtttctttgaa (NM_000474.4).

Primers for ERN1, XBP1, and ACTB were described previously (Minchenko et al. 2024b). For polyadenylation and reverse transcription of microRNAs we used Mir-X miRNA First-Strand Synthesis Kit (Takara, Japan). Primers for microRNA miR-96-5p and miR-182-5p were described previously (Minchenko et al. 2024b).

The quantitative PCR analysis was performed using a special computer program "Differential Expression Calculator" and statistical analysis using GraphPad Prism8 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. A value of $p<0.05$ was considered significant in all cases. All experimental qPCR data were analyzed for the normality of distribution using a graphical tool (normal probability plot) and a histogram as described previously (Rudnytska et al. 2021). 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).

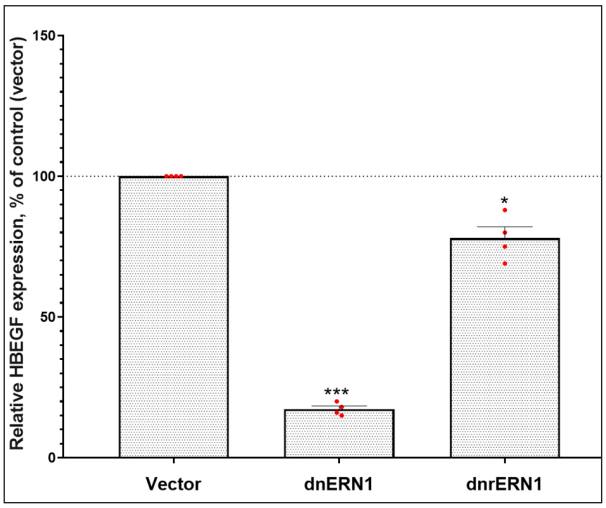

Figure 1. The expression of heparin binding epidermal growth factor like growth factor (HBEGF) in control U87MG glioblastoma cells (transfected by an empty vector; Vector), cells with suppressed endoribonuclease and protein kinase activities of ERN1 (dnERN1) or only endoribonuclease activity of ERN1 (dnrERN1) measured by quantitative RT-PCR. The values of this mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (Vector, 100%); mean±SEM; *p<0.05 and ***p<0.001 vs. control.

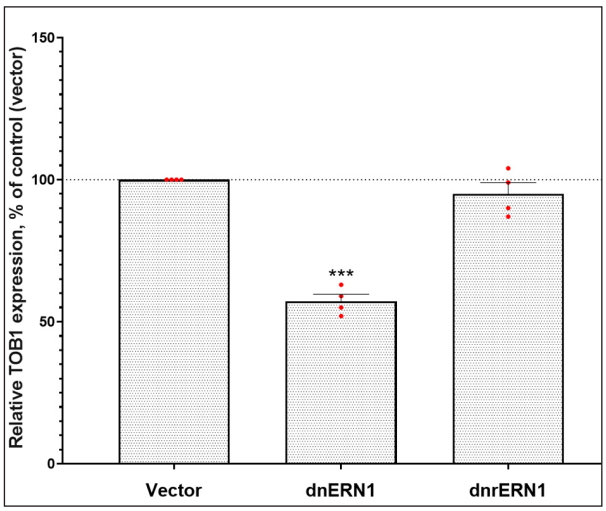

Figure 2. The expression of transducer of ERBB2, 1 (TOB1) in control U87MG glioblastoma cells (transfected by an empty vector; Vector), cells with suppressed endoribonuclease and protein kinase activities of ERN1 (dnERN1) or only endoribonuclease activity of ERN1 (dnrERN1) measured by quantitative RT-PCR. The values of this mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (Vector, 100%); mean±SEM; ***p<0.001 vs. control.

Results

The possible role of ER stress signaling mediated by ERN1 in regulating *HBEGF*, *TOB1*, and *TWIST1* gene expression in U87MG glioblastoma cells was evaluated. The expression level of the *HBEGF* gene was down-regulated (–22%; p<0.05) in glioblastoma cells with suppressed only endoribonuclease activity of the ERN1 signaling protein (Figure 1). At the same time, much stronger changes (–83%; p<0.001) were detected in the expression of this gene in glioblastoma cells with inhibited both enzymatic activities of ERN1 (protein kinase and endoribonuclease) in comparison to control (transfected by empty vector) cells (Figure 1).

We also studied the expression of the *TOB1* gene in the glioblastoma cells with ERN1 knockdown. As shown in Figure 2, the expression level of this pro-oncogenic gene is resistant to inhibition of endoribonuclease activity of ERN1 in glioblastoma cells, but is significantly decreased in cells with suppression of both enzymatic activities of ERN1 signaling protein (–43%; p<0.001). The expression of tumor suppressor gene *TWIST1* was also resistant to inhibition of ERN1 endoribonuclease activity in glioblastoma cells, but in cells with suppressed protein kinase and endoribonuclease activities of ERN1 signaling protein, the expression of this anti-proliferative gene was strongly up-regulated (+104%; p<0.001) (Figure 3).

As shown in Figure 4, the changes in the expression of *HBEGF* gene in U87MG glioblastoma cells after 48 h of XBP1 and ERN1 mRNAs silencing (–19 and –78%, correspondingly) were similar to that observed in cells with inhibited endoribonuclease activities of ERN1 and both enzymatic activities of this signaling protein (Figure 1). Moreover, the silencing of XBP1 mRNA does not significantly change the expression level of *TOB1* and *TWIST1* genes in glioblastoma cells (Figures 5 and 6). At the same time, in glioblastoma cells with ERN1 silencing, the expression level of the *TOB1* gene was decreased (–39%; p<0.01), while the level of the *TWIST1* gene was strongly up-regulated (+90%; p<0.001; Figures 5 and 6). As shown in Figure 7, ERN1 and XBP1 mRNAs were effectively suppressed (–87%; p<0.001 for ERN1 and –89%; p<0.001 for XBP1).

The impact of tunicamycin on the expression of *HBEGF*, *TOB1*, and *TWIST1* genes in U87MG glioblastoma cells with inhibited endoribonuclease activity of ERN1 was studied to clarify the role of other signaling pathways of ER stress in the control of the expression of these genes. As shown in Figure 8, tunicamycin significantly down-regulated the

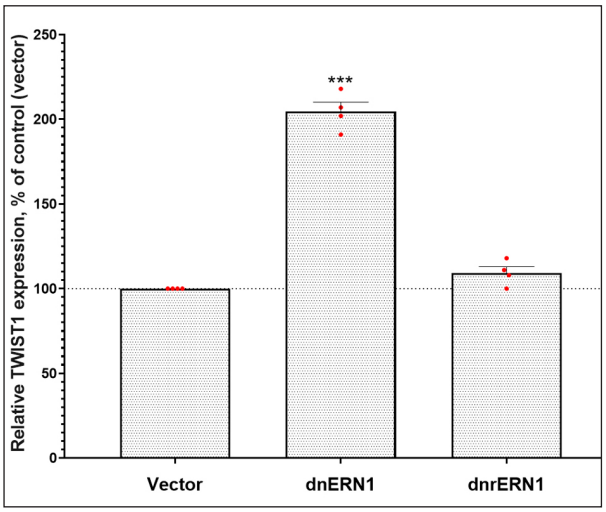

Figure 3. The expression of TWIST family bHLH transcription factor 1 (TWIST1) in control U87MG glioblastoma cells (transfected by an empty vector; Vector), cells with suppressed endoribonuclease and protein kinase activities of ERN1 (dnERN1) or only endoribonuclease activity of ERN1 (dnrERN1) measured by quantitative RT-PCR. The values of this mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (Vector, 100%); mean±SEM; ***p<0.001 vs. control.

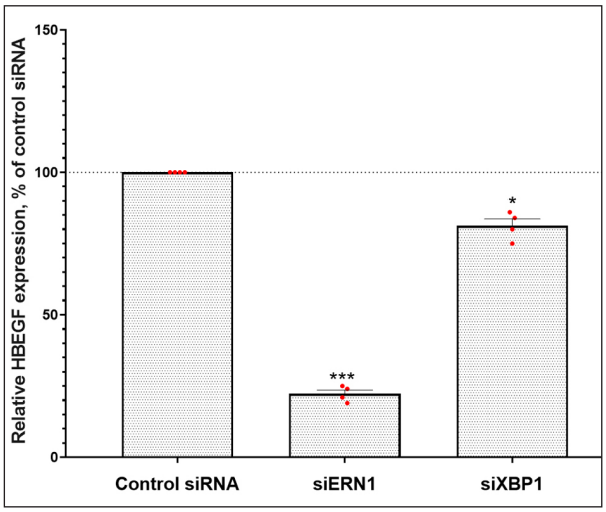

Figure 4. The impact of ERN1 and XBP1 mRNAs silencing by specific for ERN1 and XBP1 siRNAs (48 h) on the expression of HBEGF mRNA in wild-type glioblastoma cells (quantitative RT-PCR analysis). The values of mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (control siRNA; 100%); mean±SEM; *p<0.05 and ***p<0.001 vs. control siRNA.

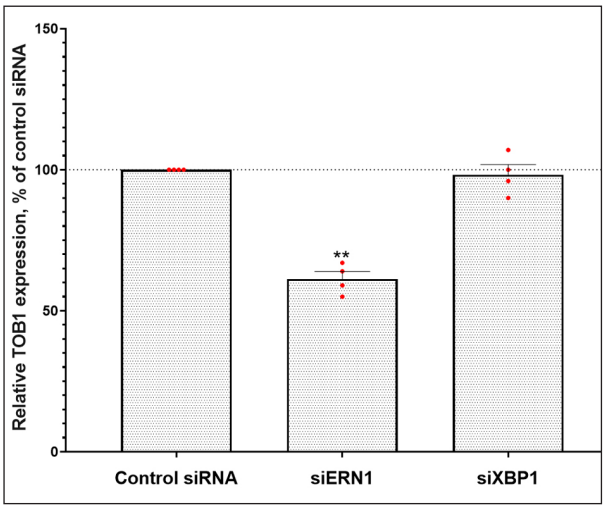

Figure 5. The impact of ERN1 and XBP1 mRNAs silencing by specific for ERN1 and XBP1 siRNAs (48 h) on the expression of TOB1 mRNA in wild-type glioblastoma cells (quantitative RT-PCR analysis). The values of mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (control siRNA; 100%); mean±SEM; **p<0.01 vs. control siRNA.

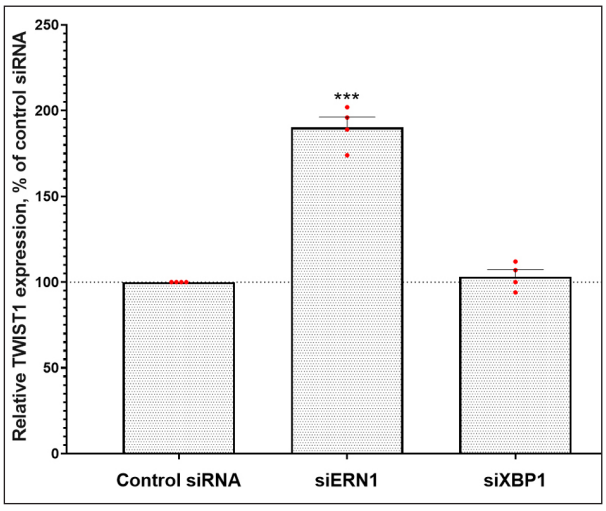

Figure 6. The impact of ERN1 and XBP1 mRNAs silencing by specific for ERN1 and XBP1 siRNAs (48 h) on the expression of TWIST1 mRNA in wild-type glioblastoma cells (quantitative RT-PCR analysis). The values of mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (control siRNA; 100%); mean±SEM; ***p<0.001 vs. control siRNA.

expression of *HBEGF* and *TOB1* genes in glioblastoma cells with inhibited ERN1 endoribonuclease activity, while strongly up-regulated *TWIST1* gene expression indicating the possible involvement of other ER stress signaling pathways in the control of *HBEGF*, *TOB1*, and *TWIST1* gene expression. Thus, the expression of *HBEGF*, *TOB1*, and *TWIST1* genes in glioblastoma cells can be controlled by ER stress

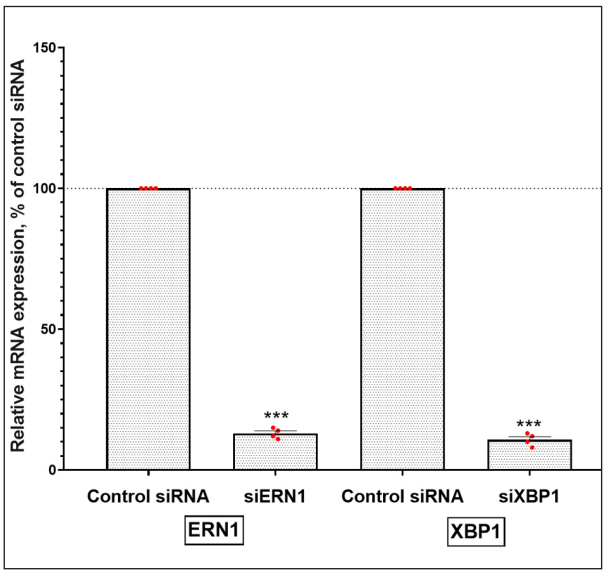

Figure 7. The impact of ERN1 and XBP1 mRNAs silencing by specific for ERN1 and XBP1 siRNAs (48 h) on the expression of ERN1 and XBP1 mRNAs in wild-type glioblastoma cells (quantitative RT-PCR analysis). The values of these mRNA expressions were normalized to beta-actin mRNA and represented as a percent of the control (control siRNA; 100%); mean±SEM; ***p<0.001 vs. control siRNA.

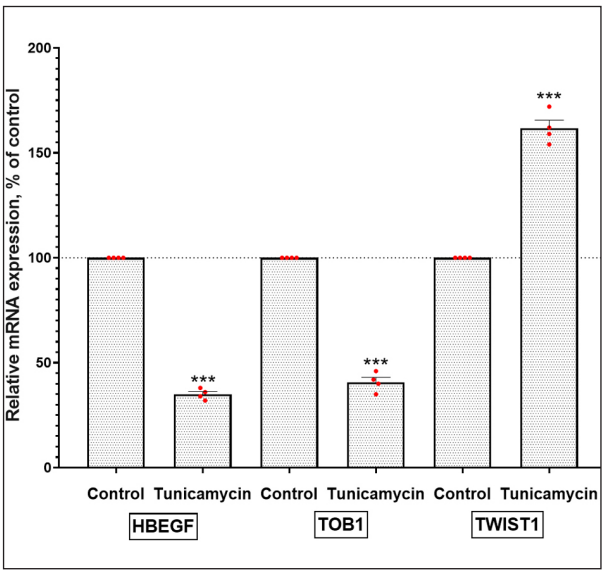

Figure 8. The impact of tunicamycin (500 ng/ml for 4 h) on the expression of HBEGF, TOB1, and TWIST1 mRNAs in glioblastoma cells without endoribonuclease activity of ERN1 (dnrERN1) measured by quantitative RT-PCR. The values of these mRNA expressions were normalized to beta-actin mRNA and represented as a percent of the control (dnrERN1 cells without tunicamycin; 100%); mean±SEM; ***p<0.001 vs. control.

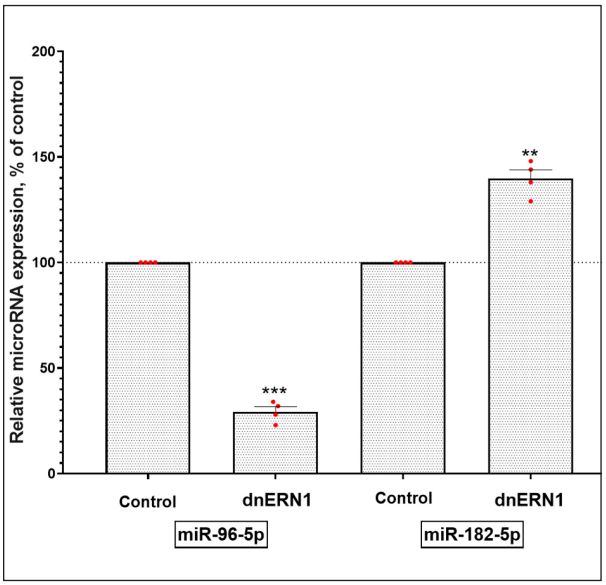

Figure 9. The expression of microRNA miR-96-5p and miR-182-5p in control U87MG glioblastoma cells (transfected by an empty vector; Vector), cells with suppressed endoribonuclease and protein kinase activities of ERN1 (dnERN1) or only endoribonuclease activity of ERN1 (dnrERN1) measured by quantitative RT-PCR. The values of this mRNA expression were normalized to beta-actin mRNA and represented as a percent of the control (Vector, 100%); mean±SEM; **p<0.01 and ***p<0.001 vs. control.

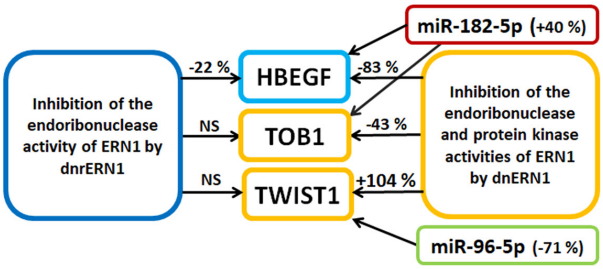

Figure 10. Schematic demonstration the changes in the expression levels of *HBEGF*, *TOB1*, and *TWIST1* genes in glioblastoma cells with inhibited ERN1 endoribonuclease activity by dnrERN1 and cells with inhibited both enzymatic activities of ERN1 (protein kinase and endoribonuclease) by dnERN1 as well as possible contribution of microRNAs in these gene expressions.

signaling pathways, especially by ERN1 signaling preferentially through ERN1 protein kinase activity in a gene-specific manner.

The binding sites for microRNA miR-182-5p in the 5'-untranslated region of HBEGF and TOB1 mRNAs and miR-96-5p – in mRNA was also identified. As shown in Figure 9, the inhibition of both endoribonuclease and protein kinase activities of ERN1 in glioblastoma cells led to a significant up-regulation

of mir-182-5p (+40 %; p<0.01) and down-regulation of miR-96-5p (–71%; p<0.001) indicating possible participation in the regulation of HBEGF, TOB1, and TWIST1 mRNAs.

Discussion

The results of this study are summarized in Figure 10. The major finding reported here is that the expression of the gene encoding the tumor suppressor protein TWIST1 was significantly up-regulated in U87MG glioblastoma cells via ERN1 protein kinase activity and relatively independent on the endoribonuclease activity of ERN1. The obtained data for the first time demonstrate the involvement of the ERN1 protein kinase in the expression level of the anti-proliferative gene increase under conditions of the signaling protein ERN1 inhibition, which is in good agreement with the data of other studies (Zhao et al. 2017; Pires et al. 2021; Pelizzari-Raymundo et al. 2023; Minchenko et al. 2024b; Peng et al. 2024; Xu et al. 2024). At the same time, the expression of the gene encoding the pro-oncogenic protein TOB1 was significantly down-regulated in U87MG glioblastoma cells also via ERN1 protein kinase activity and was relatively insensitive to inhibition of ERN1 endoribonuclease activity. These results agree well with the data concerning the biological function of TOB1 and its role in the cell proliferation control (Auf et al. 2010; Guan et al. 2017; Zhao et al. 2021; Wang et al. 2019, 2022; Minchenko et al. 2024c). Thus, suppression of cell proliferation induced by inhibition of ERN1 enzymatic activities is associated with down-regulation of pro-oncogenic TOB1 and up-regulation of tumor suppressor TWIST1 (Wang et al. 2019, 2022; Minchenko et al. 2024c; Peng et al. 2024; Xu et al. 2024).

We also showed that ERN1 knockdown down-regulated the HBEGF gene expression preferentially via ERN1 protein kinase activity, which agrees well with its pro-proliferative properties (Auf et al. 2010; Xiao et al. 2020; Park et al. 2022; Pelizzari-Raymundo et al. 2023; Minchenko et al. 2024c; Zhang et al. 2024). It is important to note that changes in *HBEGF*, *TOB1*, and *TWIST1* gene expression, received with ERN1 knockdown, were similar to those detected by silencing ERN1 and XBP1 mRNAs. This indicates for the relative independence of changes in gene expression on the method of ERN1 enzymatic activity suppression.

This study also demonstrated that inhibition of the endoribonuclease and protein kinase activities of ERN1 by dnERN1 in U87MG glioblastoma cells causes a decrease in the levels of miR-96-5p, but increases the expression levels of miR-182-5p. These changes in microRNAs may be responsible for increased expression of TWIST1 and decreased expression of TOB1 and HBEGF mRNAs. These results agree well with other data concerning the important role of microRNAs in the control of genes expression (Guo et al. 2019; Wang et al. 2020; Wu et al. 2021; Zhao et al. 2021; Minchenko et al. 2024b, c).

We identified the ERN1 protein kinase-dependent mechanism of the regulation of *HBEGF*, *TOB1*, and *TWIST1* gene expression, which are multifunctional proteins that play an important role in controlling the tumor cell proliferation and invasion (Zhou et al. 2014; Zhao et al. 2017; Wang et al. 2019, 2022; Minchenko et al. 2024a, b, c; Xu et al. 2024).

In conclusion. The data presented in this study provide unique insights into the molecular mechanisms controlling the expression of *HBEGF*, *TOB1*, and *TWIST1* genes in glioblastoma cells through ERN1 inhibition confirming the fact that ER stress is an essential factor of cancer growth and that of *HBEGF*, *TOB1*, and *TWIST1* genes participate in this process. However, the detailed molecular mechanisms of the interaction of the ERN1-mediated signaling pathways with gene expression and tumor cell proliferation warrant further study.

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

References

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

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

Bos PD, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, Minn AJ, van de Vijver MJ, Gerald WL, Foekens JA, Massague J. Genes that mediate breast cancer metastasis to the brain. Nature 459, 1005–1009, 2009.

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

Ediriweera MK, Jayasena S. The role of reprogrammed glucose metabolism in cancer. Metabolites 13, 345, 2023.

Guan R, Peng L, Wang D, He H, Wang D, Zhang R, Wang H, Hao H, Zhang J, Song H, Sui S, Meng X, Cui X, Bai J, Sun W, Fu S, Yu J. Decreased TOB1 expression and increased phosphorylation of nuclear TOB1 promotes gastric cancer. Oncotarget 8, 75243–75253, 2017.

Guo H, Zhang R, Afrifa J, Wang Y, Yu J. Decreased expression levels of DAL-1 and TOB1 are associated with clinicopathological features and poor prognosis in gastric cancer. Pathol Res Pract 215, 152403, 2019.

Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol 21, 421–438, 2020.

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

Minchenko 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 277, 6183–6187, 2002.

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 IRE1 knockdown U87 glioma cells: effect of hypoxia and glucose deprivation. Endocr Regul 54, 183–195, 2020.

Minchenko OH, Tsymbal DO, Khita OO, Minchenko DO. Inhibition of ERN1 signaling is important for the suppression of tumor growth. Clin Cancer Drugs 8, 27–38, 2021.

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 58, 206–214, 2024a.

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, 2024b.

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, 2024c.

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

Park SH, Yoon SJ, Choi S, Jung J, Park JY, Park YH, Seo J, Lee J, Lee MS, Lee SJ, Son MY, Cho YL, Kim JS, Lee HJ, Jeong J, Kim DS, Park YJ. Particulate matter promotes cancer metastasis through increased HBEGF expression in macrophages. Exp Mol Med 54, 1901–1912, 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.

Peng X, Ma L, Chen X, Tang F, Zong X. Inhibition of FBP1 expression by KMT5A through TWIST1 methylation is one of the mechanisms leading to chemoresistance in breast cancer. Oncol Rep 52, 110, 2024.

Pires BRB, Binato R, Ferreira GM, Correa S, Du Rocher B, Bulzico D, Crocamo S, Dos Santos EC, Lima LG, Abdelhay E. Twist1 influences the expression of leading members of the IL-17 signaling pathway in HER2-positive breast cancer cells. Int J Mol Sci 22, 12144, 2021.

Rudnytska OV, Khita OO, Minchenko DO, Tsymbal DO, Yefimova YV, Sliusar MY, Minchenko OH. The low doses of SWCNTs exhibit a genotoxic effect on the normal human astrocytes by disrupting the functional integrity of the genome. Curr Res Toxicol 2, 64–71, 2021.

Sun KK, Zhong N, Yang Y, Zhao L, Jiao Y. Enhanced radiosensitivity of NSCLC cells by transducer of erbB2.1 (TOB1) through modulation of the MAPK/ERK pathway. Oncol Rep 29, 2385–2391, 2013.

Wang D, Song H, Zhao T, Wang M, Wang Y, Yu L, Wang P, Yu J. Phosphorylation of TOB1 at T172 and S320 is critical for gastric cancer proliferation and progression. Am J Transl Res 11, 5227–5239, 2019.

Wang R, Huang Z, Qian C, Wang M, Zheng Y, Jiang R, Yu C. LncRNA WEE2-AS1 promotes proliferation and inhibits apoptosis in triple negative breast cancer cells via regulating miR-32-5p/TOB1 axis. Biochem Biophys Res Commun 526, 1005–1012, 2020.

Wang D, Li Y, Sui S, Cai M, Dong K, Wang P, Liang X, Fu S, Yu J. Involvement of TOB1 on autophagy in gastric cancer AGS cells via decreasing the activation of AKT/mTOR signaling pathway. PeerJ 10, e12904, 2022.

Wu YX, Lin YS, Li SC, Yao X, Cheng M, Zhu L, Liu HY. microRNA-194 is increased in polycystic ovary syndrome granulosa cell and induce KGN cells apoptosis by direct targeting heparin-binding EGF-like growth factor. Reprod Biol Endocrinol 19, 170, 2021.

Xiao W, Zheng S, Xie X, Li X, Zhang L, Yang A, Wang J, Tang H, Xie X. SOX2 promotes brain metastasis of breast cancer by upregulating the expression of FSCN1 and HBEGF. Mol Ther Oncolytics 17, 118–129, 2020.

Xu T, Yin F, Shi K. TMEM158 functions as an oncogene and promotes lung adenocarcinoma progression through the PI3K/AKT pathway via interaction with TWIST1. Exp Cell Res 437, 114010, 2024.

Zhang Y, Tang L, Liu H, Cheng Y. The multiple functions of HB-EGF in female reproduction and related cancer: Molecular mechanisms and targeting strategies. Reprod Sci 31, 2588–2603, 2024.

Zhao Z, Rahman MA, Chen ZG, Shin DM. Multiple biological functions of Twist1 in various cancers. Oncotarget 8, 20380–20393, 2017.

Zhao T, Meng W, Chin Y, Gao L, Yang X, Sun S, Pan X, He L. Identification of miR-25-3p as a tumor biomarker: Regulation of cellular functions via TOB1 in breast cancer. Mol Med Rep 23, 406, 2021.

Zhou ZN, Sharma VP, Beaty BT, Roh-Johnson M, Peterson EA, Van Rooijen N, Kenny PA, Wiley HS, Condeelis JS, Segall JE. Autocrine HBEGF expression promotes breast cancer intravasation, metastasis and macrophage-independent invasion in vivo. Oncogene 33, 3784–3793, 2014.