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Inhibition of signaling protein ERN1 increases the sensitivity of serine synthesis gene expressions to glucose and glutamine deprivations in U87MG glioblastoma cells

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Objective. Glucose and glutamine supply as well as serine synthesis and endoplasmic reticulum (ER) stress are important factors of glioblastoma growth. Previous studies showed that the knockdown of ERN1 (ER to nucleus signaling 1) suppressed glioblastoma cell proliferation and modified the sensitivity of numerous gene expressions to nutrient deprivations. The present study is aimed to investigate the impact of glucose and glutamine deprivations on the expression of serine synthesis genes in U87MG glioblastoma cells in relation to ERN1 knockdown with the intent to reveal the role of ERN1 signaling pathway on the ER stress-dependent regulation of these gene expressions. Clarification of the regulatory mechanisms of serine synthesis is a great significance for glioblastoma therapy.

Methods. The control U87MG glioblastoma cells (transfected by empty vector) and ERN1 knockdown cells (transfected by dominant-negative ERN1) were exposed under glucose and glutamine deprivation conditions for 16 h. RNA was extracted from cells and reverse transcribed. The expression level of *PHGDH* (phosphoglycerate dehydrogenase), *PSAT1* (phosphoserine aminotransferase 1), *PSPH* (phosphoserine phosphatase), *ATF4* (activating transcription factor 4), and *SHMT1* (serine hydroxymethyltransferase 1) genes was studied by real-time qPCR and normalized to ACTB.

Results. It was found that the expression level of genes responsible for serine synthesis such as *PHGDH*, *PSAT1*, *PSPH*, and transcription factor *ATF4* was up-regulated in U87MG glioblastoma cells under glucose and glutamine deprivations. Furthermore, inhibition of ERN1 significantly enhances the impact of glucose and especially glutamine deprivations on these gene expressions. At the same time, the expression of the *SHMT1* gene, which is responsible for serine conversion to glycine, was down-regulated in both nutrient deprivation conditions with more significant changes in ERN1 knockdown glioblastoma cells.

Conclusion. Taken together, the results of present study indicate that the expression of genes responsible for serine synthesis is sensitive to glucose and glutamine deprivations in gene-specific manner and that suppression of ERN1 signaling significantly modifies the impact of both glucose and glutamine deprivations on *PHGDH*, *PSAT1*, *PSPH*, *ATF4*, and *SHMT1* gene expressions and reflects the ERN1-mediated genome reprograming introduced by nutrient deprivation condition.

Key words: ERN1 knockdown, glucose deprivation, glutamine deprivation, PSAT1, PSPH, ATF4, gene expression, U87MG glioblastoma cells

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Glucose and glutamine as substrate for glycolysis as well as endoplasmic reticulum (ER) stress are very important and complementary factors to the development and for a more aggressive behavior of glioblastomas, which are highly aggressive tumors with poor prognosis (Lazo 1981; Colombo et al. 2011; Papaioannou and Chevet 2018; Ediriweera and Jayasena 2023). Glutamine is the most abundant amino acid in tissues and represents an important substrate for tumor cell metabolism (Drogat et al. 2007). Reprogrammed metabolism of glucose in malignant tumor cells is associated with cell proliferation, apoptosis, chemoresistance, and invasion. The serine synthesis pathway is often increased in tumors because it is essential for propagation and therapeutic resistance in various cancer cells (Vie et al. 2008; Engel et al. 2020; Rathore et al. 2020; Hennequart et al. 2021). It is important to note that the ER stress signaling pathways are responsible for numerous metabolic reprogramming and adaptations of tumor cells to withstand their malignancy, including an increased serine metabolism (Bravo et al. 2013; Chevet et al. 2015; Yang and Voudsen 2016; Papaioannou and Chevet 2018; Hetz et al. 2020). In many cases, extracellular serine alone is sufficient to support cancer cell proliferation, but some cancer cells need de novo serine synthesis from glucose even in the presence of abundant extracellular serine (Possemato et al. 2011; Mattaini et al. 2016; Tajan et al. 2021).

Previous studies have shown that the knockdown of ERN1/IRE1α (ER to nucleus signaling 1/inositol-requiring enzyme 1α) significantly repressed glioblastoma cell proliferation and tumor growth *in vivo* via genome reprogramming as well as enhances of chemotherapy response (Auf et al. 2010, 2013; Avril et al. 2017; Logue et al. 2018; Minchenko 2021a). The ERN1 has two enzymatic activities: endoribonuclease and protein kinase. The central role of ERN1 endoribonuclease is unusual splicing of the XBP1 (X-box binding protein 1) mRNA and creation of an active transcription factor, which controls the expression of many genes encoding proteins for degradation of unfolded proteins and protein folding (Acosta-Alvear et al. 2007; Hetz et al. 2020). Furthermore, ERN1 endoribonuclease is also responsible for the selective degradation of mRNAs by special RIDD (ERN1-dependent decay of mRNA) mechanism (Hollien et al. 2007; Maurel et al. 2014).

The protein kinase of ERN1 is also involved in the regulation of gene expression (Auf et al. 2013; Minchenko et al. 2015, 2019, 2020). Thus, the expression of epiregulin (*EREG*), endothelin 1 (*EDN1*), activating transcription factor 3 (*ATF3*) and other genes is controlled by ERN1 protein kinase. It is interesting to note that transcription factor ATF3 promotes the expression of serine biosynthesis genes by binding to the promoters of *PHGDH* (phosphoglycerate dehydrogenase), *PSAT1* (phosphoserine aminotransferase 1), and *PSPH* (phosphoserine phosphatase) genes (Li et al. 2021; Luo et al. 2022).

A basic characteristic of tumor cells is metabolic reprogramming, which is linked with glycolysis activation through PFKFBs and *de novo* synthesis of serine, an important branch of glycolysis (Minchenko et al. 2002, 2005; Possemato et al. 2011; Labuschagne et al. 2014). Serine is a non-essential amino acid that is critical for tumor proliferation and the reduction of circulating serine results in reduced cancer growth and increased survival in various cancers (Engel et al. 2020; Hennequart et al. 2021). In serine synthesis, three important metabolic enzymes such as PHGDH, PSAT1, and PSPH are involved. The PHGDH is a rate-limiting enzyme for the de novo synthesis of serine, which is highly expressed in a variety of cancers (Reid et al. 2018; Itoyama et al. 2021). It is necessary for the rapid growth of tumor cells and chemotherapy resistance (Vie et al. 2008; Jin et al. 2022). It was also shown that over-expressed PSAT1 stimulates cell growth and increases chemoresistance of cancer cells via the modulation of the cell cycle and that microRNA-145-5p represses cancer cell proliferation and cell cycle progression through targeting of PSAT1, which enhances the proliferation of cancer cells through cyclin D1 (Vie et al. 2008; Gao et al. 2017; Ding et al. 2022). The PSPH enzyme catalyzes the last irreversible step in the biosynthesis of serine and also promotes cancer development, cell proliferation and metastasis via an independent from L-serine synthesis pathways through the dephosphorylation of IRS1 (insulin receptor substrate 1) and MAPK signaling pathways (Liao et al. 2019; Park et al. 2019). An important role in the control of serine level in the cell plays also serine hydroxymethyltransferase 1, which is responsible for the reversible conversion of serine to glycine (Pikman et al. 2022).

The transcription factor ATF4 has relation to the control of serine synthesis through the regulation of *PSAT1* gene expression (Gao et al. 2017). Furthermore, it is the main regulator of the mitochondrial stress response and possibly controls ER stress dependent *PSAT1* gene expression (Quiros et al. 2017). Furthermore, there are data that ATF4 participates in the control of neuroblastoma cell death upon glutamine deprivation (Qing et al. 2012).

Thus, the synthesis of serine is an important factor of most malignant tumor progression, metabolic

reprogramming, and therapeutic resistance, but there is no data as of yet concerning sensitivity of serine synthesis gene expressions to glucose and glutamine deprivations and its dependence on ERN1 activity. In this study, we have demonstrated that the expression level of serine synthesis genes was up-regulated by glucose and especially glutamine deprivations in U87MG glioblastoma cells in an ERN1-dependent manner. Significant changes in the expression of serine synthesis genes in ERN1 knockdown cells under nutrient deprivation may reflect the metabolic reprogramming of these cells introduced by inhibition of ERN1 signaling.

Materials and Methods

Cell lines and culture conditions. In this work, we used two sublines of stably transfected U87MG glioblastoma cells (control, transfected by empty vector, and ERN1 knockdown, transfected by dnERN1), which were kindly provided by Prof. M. Moenner (University de Bordeaux, Talence, France) and grown in high glucose Dulbecco's modified Eagle's minimum essential medium (DMEM) (Sigma-Aldrich, St. Louis, MO, U.S.A.) in the presence of geneticin (G418) as described previously (Auf et al. 2013). Previously, it has been shown that glioblastoma cells with dnERN1 have a significantly lower proliferation rate, do not express spliced variant of XBP1 (XBP1s), a key transcription factor in ERN1 signaling, and do not have the phosphorylated isoform of ERN1 after induction of ER stress by tunicamycin (Auf et al. 2010, 2013; Minchenko et al. 2015, 2021a).

Glucose and glutamine deprivation conditions were created by changing the complete DMEM medium in the culture plates (Thermo Fisher Scientific, Waltham, U.S.A.) on DMEM medium without glucose or glutamine, and plates were exposed to this condition for 16 h. The impact of glucose and glutamine deprivations on the expression of genes encoding enzymes and transcription factor ATF4, which are responsible for serine synthesis, in control and ERN1 knockdown glioblastoma cells was compared with corresponding control.

RNA isolation. Total RNA was extracted from glioblastoma cells using the TRIZOL reagent according to manufacturer's protocols (Invitrogen, Carlsbad, CA, USA) essentially as described previously (Bobarykina et al. 2006; Rudnytska et al. 2021). The RNA pellets were washed with 75% ethanol and dissolved in ribonuclease-free water. For additional purification RNA samples were re-precipitated with 95% ethanol with sodium acetate, washed with 75% ethanol, and re-dissolved again in ribonuclease-free water. An RNA quantity and spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis of mRNA expression. For the synthesis of cDNA from total RNA we used Thermo Scientific Verso cDNA Synthesis Kit (Germany) as described previously (Rudnytska et al. 2021). The expression of *PHGDH*, *PSAT1*, *PDPH*, *SHMT1*, *ATF4*, and *ACTB* mRNAs was studied by real-time qPCR using "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). The values of beta-actin mRNA were used for normalization of studied mRNA expressions. The results represented as percent of control (vector, 100%). The impact of glucose and glutamine deprivations on the expression of genes responsible for serine synthesis in control and ERN1 knockdown glioblastoma cells was compared to these non-treated cells. Polymerase chain reaction was performed in triplicate. Specific for each mRNA

Table 1
Characteristics of the primers used for quantitative real-time polymerase chain reaction

Gene symbol	Gene name	Primer's sequence	Nucleotide number in sequence	GeneBank accession number
PHGDH	Phosphoglycerate dehydrogenase	F: 5'- tcagttcgtggacatggtga R: 5'- tctttcaggaggccgacaat	992-1011 1231-1212	NM_006623.4
PSAT1	Phosphoserine aminotransferase 1	F: 5'- tgtcaagggagcagtactgg R: 5'- gcctgcaccttgtattccag	587-606 787-768	NM_021154.5
PSPH	Phosphoserine phosphatase	F: 5'- agcactggagaaacgaggaa R: 5'- agagcagctttgaaaggcac	566-585 783-764	NM_004577.4
SHMT1	Serine hydroxymethyltransferase 1	F: 5'- tctgccacgtccatcttctt R: 5'- cgggcatattccaggtttcg	679-698 836-817	NM_004169.5
ATF4	Activating transcription factor 4	F: 5'- gtccctccaacaacagcaag R: 5'- actttctgggagatggccaa	1093–1112 1328–1309	NM_005749.4
ACTB	beta-actin	F: 5'- catccgcaaagacctgtacg R: 5'- cctgcttgctgatccacatc	948–967 1165–1146	NM_001101.5

primers were received from Sigma-Aldrich (St. Louis, MO, U.S.A.) and used for quantitative polymerase chain reaction (Table 1).

Data and statistical analysis. The analysis of all quantitative PCR data was performed using a special computer program "Differential expression calculator" and statistical analysis using GraphPad Prism8 program. The values of studied genes 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 3 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 3% agarose gel and then visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

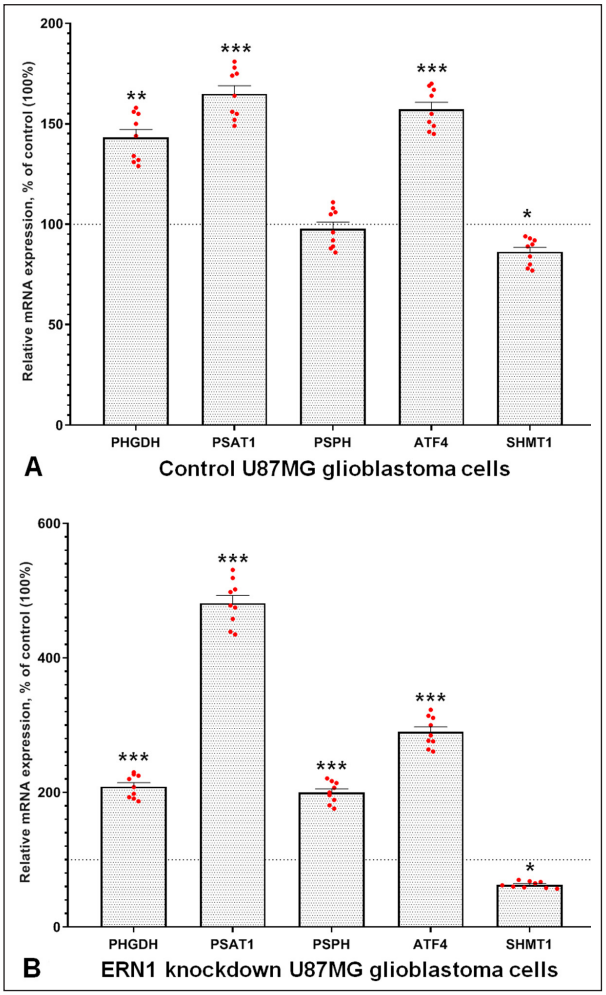


Figure 1. The impact of glucose deprivation on the expression level of PHGDH (phosphoglycerate dehydrogenase), PSAT1 (phosphoserine aminotransferase 1), PSPH (phosphoserine phosphatase), ATF4 (activating transcription factor 4), and SHMT1 (serine hydroxymethyltransferase 1) in control glioblastoma cells (stable transfected by empty vector **(A)** and glioblastoma cells with ERN1/IRE1α knockdown by dnERN1 **(B)** measured by qPCR. The mRNA expression values were normalized to beta-actin mRNA and represented as percent of control (non-treated cells; 100%). Data are presented as mean±SEM; *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. control.

Results

A possible role of the ER stress signaling mediated by ERN1 in the regulation of *PHGDH*, *PSAT1*, *PSPH*, *SHMT1*, and *ATF4* gene expressions in control and ERN1 knockdown glioblastoma cells under glucose and glutamine deprivations was evaluated.

The impact of glucose deprivation on the expression of PHGDH, PSAT1, PSPH, ATF4, and SHMT1 mRNAs in control and ERN1 knockdown U87MG glioblastoma cells. As shown in Figure 1A, the expression of PHGDH mRNA is up-regulated (+43%; $p<0.01$) in control glioblastoma cells exposed to glucose deprivation in comparison with control (non-treated) cells. At the same time, the expression level of this mRNA in glioblastoma cells with knockdown of ERN1 is increased to a much greater extent (+109%; $p<0.001$) in comparison with corresponding control (non-treated ERN1 knockdown cells) (Figure 1B). More pronounced changes were observed in PSAT1 mRNA expression (+65%; $p<0.001$) in control glioblastoma cells exposed to glucose deprivation (Figure 1A). As shown in Figure 1B, the inhibition of ERN1 signaling strongly enhances the effect of glucose deprivation (+385%; $p<0.001$) as compared to non-treated ERN1 knockdown cells. At the same time, glucose deprivation of control glioblastoma cells did not significantly change the expression level of PSPH mRNA, but in cells with suppressed ERN1, we detected a strong increase in the expression of this mRNA (+100%; $p<0.001$) (Figures 1A and 1B). Furthermore, we have shown that the expression level of transcription factor ATF4 mRNA is also up-regulated (+57%; $p<0.001$) in control glioblastoma cells after glucose deprivation (Figure 1A). However, higher induction of ATF4 mRNA expression (+188%; $p<0.001$) was observed in U87MG glioblastoma cells with suppressed ERN1 after glucose deprivation (Figure 1B). Next, we studied the expression of SHMT1 mRNA in

both types of glioblastoma cells and detected down-regulation of this mRNA expression in control and ERN1 knockdown glioblastoma cells (–14 and –37%, respectively; both $p<0.05$) compared to corresponding controls (Figures 1A and 1B).

The impact of glutamine deprivation on the expression of PHGDH, PSAT1, PSPH, ATF4, and SHMT1 mRNAs in control and ERN1 knockdown U87MG glioblastoma cells. Glutamine deprivation induced more significant changes in the expression of genes controlled serine synthesis and metabolism in both control and ERN1 knockdown glioblastoma cells. Thus, the expression level of PHGDH mRNA is increased (+145%; $p<0.001$) in control glioblastoma cells upon glutamine deprivation in comparison with non-treated control cells (Figure 2A). Significantly higher impact of glutamine deprivation on the expression level of PHGDH mRNA was observed in cells with ERN1 knockdown (+264%; $p<0.001$) as compared to non-treated ERN1 knockdown cells (Figure 2B). Even more pronounced changes were induced by glutamine deprivation on the expression of PSAT1 mRNA (+170%; $p<0.001$) in control glioblastoma cells (Figure 2A). The expression of PSAT1 mRNA became more distinct in ERN1 knockdown glioblastoma cells under the condition of glutamine deprivation (+477%; $p<0.001$) (Figure 2B).

Figures 2A and 2B also presents the expression data of PSPH mRNA in control and ERN1 knockdown glioblastoma cells after glutamine deprivation. A significant increase of this mRNA expression was found in both type of U87MG cells upon glutamine deprivation, but in cells with suppressed ERN1 we detected a stronger induction of this gene expression: +96% ($p<0.001$) in control cells and +183% ($p<0.001$) in ERN1 knockdown cells (Figures 2A and 2B). Furthermore, we analysed the expression of ATF4 mRNA in both types of glioblastoma cells exposure under glutamine deprivation condition. As shown in Figure 2A, treatment of control glioblastoma cells by glutamine deprivation also led to up-regulation of ATF4 mRNA expression (+99%; $p<0.001$). However, more marked changes in the expression of ATF4 mRNA (+165%; $p<0.001$) were establish in glioblastoma cells with ERN1 knockdown under glutamine deprivation (Figure 2B). At the same time, the expression level of SHMT1 mRNA was down-regulated in both control and ERN1 knockdown glioblastoma cells after glutamine deprivation compared to corresponding controls (–88% in control glioblastoma cells and –70% in ERN1 knockdown glioblastoma cells; both $p<0.001$) (Figures 2A and 2B).

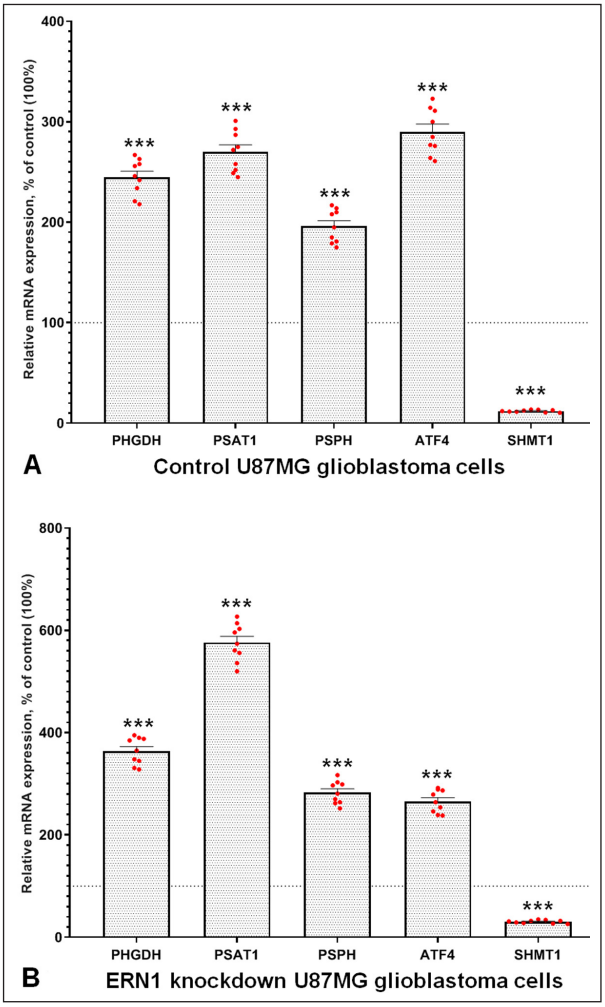


Figure 2. The impact of glutamine deprivation on the expression level of PHGDH (Phosphoglycerate dehydrogenase), PSAT1 (Phosphoserine aminotransferase 1), PSPH (Phosphoserine phosphatase), ATF4 (Activating transcription factor 4), and SHMT1 (Serine hydroxymethyltransferase 1) in control glioblastoma cells (transfected by empty vector) **(A)** and glioblastoma cells with ERN1/IRE1α knockdown by dnERN1 **(B)** measured by qPCR. The mRNA expression values were normalized to beta-actin mRNA and represented as percent of control (non-treated cells; 100%). Data are presented as mean±SEM; ***$p<0.001$ vs. control.

Discussion

The results of this study are summarized in Figure 3. The major finding reported here is that the expression of genes encoding the PHGDH and PSAT1, key enzymes of serine biosynthesis, are up-regulated in control and ERN1 knockdown U87MG glioblastoma cells under glucose and glutamine deprivations and that more significant changes were observed in glioblastoma cells upon

glutamine deprivation. Furthermore, we have also shown that the expression of transcription factor ATF4, which controls *PSAT1* gene expression, is also increased by glucose and glutamine deprivations in both control and ERN1 knockdown U87MG glioblastoma cells in an ERN1-dependent manner. The results agree well with the prooncogenic role of these genes, which are responsible for serine synthesis and play an important role in tumor growth, cell survival, metastasis, and chemoresistance (Vie et al. 2008; Qing et al. 2012; Gao et al. 2017; Engel et al. 2020; Rathore et al. 2020; Zhao et al. 2020; Hennequart et al. 2021; Itoyama et al. 2021; Ding et al. 2022). Moreover, the results of this study clearly demonstrated that glioblastoma cells are sensitive to glucose and glutamine deprivations in an ERN1-dependent manner and that both glucose and glutamine as substrates for glycolysis are very important factors for glioblastoma cells together with ER stress, which is consistent with the data on their importance for tumor development and for a more aggressive behavior of glioblastomas (Lazo 1981; Drogat et al. 2007; Colombo et al. 2011; Almanza et al. 2019; Ediriweera and Jayasena 2023).

In this study, we have shown that the expression level of genes responsible for serine synthesis was up-regulated by glucose and glutamine deprivations in U87MG cells preferentially in an ERN1-dependent manner and that these changes possibly reflect the metabolic reprogramming of glioblastoma cells by this type of nutrient deficiency. These results are consistent with numerous data regarding the important role of glucose and glutamine as well as serine synthesis and ER stress in tumor growth, metastasis, cell survival, and chemoresistance (Colombo et al. 2011; Papaioannou and Chevet 2018; Almanza et al. 2019; Engel et al. 2020; Minchenko et al. 2020, 2021a, b; Rathore et al. 2020; Ediriweera and Jayasena 2023). Therefore, Hennequart et al. (2021) demonstrate that non-essential amino acid serine is important for cancer cell proliferation and that depletion of this amino acid results in reduced malignant tumor growth. Many cancer cells are highly dependent on serine because this amino acid supports numerous metabolic processes, which are important for cell proliferation and survival of malignant cells, including glutathione and nucleotide

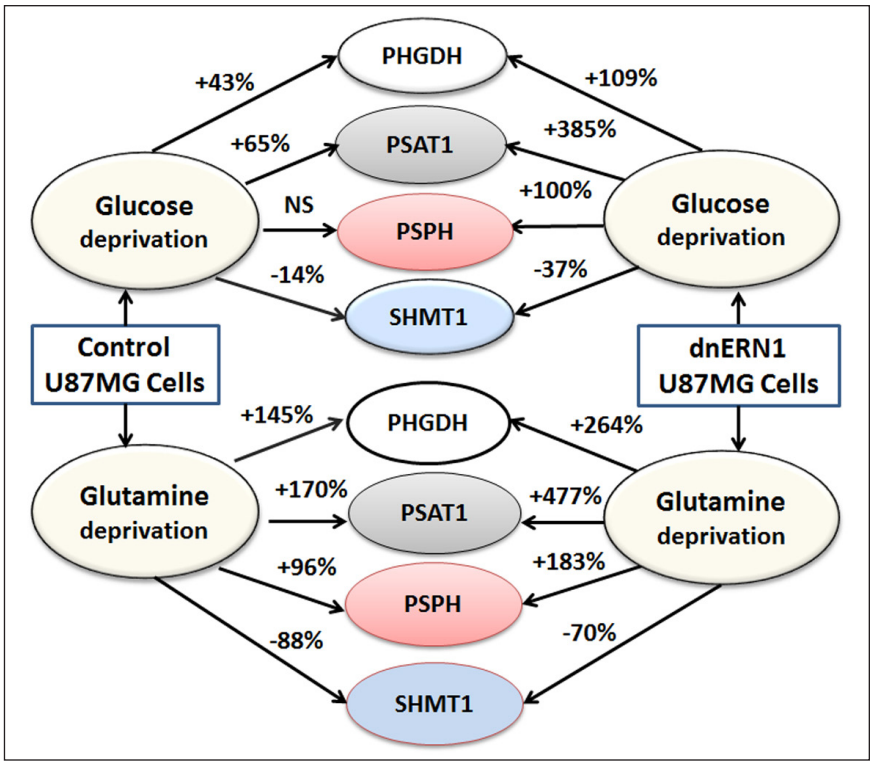


Figure 3. Schematic demonstration of changes in the expression profile of *PHGDH*, *PSAT1*, *PSPH*, *SHMT1*, and *ATF4* genes introduced by glucose and glutamine deprivations in control glioblastoma cells (stable transfected by empty vector) and ERN1 knockdown glioblastoma cells (dnERN1) in comparison to corresponding non-treated control cells with empty vector or dnERN1.

synthesis, and contributes to methylation reactions as well as the generation of NADPH (Possemato et al. 2011; Reid et al. 2018; Engel et al. 2020; Luo et al. 2021).

We have also shown that the expression of the *SHMT1* gene, which catalyzes the conversion of serine to glycine, is meaningfully down-regulated in both control glioblastoma cells and cells with ERN1 knockdown upon glutamine deprivation condition. These data may contribute to the up-regulation of serine level upon glutamine deprivation. Recently, we have shown that glutamine deprivation down-regulated the expression of endothelin 1 (EDN1) and its receptor (EDNRA) in both control and ERN1 knockdown glioblastoma cells (Minchenko et al. 2021b). Furthermore, our data concerning up-regulation of the expression level of transcription factor ATF4 both upon glucose and glutamine deprivation conditions correlate with strong induction of PSAT1 gene expression. These results are consistent with those published by Gao et al. (2017). They have demonstrated that ATF4 is responsible for the activation of *PSAT1* gene transcription. Furthermore, enhanced expression of ATF4 in these cells also agrees well with the data of Qing et al. (2012), who have found that ATF4 participates in the control of neuroblastoma cell death upon glutamine deprivation. There are data that ATF3 also promotes the serine synthesis pathway and tumor growth and its expression is also ERN1-dependent in glioblastoma cells (Minchenko et al. 2015; Li et al. 2021; Luo et al. 2022).

It is possible that glucose and glutamine deprivation conditions reprogram the serine biosynthesis, which strongly enhances in various cancer cells, and that ERN1 signaling is very important for the control of serine synthesis genes under nutrient deprivation, especially glutamine deprivation (Reid et al. 2018; Engel et al. 2020; Li and Ye 2020; Hennequart et al. 2021). The results of this investigation are important for the evaluation of the possible significance of genes responsible for serine synthesis and its regulation in ERN1-dependent control of glioblastoma growth by nutrient supply, particularly by glucose and glutamine. Furthermore, the detailed mechanisms of ERN1 mediated control of the expression of serine synthesis genes are not clear yet and warrant further deeper investigations, because this ER stress signaling pathway is involved in many metabolic pathways and ERN1 inhibition had clear anti-tumor effects (Auf et al. 2010, 2013; Avril et al. 2017; Minchenko et al. 2021a). This study provides unique insights into the molecular mechanisms controlling the expression of serine synthesis genes in glioblastoma cells under glucose and glutamine deprivations in response to knockdown of ERN1, confirming to the fact that ER stress is an essential factor of cancer growth. In this regards, ERN1 as a major signaling pathway of ER stress is an important target for glioblastoma therapy (Auf et al. 2010, 2013; Avril et al. 2017; Obacz et al. 2017; Logue et al. 2018; Almanza et al. 2019; Minchenko et al. 2021a).

In conclusion, the data presented in this study demonstrate that glucose and glutamine deprivations up-regulate the expression of genes responsible for serine biosynthesis (*PHGDH*, *PSAT1*, and *PSPH*) and its regulation (*ATF4*) in U87 glioblastoma cells in the gene-specific manner and that knockdown of ERN1 signaling protein function strongly enhances this effect of nutrient deprivation and reflects the ERN1-mediated genome reprograming, which is needed for cell surviving. Furthermore, the impact of glucose and glutamine deprivations on the expression of *SHMT1* gene, which is responsible for conversion of serine to glycine, has opposite direction and possibly contributes to the level of serine in the cells.

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

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.

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.

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

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

Colombo SL, Palacios-Callender M, Frakich N, Carcamo S, Kovacs I, Tudzarova S, Moncada S. Molecular basis for the differential use of glucose and glutamine in cell proliferation as revealed by synchronized HeLa cells. Proc Natl Acad Sci USA 108, 21069–21074, 2011.

Ding R, Hong W, Huang L, Shao J, Yu W, Xu X. Examination of the effects of microRNA-145-5p and phosphoserine aminotransferase 1 in colon cancer. Bioengineered 13, 12794–12806, 2022.

Drogat B, Bouchecareilh M, North S, Petibois C, Deleris G, Chevet E, Bikfalvi A, Moenner M. Acute L-glutamine deprivation compromises VEGF-a upregulation in A549/8 human carcinoma cells. J Cell Physiol 212, 463–472, 2007.

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

Engel AL, Lorenz NI, Klann K, Munch C, Depner C, Steinbach JP, Ronellenfitsch MW, Luger AL. Serine-dependent redox homeostasis regulates glioblastoma cell survival. Br J Cancer 122, 1391–1398, 2020.

Gao S, Ge A, Xu S, You Z, Ning S, Zhao Y, Pang D. PSAT1 is regulated by ATF4 and enhances cell proliferation via the GSK3beta/beta-catenin/cyclin D1 signaling pathway in ER-negative breast cancer. J Exp Clin Cancer Res 36, 179, 2017.

Hennequart M, Labuschagne CF, Tajan M, Pilley SE, Cheung EC, Legrave NM, Driscoll PC, Vousden KH. The impact of physiological metabolite levels on serine uptake, synthesis and utilization in cancer cells. Nat Commun 12, 6176, 2021.

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

Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol 186, 323–331, 2009.

Itoyama R, Yasuda-Yoshihara N, Kitamura F, Yasuda T, Bu L, Yonemura A, Uchihara T, Arima K, Hu X, Jun Z, Okamoto Y, Akiyama T, Yamashita K, Nakao Y, Yusa T, Kitano Y, Higashi T, Miyata T, Imai K, Hayashi H, Yamashita YI, Mikawa T, Kondoh H, Baba H, Ishimoto T. Metabolic shift to serine biosynthesis through 3-PG accumulation and PHGDH induction promotes tumor growth in pancreatic cancer. Cancer Lett 523, 29–42, 2021.

Jin L, Kiang KM, Cheng SY, Leung GK. Pharmacological inhibition of serine synthesis enhances temozolomide efficacy by decreasing O(6)-methylguanine DNA methyltransferase (MGMT) expression and reactive oxygen species (ROS)-mediated DNA damage in glioblastoma. Lab Invest 102, 194–203, 2022.

Labuschagne CF, van den Broek NJ, Mackay GM, Vousden KH, Maddocks OD. Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. Cell Rep 7, 1248–1258, 2014.

Lazo PA. Amino acids and glucose utilization by different metabolic pathways in ascites-tumor cells. Eur J Biochem 117, 19–25, 1981.

Li AM, Ye J. Reprogramming of serine, glycine and one-carbon metabolism in cancer. Biochim Biophys Acta Mol Basis Dis 1866, 165841, 2020.

Li X, Gracilla D, Cai L, Zhang M, Yu X, Chen X, Zhang J, Lon X, Ding H-F, Yan C. ATF3 dietary serine restriction. Cell Rep 36, 109706, 2021.

Liao L, Ge M, Zhan Q, Huang R, Ji X, Liang X, Zhou X. PSPH mediates the metastasis and proliferation of non-small cell lung cancer through MAPK signaling pathways. Int J Biol Sci 15, 183–194, 2019.

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.

Luo X, Ge J, Liu J, Liu Z, Bi C, Lan S. TFCP2, a binding protein of ATF3, promotes the progression of glioma by activating the synthesis of serine. Exp Cell Res 416, 113136, 2022.

Mattaini KR, Sullivan MR, Vander Heiden MG. The importance of serine metabolism in cancer. J Cell Biol 214, 249–257, 2016.

Maurel M, Chevet E, Tavernier J, Gerlo S. Getting RIDD of RNA: IRE1 in cell fate regulation. Trends Biochem Sci 39, 245–254, 2014.

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 OH, Ochiai A, Opentanova IL, Ogura T, Minchenko DO, Caro J, Komisarenko SV, Esumi H. Overexpression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 in the human breast and colon malignant tumors. Biochimie 87, 1005-1010, 2005.

Minchenko OH, Tsymbal DO, Moenner M, Minchenko DO, Kovalevska OV, Lypova NM. Inhibition of the endoribonuclease of ERN1 signaling enzyme affects the expression of proliferation-related genes in U87 glioma cells. Endoplasm Reticul Stress Dis 2, 18–29, 2015.

Minchenko DO, Tsymbal DO, Riabovol OO, Viletska YM, Lahanovska YO, Sliusar MY, Bezrodnyi BH, Minchenko OH. Hypoxic regulation of EDN1, EDNRA, EDNRB, and ECE1 gene expressions in IRE1 knockdown U87 glioma cells. Endocr Reg 53, 250–262, 2019.

Minchenko 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 Reg 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, 2021a.

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 Reg 55, 72-82, 2021b.

Obacz J, Avril T, Le Reste PJ, Urra H, Quillien V, Hetz C, Chevet E. Endoplasmic reticulum proteostasis in glioblastoma-from molecular mechanisms to therapeutic perspectives. Sci Signal 10, eaal2323, 2017.

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

Park SM, Seo EH, Bae DH, Kim SS, Kim J, Lin W, Kim KH, Park JB, Kim YS, Yin J, Kim SY. Phosphoserine phosphatase promotes lung cancer progression through the dephosphorylation of IRS-1 and a noncanonical L-serine-independent pathway. Mol Cells 42, 604–616, 2019.

Pikman Y, Ocasio-Martinez N, Alexe G, Dimitrov B, Kitara S, Diehl FF, Robichaud AL, Conway AS, Ross L, Su A, Ling F, Qi J, Roti G, Lewis CA, Puissant A, Vander Heiden MG, Stegmaier K. Targeting serine hydroxymethyltransferases 1 and 2 for T-cell acute lymphoblastic leukemia therapy. Leukemia 36, 348–360, 2022.

Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, Sethumadhavan, Woo HK, Jang HG, Jha AK, Chen WW, Barrett FG, Stransky N, Tsun ZY, Cowley GS, Barretina J, Kalaany NY, Hsu PP, Ottina K, Chan AM, Yuan B, Garraway LA, Root DE, Mino-Kenudson M, Brachtel EF, Driggers EM, Sabatini DM. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. Nature 476, 346–350, 2011.

Qing G, Li B, Vu A, Skuli N, Walton ZE, Liu X, Mayes PA, Wise DR, Thompson CB, Maris JM, Hogarty MD, Simon MC. ATF4 regulates MYC-mediated neuroblastoma cell death upon glutamine deprivation. Cancer Cell 22, 631–644, 2012.

Quiros PM, Prado MA, Zamboni N, D'Amico D, Williams RW, Finley D, Gygi SP, Auwerx J. Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. J Cell Biol 216, 2027–2045, 2017.

Rathore R, Schutt CR, Van Tine BA. PHGDH as a mechanism for resistance in metabolically-driven cancers. Cancer Drug Resist 3, 762–774, 2020.

Reid MA, Allen AE, Liu S, Liberti MV, Liu P, Liu X, Dai Z, Gao X, Wang Q, Liu Y, Lai L, Locasale JW. Serine synthesis through PHGDH coordinates nucleotide levels by maintaining central carbon metabolism. Nat Commun 9, 5442, 2018.

Rudnytska OV, Khita OO, Minchenko, 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.

Tajan M, Hennequart M, Cheung EC, Zani F, Hock AK, Legrave N, Maddocks ODK, Ridgway RA, Athineos D, Suarez-Bonnet A, Ludwig RL, Novellasdemunt L, Angelis N, Li VSW, Vlachogiannis G, Valeri N, Mainolfi N, Suri V, Friedman, Manfredi M, Blyth K, Sansom OJ, Vousden KH. Serine synthesis pathway inhibition cooperates with dietary serine and glycine limitation for cancer therapy. Nat Commun 12, 366, 2021.

Vie N, Copois V, Bascoul-Mollevi C, Denis V, Bec N, Robert B, Fraslon C, Conseiller E, Molina F, Larroque C, Martineau P, Del Rio M, Gongora C. Overexpression of phosphoserine aminotransferase PSAT1 stimulates cell growth and increases chemoresistance of colon cancer cells. Mol Cancer 7, 14, 2008.

Yang M, Vousden KH. Serine and one-carbon metabolism in cancer. Nat Rev Cancer 16, 650–662, 2016.

Zhao X, Fu J, Du J, Xu W. The role of D-3-phosphoglycerate dehydrogenase in cancer. Int J Biol Sci 16, 1495–1506, 2020.