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Endoplasmic Reticulum Stress-Dependent Sensitivity of *EGFR, ERBB2, TOB1*, and *CEBPB* Gene Expressions to Glutamine Deprivation in U87MG Glioblastoma Cells

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ABSTRACT

Glutamine is a necessary substrate for glioblastoma cells and is important for tumor growth. We studied the impact of ERN1 knockdown on *EGFR, ERBB2, TOB1*, and *CEBPB* gene expressions in U87MG glioblastoma cells in response to glutamine deficiency. It was shown that the expression level of *EGFR* and *ERBB2* genes was resistant to glutamine deprivation in control glioblastoma cells but ERN1 knockdown lead to up-regulation these gene expressions. Moreover, *TOB1* and *CEBPB* gene expressions are sensitive to glutamine deprivation in both control and ERN1 knockdown glioblastoma cells but inhibition of ERN1 significantly increases the sensitivity of these genes to glutamine deprivation, especially *CEBPB* gene. These results demonstrated that ERN1, a major signalling pathway of endoplasmic reticulum stress, controls the sensitivity of all studied genes to glutamine deprivation in glioblastoma cells in a gene-specific manner and that the lower sensitivity of the expression of the studied genes in control glioblastoma cells to glutamine deficiency is due to endoplasmic reticulum stress, its ERN1 signalling pathway.

Keywords: ERN1 Knockdown; *EGFR*; *TOB1*; *CEBPB*; mRNA Expression; Glutamine Deprivation; U87MG Glioblastoma Cells

Introduction

Glutamine is an essential substrate for tumor cell metabolism as a substrate for glycolysis, but the requirements for glutamine in cancer are heterogeneous [1-5]. Glutamine metabolism is vital for all cellular processes, including cancer development [6,7]. It is interesting to note that ERN1/IRE1 (endoplasmic reticulum to nucleus signalling 1/inositol requiring enzyme 1) mediated stress signalling modifies the effects of glutamine deficiency on the expression of many genes [8-13]. It has also been shown that the ERN1/XBP1 pathway is essential for the glutamine response and protection of β cells [14]. Transcription factor *CEBPB* (CCAAT enhancer binding protein) controls numerous metabolic processes including gluconeogenesis and immune response [15-18]. Epidermal growth factor receptor (*EGFR*/ErbB1) is activated after dimerization. The activated ERBB receptors bind to many signalling proteins and activate cancer-related signalling pathways [19-21]. *ERBB2*/HER2 is overexpressed in invasive carcinomas and increases tumour growth via cell cycle signalling [22,23]. It is important to note that the transducer of *ERBB2*, 1 (*TOB1*) function as a tumour suppressor and shows anti-proliferative properties that regulate cell growth via reducing the activation of the AKT/mTOR signalling pathway [24]. Furthermore, a decreased *TOB1* expression and up-regulated phosphorylation of *TOB1* in the nucleus supports stomach cancer and microRNA participates in this process [25,26].

Malignant tumors use endoplasmic reticulum unfolded protein response and its signalling pathways to increase cell proliferation under stressful environmental conditions including low glutamine [27-29]. Moreover, the activation of the ERN1 signalling pathway of the unfolded protein response is tightly linked to cell death, and inhibi-

tion of ERN1 function leads to significant suppression of glioblastoma cell proliferation and tumour growth [29-33]. Thus, the endoplasmic reticulum stress and glutamine supply are important factors of cancer progression. Still, the molecular mechanisms of stress-dependent regulation of the expression of tumour-related genes under glutamine deficiency are complex and have not yet been sufficiently elucidated. This study examined the expression of important regulatory genes in response to glutamine deficiency in control and ERN1 knockdown glioblastoma cells for evaluation of the possible significance of this nutrient supply in the controlling ERN1-mediated cell proliferation.

Materials and Methods

Cell Lines and Culture Conditions

In this study, we used two sublines of U87MG glioblastoma cells: control cells – stably transfected clone with overexpression of vector pcDNA3.1, and ERN1 knockdown cells – stably transfected clone overexpressed dnERN1, described previously [34]. Glutamine deprivation and growing conditions were described previously [13].

RNA Isolation

Total RNA was isolated from cells using the Trizol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA) as described previously [34].

Reverse Transcription and Quantitative PCR Analysis

The expression levels of, *EGFR*, *ERBB2*, *TOB1*, *CEBPB*, and ACTB mRNAs were measured in glioblastoma cells by quantitative polymerase chain reaction as described previously [35]. Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription as described previously [35]. Polymerase chain reaction was performed in triplicate. The expression of ACTB mRNA was used for normalization. The pair of primers specific for *EGFR*, *ERBB2*, *TOB1*, and *CEBPB* mRNAs was received from Sigma-Aldrich (St. Louis, MO, U.S.A.) and used for quantitative polymerase chain reaction: *EGFR*/HER1 forward 5'- aggtgaaaacagctgcaagg and reverse 5'- aggtgatgttcatggcctga (NM_005228.5); *ERBB2*/HER2 forward 5'- ggtggtctttggatcctca and reverse 5'- accttcaccttcctcagctc (NM_004448.4); *TOB1* forward 5'- agcccgaacaagatcactca and reverse 5'- cacgtctcctgggaagctta (NM_005749.4); *CEBPB* forward 5'- caagaagaccgtggacaagc and reverse 5'- agctgctccaccttcttctg (NM_005194.4). Primers ACTB were described previously [35]. Quantitative PCR analysis was performed as described previously [33]. The values of studied gene expressions were normalized to the expression of beta-actin mRNA and represented as percent of control (100%). All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments.

Results

To investigate a possible role of endoplasmic reticulum stress response in the control of *EGFR*, *ERBB2*, *TOB1*, and *CEBPB* gene expressions in U87MG glioblastoma cells under low glutamine conditions we studied the impact of glutamine deprivation on the expression of these genes in control glioblastoma cells (transfected by empty vector) and cells with ERN1 knockdown. As shown in Figure 1, the expression of *EGFR* mRNA does not significantly change in control glioblastoma cells after exposure to glutamine deprivation in comparison with the cells growing in a complete DMEM medium. However, inhibition of enzymatic activity of ERN1 signalling protein by dnERN1 is enhanced the sensitivity of *EGFR* gene expression to this experimental condition. Thus, the level of this mRNA expression is increased by 27 % ($p < 0.05$) in cells without the enzymatic activities of the ERN1 signalling protein (Figure 1). Next, we investigated the effect of glutamine deprivation on the expression of gene encoding *ERBB2* with inhibition of ERN1 function. As shown in Figure 2, the expression of this gene is resistant to glutamine deprivation resulting in control glioblastoma cells in comparison with no treated cells (control 1). At the same time, ERN1 knockdown also increases the sensitivity of *ERBB2* gene expression to glutamine deficiency (+21 %; Figure 2). We also investigated the effect of glutamine deprivation condition on the expression of *TOB1* (transducer of *ERBB2*, 1) gene in U87MG glioblastoma cells with ERN1 knockdown.

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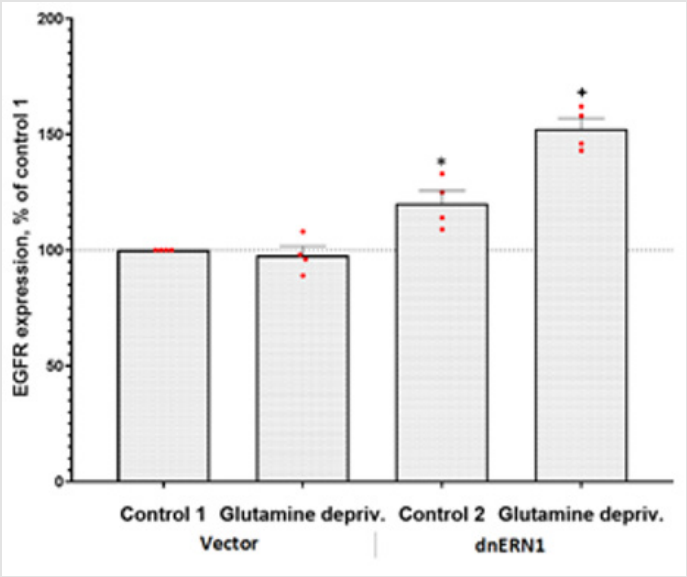

Figure 1: Effect of glutamine deprivation on the expression level of epidermal growth factor receptor (EGFR) mRNA in control U87 glioblastoma cells (Vector) and cells with a blockade of the ERN1 (dnERN1) measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4; *- $p < 0.05$ vs control 1; +- $p < 0.05$ vs control 2.

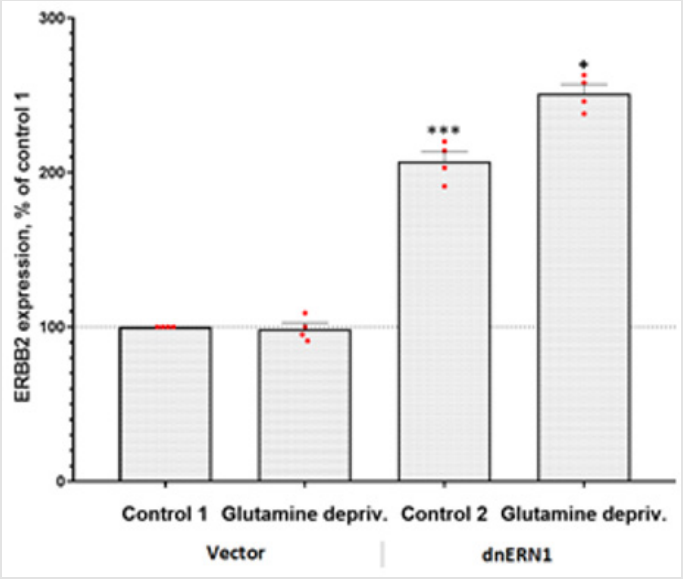

Figure 2: Effect of glutamine deprivation on the expression level of erb-b2 receptor tyrosine kinase 2 (ERBB2) mRNA in control U87 glioblastoma cells (Vector) and cells with a blockade of the ERN1 (dnERN1) measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4; ***- $p < 0.001$ vs control 1; +- $p < 0.05$ vs control 2.

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As shown in Figure 3, the expression of this mRNA is up-regulated in control glioblastoma cells treated by glutamine deprivation (+27 %) and inhibition of enzymatic activities of ERN1 signalling protein significantly increased the sensitivity of *TOB1* mRNA expression to glutamine deprivation (+65 %) in comparison with cells growing with glutamine. We have also studied the effect of glutamine deprivation on the expression of gene encoding transcription factor *CEBPB* (CCAAT enhancer binding protein beta) in control and ERN1 knockdown glioblastoma cells. As shown in Figure 4, exposure of control glioblastoma cells under glutamine deprivation conditions leads to up-regulation of this mRNA expression (+17 %) in comparison with control cells growing under conditions with glutamine. Furthermore, the inhibition of ERN1 strongly induced the sensitivity of this gene expression (+182 %) to glutamine deprivation in glioblastoma cells (Figure 4). Thus, exposure of control glioblastoma cells under glutamine deficiency affects the expression of *CEBPB* and *TOB1* genes but ERN1 knockdown significantly increases the sensitivity of all studied gene expressions to glutamine deprivation conditions, especially the *CEBPB* gene.

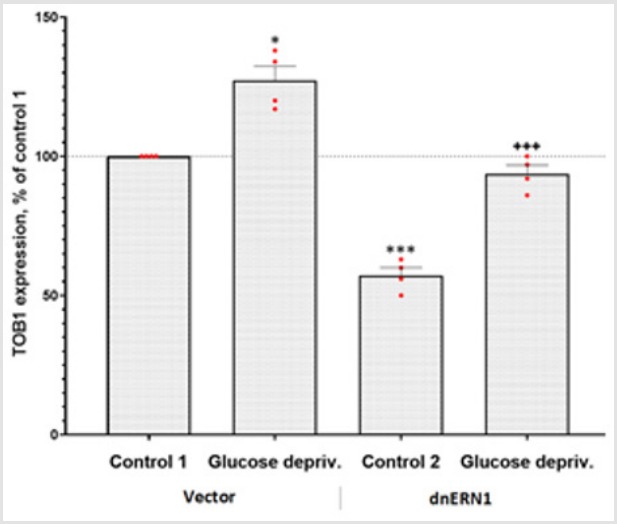

Figure 3: Effect of glutamine deprivation on the expression level of transducer of ERBB2, 1 (TOB1) mRNA in control U87 glioblastoma cells (Vector) and cells with a blockade of the ERN1 (dnERN1) measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4; *- $p < 0.05$ and ***- $p < 0.001$ vs control 1; +++- $p < 0.001$ vs control 2.

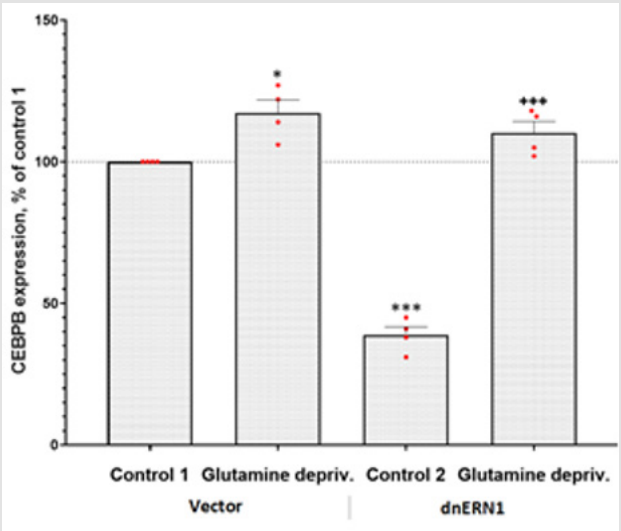

Figure 4: Effect of glutamine deprivation on the expression level of CCAAT enhancer binding protein beta (CEBPB) mRNA in control U87 glioblastoma cells (Vector) and cells with a blockade of the ERN1 (dnERN1) measured by qPCR. Values of this mRNA expression were normalized to beta-actin mRNA level and represented as percent for control 1 (100%); n=4; *- $p < 0.05$ and ***- $p < 0.001$ vs control 1; +++- $p < 0.001$ vs control 2.

Discussion

In this work, we studied the effect of glutamine deprivation on the expression of *EGFR*, *ERBB2*, *TOB1*, and *CEBPB* genes encoding important tumour-related factors in glioblastoma cells with the inhibition of ERN1, the major signalling pathway of the unfolded protein response. The results of our study clarify the possible mechanism of changed sensitivity to glutamine deficiency of the expression of genes encoding important factors associated with tumour growth in ERN1 knockdown glioblastoma cells [11-13]. Thus, our results indicated that ERN1, a major signalling pathway of unfolded protein response, controls the sensitivity of all studied genes to glutamine supply in glioblastoma cells in a gene-specific manner and that the lower sensitivity of the expression of *EGFR*, *ERBB2*, *TOB1*, and *CEBPB* genes in control glioblastoma cells to glutamine deficiency is due to stress-mediated polyresistance, in particular its ERN1 signalling pathway [13,29]. Results of this study summarized in Figure 5, clearly demonstrate the differential effect of decreased glutamine level on the expression of *EGFR*, *ERBB2*, *TOB1*, and *CEBPB* genes and ERN1-dependent character of changes in their expression profile in glioblastoma cells under glutamine deficiency in a gene-specific manner. These results are consistent with previously obtained data on ERN1-mediated dependence of many other gene expressions on glutamine deficiency [10-13]. Furthermore, we showed that the expression of tumour suppressor *TOB1* and especially multi-functional transcription factor *CEBPB* has increased sensitivity to glutamine deficiency in glioblastoma cells with inhibited enzymatic activities of ERN1 compared to control cells (Figure 5).

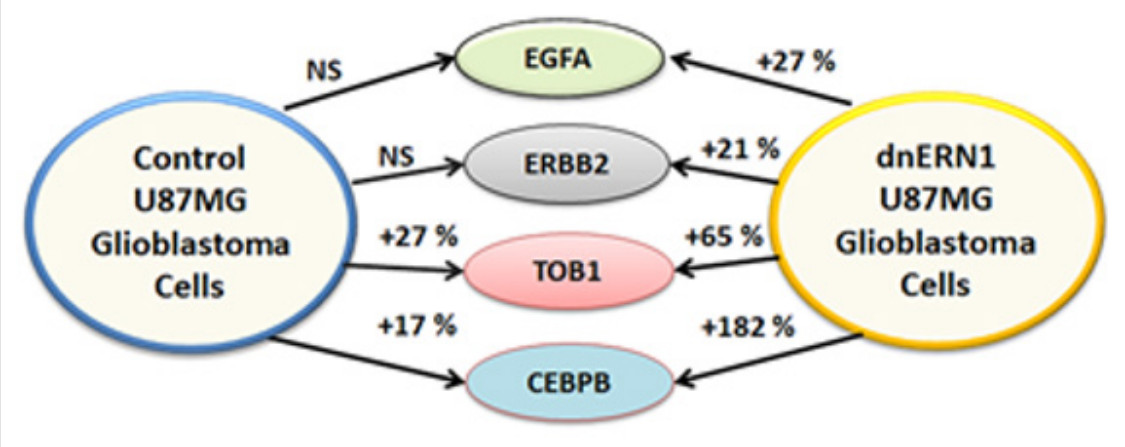

Figure 5: Schematic demonstration of changes in the expression profile of EGFR, ERBB2, TOB1, and CEBPB genes in the control and ERN1 knockdown (dnERN1) glioblastoma cells treated by glutamine deprivation; NS – no significant changes.

Thus, increased expression of tumour suppressor *TOB1* and especially multi-functional transcription factor *CEBPB*, which exerts multiple biological functions and also has anti-proliferative properties, agrees well with numerous data that inhibition of ERN1 suppresses the proliferation of cancer cells [17,18,24-26,30-33,36]. This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding important proteins related to tumour growth in response to glutamine deprivation and their correlation with inhibition of ERN1 activity and reduced cell proliferation in cells harbouring dnERN1, attesting to the fact that endoplasmic reticulum stress, as well as glutamine, is a necessary component of malignant tumour growth and cell survival. Furthermore, our results validate the tight interaction of endoplasmic reticulum stress signalling pathway ERN1 with glutamine deprivation in gene expression regulation of tumour growth-related proteins. However, the detailed molecular mechanisms of ERN1-mediated dependence of gene expression on glutamine deficiency are not yet clearly defined and require further investigation.

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Competing Interest

All authors declare that they reviewed the manuscript and have no conflict of interest.

References

1. Colombo SL, Palacios Callender M, Frakich N, Carcamo S, Kovacs I, et al. (2011) 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(52): 21069-21074.
2. Cluntun AA, Lukey MJ, Cerione RA, Locasale JW (2017) Glutamine Metabolism in Cancer: Understanding the Heterogeneity. Trends Cancer 3(3): 169-180.

3. Kodama M, Oshikawa K, Shimizu H, Yoshioka S, Takahashi M, et al. (2020) A shift in glutamine nitrogen metabolism contributes to the malignant progression of cancer. Nat Commun 11: 1320.
4. Bhowmick N, Posadas E, Ellis L, Freedland SJ, Vizio DD, et al. (2023) Targeting Glutamine Metabolism in Prostate Cancer. Front Biosci (Elite Ed) 15(1): 2.
5. Li S, Zeng H, Fan J, Wang F, Xu C, et al. (2023) Glutamine metabolism in breast cancer and possible therapeutic targets. Biochem Pharmacol 210: 115464.
6. Fasoulakis Z, Koutras A, Ntounis T, Prokopakis I, Perros P, et al. (2023) Ovarian Cancer and Glutamine Metabolism. Int J Mol Sci 24(5): 5041.
7. Jin J, Byun JK, Choi YK, Park KG (2023) Targeting glutamine metabolism as a therapeutic strategy for cancer. Exp Mol Med 55(4): 706-715.
8. Minchenko OH, Kharkova AP, Hnatiuk OS, Luzina OY, Kryvdiuk IV, et al. (2018) ERN1 modifies effect of glutamine deprivation on tumor growth related factors expression in U87 glioma cells. Ukr Biochem J 90(3): 49-61.
9. Jiang J, Srivastava S, Zhang J (2019) Starve Cancer Cells of Glutamine: Break the Spell or Make a Hungry Monster? Cancers (Basel) 11(6): 804.
10. Krasnytska DA, Khita OO, Tsymbal DO, Luzina OY, Cherednychenko AA, et al. (2022) The impact of glutamine deprivation on the expression of MEIS3, SPAG4, LHX1, LHX2, and LHX6 genes in ERN1 knockdown U87 glioma cells. Endocr Regul 56(1): 38-47.
11. Minchenko DO, Kharkova AP, Hubenia OV, Minchenko OH (2013) Insulin receptor, IRS1, IRS2, INSIG1, INSIG2, RRAD, and BAIAP2 gene expressions in glioma U87 cells with ERN1 loss of function: effect of hypoxia and glutamine or glucose deprivation. Endocr Regul 47(1): 15-26.
12. Minchenko DO, Khita OO, Tsymbal DO, Danilovskyi SV, Rudnytska et al. (2020) Expression of IDE and PITRM1 genes in IRE1 knockdown U87 glioma cells: effect of hypoxia and glucose deprivation. Endocr Regul 54(3): 183-195.
13. Minchenko OH, Sliusar MY, Khita OO, Minchenko DO, Viletska YM, et al. (2024) 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(1): 91-100.
14. Hassler JR, Scheuner DL, Wang S, Han J, Kodali VK, et al. (2015) The IRE1α/XBP1s pathway is essential for the glutamine response and protection of β cells. PLoS Biol 13(10): e1002277.
15. Li W, Tanikawa T, Kryczek I, Xia H, Li G, et al. (2018) Aerobic Glycolysis Controls Myeloid-Derived Suppressor Cells and Tumor Immunity via a Specific CEBPB Isoform in Triple-Negative Breast Cancer. Cell Metab 28(1): 87-103.e6.
16. Huber R, Pietsch D, Panterodt T, Brand K (2012) Regulation of C/EBPbeta and resulting functions in cells of the monocytic lineage. Cell Signal 24(6): 1287-1296.
17. Ma Y, Chen Y, Zhan L, Dong Q, Wang Y, et al. (2024) CEBPB-mediated upregulation of SERPINA1 promotes colorectal cancerprogression by enhancing STAT3 signaling. Cell Death Discov 10: 219.
18. Zhang Y, Li L, Chu F, Wu H, Xiao X, et al. (2024) Itraconazole inhibits tumor growth via CEBPB-mediated glycolysis in colorectal cancer. Cancer Sci 115(4): 1154-1169.
19. Rajaram P, Chandra P, Ticku S, Pallavi BK, Rudresh KB, et al. (2017) Epidermal growth factor receptor: Role in human cancer. Indian J Dent Res 28(6): 687-694.
20. Wang X, Men C, Shan S, Yang J, Zhang S, et al. (2025) EGFR upregulates miRNA subset to inhibit CYBRD1 and cause DDP resistance in gastric cancer. Gene 933: 149005.
21. Wang Z (2017) ErbB Receptors and Cancer. Methods Mol Biol 1652: 3-35.
22. Moasser MM (2007) The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. Oncogene 26(45): 6469-6487.
23. Han D, Zhu S, Li X, Li Z, Huang H, et al. (2022) The NF-kappaB/miR-488/ERBB2 axis modulates pancreatic cancer cell malignancy and tumor growth through cell cycle signaling. Cancer Biol Ther 23(1): 294-309.
24. Wang D, Li Y, Sui S, Cai M, Dong K, et al. (2022) Involvement of TOB1 on autophagy in gastric cancer AGS cells via decreasing the activation of AKT/mTOR signaling pathway. PeerJ 10: e12904.
25. Wang D, Song H, Zhao T, Wang M, Wang Y, et al. (2019) Phosphorylation of TOB1 at T172 and S320 is critical for gastric cancer proliferation and progression. Am J Transl Res 11(8): 5227-5239.
26. Wang R, Huang Z, Qian C, Wang M, Zheng Y, et al. (2020) 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.
27. Huber AL, Lebeau J, Guillaumot P, Petrilli V, Malek M, et al. (2013) p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows malignant progression under low glucose. Mol Cell 49(6): 1049-1059.
28. Papaioannou A, Chevet E (2018) Driving cancer tumorigenesis and metastasis through UPR signaling. Curr Top Microbiol Immunol 414: 159-192.
29. Almanza A, Carlesso A, Chintha C, Creedican S, Doultsinos D, et al. (2019) Endoplasmic reticulum stress signalling - from basic mechanisms to clinical applications. FEBS J 286(2): 241-278.
30. Auf G, Jabouille A, Guérit S, Pineau R, Delugin M, et al. (2010) 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 U S A 107: 15553-15558.
31. Pelizzari Raymundo D, Doultsinos D, Pineau R, Sauzay C, Koutsandreas T, et al. (2023) A novel IRE1 kinase inhibitor for adjuvant glioblastoma treatment. iScience 26(5): 106687.
32. Minchenko OH, Tsymbal DO, Khita OO, Minchenko DO (2021) Inhibition of ERN1 signaling is important for the suppression of tumor growth. Clin Cancer Drugs 8(1): 27-38.
33. Minchenko OH, Sliusar MY, Khikhlo YP, Halkin OV, Viletska YM, et al. (2024) Knockdown of ERN1 disturbs the expression of phosphoserine aminotransferase 1 and related genes in glioblastoma cells. Arch Biochem Biophys 759: 110104.
34. Auf G, Jabouille A, Delugin M, Guérit S, Pineau R, et al. (2013) High epiregulin expression in human U87 glioma cells relies on IRE1alpha and promotes autocrine growth through EGF receptor. BMC Cancer 13: 597.
35. Rudnytska OV, Khita OO, Minchenko DO, Tsymbal DO, Yefimova YV, et al. (2021) 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.
36. Suleiman S, Di Fiore R, Cassar A, Formosa MM, Calleja Agius J, et al. (2020) Anticancer effects of an extract from a local planarian species on human acute myeloid leukemia HL-60 cells in vitro. Biomed Pharmacother 130: 110549.

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