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Acute L-glutamine deprivation affects the expression of TP53-related protein genes in U87 glioma cells

We have studied the effect of acute L-glutamine deprivation on the expression of tumor protein 53 (TP53)-related genes such as TOPORS (topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase), TP53BP1 (TP53 binding protein 1), TP53TG1 (TP53 inducible gene 1), SESN1 (p53 regulated PA26 nuclear protein), NME6 (NME/NM23 nucleoside diphosphate kinase 6), and ZMAT3 (zinc finger, Matrin-type 3) in glioma cells with ERN1 knockdown. It was shown that blockade of ERN1 function in U87 glioma cells is induced the expression of RYBP and SESN1 genes, but decreased the expression of TP53BP1, TP53TG1, TOPORS, NME6, genes. Moreover, the expression levels of RYBP, SESN1, TP53BP1, and ZMAT3 genes is increased in control glioma cells by L-glutamine deprivation condition but blockade of ERN1 signaling enzyme function significantly enhances this effect on the expression all of these genes. At the same time, the ERN1 knockdown eliminates the response TP53TG1 and TOPORS genes to L-glutamine deprivation condition. Results of this investigation clearly demonstrate that the expression most of genes encoding TP53-related factors depends upon acute L-glutamine deprivation condition as well as upon ERN1, the major signaling system of the endoplasmic reticulum stress.

Key words: glutamine deprivation; endoplasmic reticulum stress, ERN1, RYBP, TP53BP1, TP53TG1, TOPORS, SESN1, ZMAT3, NME6, gene expressions.

INTRODUCTION

Malignant tumors use endoplasmic reticulum stress for activation of proliferation [1–3]. Moreover, the endoplasmic reticulum stress response-signalling ERN1 pathway is linked to apoptosis and cell death processes and suppression of ERN1 gene function significantly decreases tumor growth [1, 4, 5]. Malignant gliomas are highly aggressive tumors and are characterized by marked angiogenesis, extensive tumor cell invasion into the normal brain parenchyma and to date there is no efficient treatment available. The very poor prognosis and the moderate efficacy of conventional clinical approaches therefore underline the need for new therapeutic strategies. Ischaemia as well as hypoxia is associated with glioma development and locally induce an adaptive response which confers to tumor cells an enhanced survival and a more agressive behaviour. A better knowledge of tumor responses to hypoxia is required to elaborate therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms [1, 5].

The endoplasmic reticulum is a key organelle in the cellular response to nutrient deprivation, hypoxia, and some chemicals which activate a complex set of signaling pathways named the unfolded protein response. This adaptive response is activated upon the accumulation of misfolded proteins in the endoplasmic reticulum and is mediated by three endoplasmic reticulum-resident sensors named PERK (PRK-like ER kinase), IRE1/ERN1 (Inositol Requiring Enzyme-1/Endoplasmic Reticulum – Nuclei-1) and ATF6 (Activating Transcription Factor 6), however, ERN1 is the dominant sensor of the unfolded protein response to the accumulation of misfolded proteins and represents a key regulator of the life and death processes [1,

6, 7]. Misfolded proteins in the endoplasmic reticulum lumen activate two distinct catalytic domains of ERN1, which display serine/threonine trans-autophosphorylation and endoribonuclease activities, respectively. ERN1-associated endoribonuclease activity is involved in the degradation of a specific subset of mRNA and also initiates the cytosolic splicing of the pre-XBP1 (X-box binding protein 1) mRNA whose mature transcript encodes a transcription factor that stimulates the expression of unfolded protein response specific genes [2, 8].

The tumor protein 53 (TP53) is one of the best-known tumor suppressors. TP53 responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism [9, 10]. This protein is involved in cell cycle regulation as a transactivator that acts to negatively regulate cell division by controlling a set of genes required for this process, including an inhibitor of cyclin-dependent kinases, via binding to gene specific response elements, but induction of necrosis by TP53 seems to be largely independent of transcription regulation [11].

Stability as well as activity of TP53 depends upon many factors. Thus, death effector domain-associated factor RYBP (RING1 and YY1-binding protein) also known as YEAF (YY1 and E4TF1-associated factor 1) or DADAF (DAD-associated factor) inhibits ubiquitination and subsequent degradation of TP53, and thereby plays a role in regulating transcription of TP53 target genes. This factor interacts with MDM2 and decreases MDM2-mediated TP53 ubiquitination, stabilizing TP53 and increasing its activity [12]. RYBP is decreased in human cancer tissues and implicated in the regulation of the transcription as a repressor of the transcriptional activity of E4TF1 and promotes apoptosis [12, 13].

There is data that TP53 binding proteins (TP53BP1, TP53BP2, and TP53BP3) modulate p53 function and suppresses tumor growth and promotes susceptibility to apoptosis, but its activity depends upon different factors [3, 14 – 18]. Thus, TP53BP1 has a role in checkpoint signaling during mitosis, enhances TP53-mediated transcriptional activation and plays a role in the response to DNA damage [18, 19]. TP53BP2 is a member of the ASPP (apoptosis-stimulating protein of p53) family of TP53 interacting proteins which are down regulated in tumor tissues and is required for the induction of apoptosis by TP53-family proteins [16]. TP53BP2 protein promotes DNA binding and transactivation of TP53-family proteins on the promoters of pro-apoptotic genes. TP53BP3, also known as SUMO1-protein E3 ligase TOPORS, contains a RING-type zinc finger domain and functions as an ubiquitin-protein E3 ligase. It regulates TP53 stability through ubiquitin-dependent degradation and is involved in cell growth, cell proliferation and apoptosis [3, 21, 22].

There is data that NME6 (non-metastatic cells 6) also known as NME/NM23 nucleoside diphosphate kinase 6 inhibits of p53-induced apoptosis and also participates in oncogenesis [23, 24]. SESN1 (sestrin 1 or TP53 regulated PA26 nuclear protein) and ZMAT3 (zinc finger, Matrin-type 3 or TP53 target zinc finger protein) are TP53 target genes and have a role in the TP53-dependent growth regulatory pathway [25 – 27]. It is known that cell growth is positively regulated by mTOR, whose activity is inhibited by Sestrin1 and sestrin2 [25]. Thus, SESN proteins provide an important link between genotoxic stress, TP53 and the mTOR signaling pathway. Recently was shown that ZMAT3 is an mRNA stability-regulating protein which prevents cellular senescence by regulating p21 mRNA decay through control of RISC recruitment [28]. ZMAT3 can bind different types of double-stranded RNAs, including small interfering RNAs and microRNAs [26, 27].

Because inhibition of ERN1 signaling enzyme function significantly decreases tumor growth [4, 5] and TP53 is one of the best-known tumor suppressors, the aim of this study was investigation of the effect of L-glutamine deprivation on the expression of TP53-related genes

(RYBP, TP53BP1, TP53TG1, TOPORS, SESN1, ZMAT3, and NME6), which participate in the regulation of cell proliferation and apoptosis, in glioma cells in relation to knockdown of ERN1. Moreover, using bioinformatics screening we have identified the potential XBP1-response elements in TOPORS and SESN1 genes.

EXPERIMENTAL PROCEDURES

The glioma cell line U87 was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (DMEM; Gibco, Invitrogen, 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 a 5% CO_2 incubator. In this work we used two sublines of this glioma cell line. One subline was obtained by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dominant-negative constructs (dnERN1). This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of L-glutamine deprivation action on the expression level of RYBP, TP53BP1, TP53TG1, TOPORS, SESN1, ZMAT3, and NME6 genes. Second subline was obtained by selection of stable transfected clones with overexpression of dnERN1 and has suppressed both protein kinase and endoribonuclease activities of this bifunctional sensing and signaling enzyme of endoplasmic reticulum stress. These cells were obtained from prof. M. Moenner (France) [1]. For creation of acute L-glutamine deprivation condition, control glioma cells and ERN1 knockdown glioma cells were washed in DMEM and then incubated in the medium without glutamine for 16 hrs. The cells which over express dnERN1 were used as control 2 for investigation the effect of L-glutamine deprivation condition on gene expressions under blockade the ERN1 function.

The blockade of both kinase and endoribonuclease enzymatic activity of ERN1 in glioma cells that over express a dominant-negative construct of endoplasmic reticulum–nuclei-1 (dnERN1) was studied by analysis of mRNA expression of XBP1 alternative splice variant (XBP1s), a key transcription factor in ERN1 signaling, using cells treated by tunicamycin (0.01 mg/ml for 2 hours). The proliferation rate of control glioma cells and ERN1 knockdown cells were measured in triplicates after 3 days by using a cell counter (Coultronics, Margency, France).

Total RNA was extracted from glioma cells using Trizol reagent according to manufacturer protocols (Invitrogen, USA). The RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water. For additional purification RNA samples were re-precipitated with 95 % ethanol and re-dissolved again in nuclease-free water.

QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis according to manufacturer protocol. The expression level of RYBP, TP53BP1, TP53TG1, TOPORS, SESN1, ZMAT3, NME6, and ACTB mRNA were measured in glioma cell line U87 and its subline (clone 1C5) by real-time quantitative polymerase chain reaction using „Mx 3000P QPCR" (Stratagene, USA) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, ABgene House, UK). Polymerase chain reaction was performed in triplicate.

For amplification the cDNA for RYBP (RING1 and YY1-binding protein; ring1 interactor RYBP), which also known as YEAF (YY1 and E4TF1-associated factor 1) and DADAF (DAD-associated factor), we used next primers: forward 5'– TGACATTGCAGTGGTGGTTT –3' and reverse 5'– TACGAGCTTTCCCTGCAACT –3'. The nucleotide sequences of these primers correspond to sequences 1944 – 1963 and 2221 – 2202 of human RYBP cDNA (GenBank accession number NM_012234). The size of amplified fragment is 278 bp. The amplification of cDNA for TP53BP1 (tumor protein p53 binding protein 1) was performed using forward primer (5'– CAGTCCCCAGAAGACCATGT–3') and reverse primer (5'– CTGGGAAGCAGGAGAAACAG –3'). These oligonucleotides correspond to

sequences 3229 – 3248 and 3463 – 3444 of human TP53BP1 cDNA (GenBank accession number NM_005657). The size of amplified fragment is 235 bp. The amplification of cDNA for TP53TG1 (TP53 target 1) also known as tumor protein p53-activated protein 1 or TP53-inducible gene 1 was performed using forward primer (5'– ACGAAGGTACCCAACCCTCT –3') and reverse primer (5'– TGTTCTTTTGCCAAGACACG –3'). These oligonucleotides correspond to sequences 206 – 225 and 406 – 387 of human TP53TG1 cDNA (GenBank accession number NM_007233). The size of amplified fragment is 201 bp. For amplification the cDNA for TOPORS (topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase) also known as tumor suppressor p53-binding protein 3 was used next oligonucleotide primers: forward – 5'– TTCGCTGTGTACAGGAGTGG –3' and reverse – 5'– CCGGTGGAGTTGTTGTTCTT –3'. The nucleotide sequences of these primers correspond to sequences 566 – 585 and 814 – 595 of human USP7 cDNA (GenBank accession number NM_005802). The size of amplified fragment is 249 bp. The amplification of cDNA for NME6 (non-metastatic cells 6) also known as NME/NM23 nucleoside diphosphate kinase 6 was performed using forward primer (5'– TTCTATCCGTGGGAGTTTCG –3') and reverse primer (5'– TAGGCCTCCTGTTCCAGCTA –3'). These oligonucleotides correspond to sequences 409 – 428 and 619 – 600 of human NME6 cDNA (GenBank accession number NM_005793). The size of amplified fragment is 211 bp. The amplification of cDNA for SESN1 (sestrin 1) also known as p53-activated gene 26 and p53 regulated PA26 nuclear protein was performed using forward - 5'– GCATGTTCCAACATTTCGTG –3' and reverse - 5'– GTTCCAAATTGCCCGTCTAA –3' primers. These primers nucleotide sequences correspond to 1641 – 1660 and 1830 – 1811 of human SESN1 cDNA (GenBank accession number NM_014454). The size of amplified fragment is 190 bp. For amplification of cDNA for ZMAT3 (zinc finger, Matrin-type 3) also known as p53-activated gene 608 protein and p53 Target Zinc Finger Protein) we used forward 5'– GAATTCCGGCAGCATTTAGA –3' and reverse 5'– ACGTTCTTCACACCCACCTC –3' primers. The nucleotide sequences of these primers correspond to sequences 1109 – 1128 and 1396 – 1377 of human ZMAT3 cDNA (GenBank accession number NM_022470). The size of amplified fragment is 288 bp. The amplification of beta-actin (ACTB) cDNA was performed using forward - 5'– GGACTTCGAGCAAGAGATGG –3' and reverse - 5'– AGCACTGTGTTGGCGTACAG –3' primers. These primers nucleotide sequences correspond to 747 – 766 and 980 – 961 of human ACTB cDNA (GenBank accession number NM_001101). The size of amplified fragment is 234 bp. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The primers were received from "Sigma" (USA).

An analysis of quantitative PCR was performed using special computer program "Differential expression calculator" and statistical analysis – in Excel program. The values of RYBP, TP53BP1, TOPORS, TP53TG1, SESN1, NME6, and ZMAT3 mRNA expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (100 %). All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments.

RESULTS

We have studied the efficiency of inhibition of enzymatic activity of ERN1 by analysis of mRNA expression of XBP1 and its splice variant (XBP1**s**) in U87 glioma cells that overexpress a dominant-negative construct of endoplasmic reticulum–nuclei-1 as compared to control glioma cells transfected with a vector. As shown in Fig. 1, inductor of endoplasmic reticulum stress, tunicamycin (0,01 mg/ml), strongly induces the alternative splicing of XBP1 only in control glioma cells, while having no effect on this process

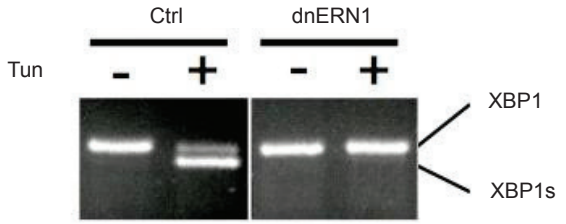

Fig. 1. Effect of tunicamicin (Tun; 0,01 mg/ml) on mRNA level of transcription factor XBP1 and its alternative splice variant (XBP1s) in glioma cell line U87 stable transfected with vector (Ctrl) and its subline stable transfected with dominant-negative construct of endoplasmic reticulum–nuclei-1 (dnERN1)

in cells, transfected by dnERN1. Moreover, inhibition of ERN1 results in suppression of the proliferation rate of cells, expressing dnERN1 (two fold lower after 3 days in culture as compared to control U87 glioma cells; Fig. 2). It is possible that inhibition of ERN1 can result in suppression of malignant cell proliferation possibly though deregulation of TP53 signaling.

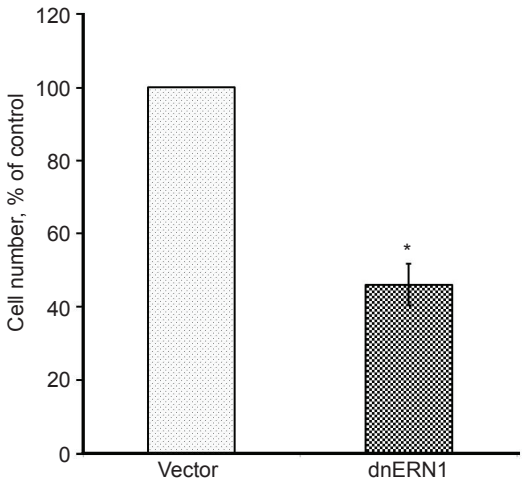

Fig. 2. The proliferation rate of control U87glioma cells, transfected with vector, (Vector) and ERN1 knockdown cells (dnERN1). Cell number was measured in triplicates after 3 days growing by using a cell counter (Coultronics, Margency, France) and represent as percent of control (100 %); mean ± SEM; n = 4; *P < 0.05 as compared to control

As shown in Fig. 3, the blockade of ERN1 enzyme function in U87 glioma cells affects the expression of several TP53-related genes, being more significant for RYBP, TOPORS, and

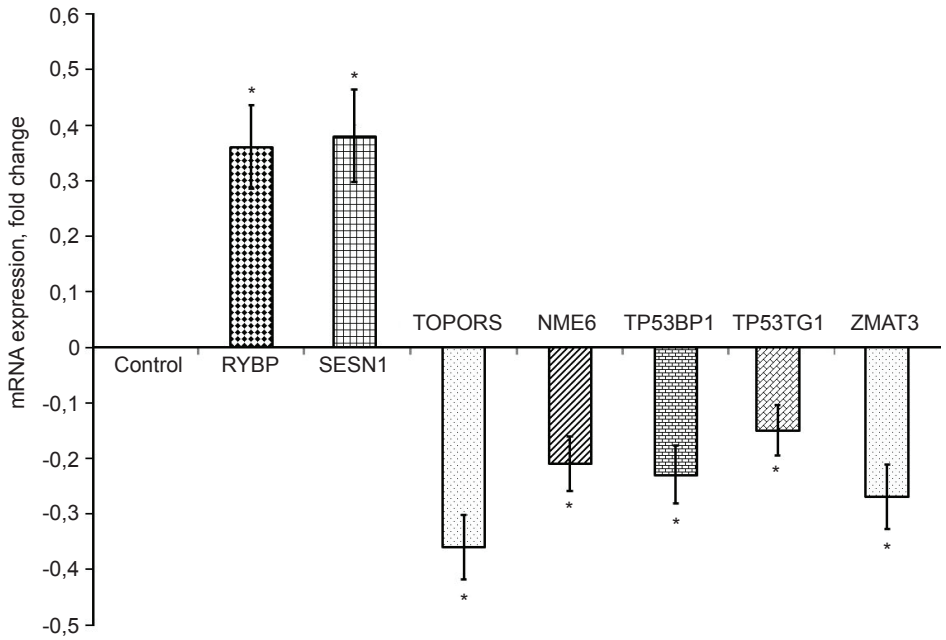

Fig. 3. The expression of RYBP, SESN1, TOPORS, NME6, TP53BP1, TP53TG1, and ZMAT3 mRNA in glioma cell line U87, transfected with vector, and its subline with knockdown of the signaling enzyme ERN1 (dnERN1). Values of these mRNA expressions were measured by qPCR, normalized to beta-actin mRNA and represent as fold changes of control (0); n = 4; *P < 0.05 as compared to control

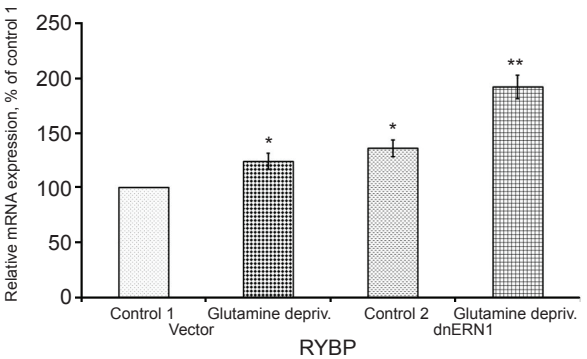

Fig. 4. Expression of RYBP (RING1 and YY1-binding protein; YY1 and E4TF1 associated factor 1) mRNA in glioma cell line U87 (Vector) and its subline with a deficiency of the signaling enzyme ERN1 (dnERN1): effect of L-glutamine deprivation (16 hrs). Values of RYBP mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; * – P < 0.05 as compared to control 1; **P < 0.05 as compared to control 2

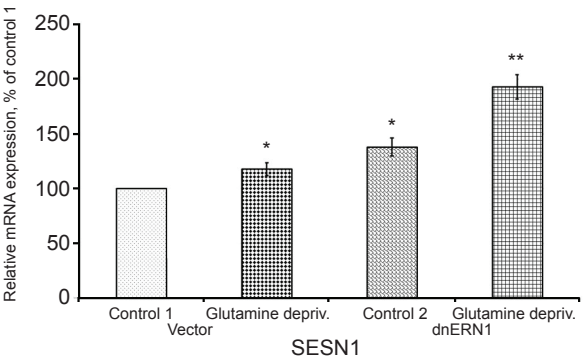

Fig. 5. Expression of SESN1 (sestrin 1; p53 regulated PA26 nuclear protein) mRNA in glioma cell line U87 (Vector) and its subline with a deficiency of the signaling enzyme ERN1 (dnERN1): effect of L-glutamine deprivation. Values of SESN1 mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; * – P < 0.05 as compared to control 1; **P < 0.05 as compared to control 2

SESN1 genes. It should be noted that the expression of RYBP and SESN1 genes is increased in glioma cells at this experimental condition. At the same time, the expression of TOPORS, NME6, TP53BP1, TP53TG1, and ZMAT3 genes is suppressed in glioma cells with ERN1 knockdown (Fig. 3). Thus, the blockade of ERN1 gene function in U87 glioma cells changes the expression of studied TP53-related genes in different ways.

As shown in Fig. 4, the expression level of RYBP (RING1 and YY1-binding protein) mRNA is increased under acute L-glutamine deprivation condition in control glioma cells (stable transfected by vector) as well as in cells with suppressed function of ERN1 signaling enzyme (stable transfected by dnERN1); however, ERN1 knockdown enhance effect of glutamine deprivation on the expression of this gene in U87 glioma cells. Similar results were received with TP53 target gene SESN1, TP53 regulated PA26 nuclear protein. It was shown that the expression of this gene is increased in control and ERN1 knockdown glioma cells treated with L-glutamine deprivation condition, but this effect was more significant in cells with ERN1 knockdown (Fig. 5).

It was also shown that L-glutamine deprivation also increased the expression of TOPORS (topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase) and TP53BP1 (tumor protein TP53 binding protein 1) genes in control glioma cells (Fig. 6 and 7). At the same time, the expression of TOPORS gene did not change significantly by L-glutamine deprivation in glioma cells with suppressed function of ERN1, but expression of TP53BP1 gene is increased in these cells, being more significant in ERN1 knockdown cells as compared to con-

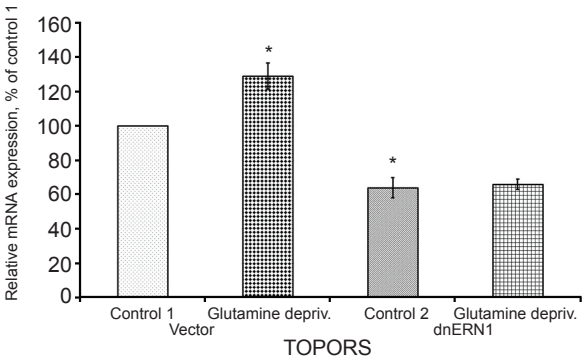

Fig. 6. Effect of L-glutamine deprivation on the expression of TOPORS (topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase; tumor suppressor P53-binding protein 3) mRNA in glioma cell line U87 (Vector) and its subline with a deficiency of ERN1 (dnERN1). Values of TOPORS mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; *P < 0.05 as compared to control 1

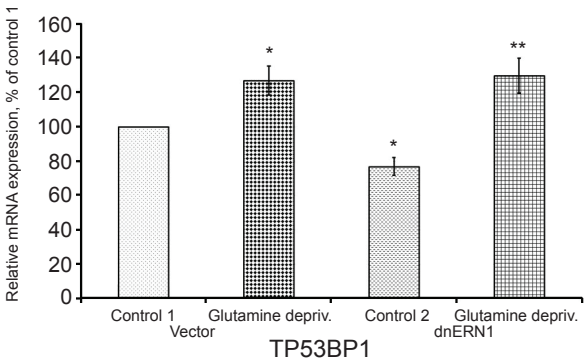

Fig. 7. TP53 binding protein 1 (TP53BP1) mRNA expression in glioma cell line U87 (Vector) and its subline with knockdown of the signaling enzyme ERN1 (dnERN1): effect of L-glutamine deprivation. Values of TP53BP1 mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; *P < 0.05 as compared to control 1; **P < 0.05 as compared to control 2

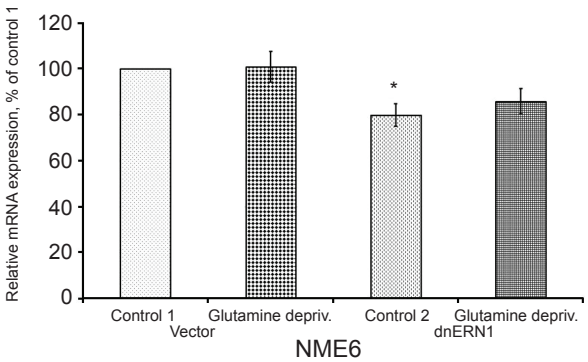

Fig. 9. Non-Metastatic Cells 6 (NME6; NME/NM23 Nucleoside Diphosphate Kinase 6) mRNA expression in glioma cell line U87 (Vector) and its subline with knockdown of the signaling enzyme ERN1 (dnERN1): effect of L-glutamine deprivation. Values of NME6 mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; *P < 0.05 as compared to control 1

trol glioma cells (Figure 6 and 7). Thus, ERN1 knockdown modifies the effect of L-glutamine deprivation on the expression of TOPORS and TP53BP1 genes.

As shown in figure 8, the expression level of tumor protein TP53 target 1 gene (TP53TG1) also known as TP53-activated protein 1 or TP53-inducible gene 1 is increased under L-glutamine deprivation condition in control glioma cells but did not change significantly in cells with suppressed function of ERN1 signaling enzyme. Moreover, the expression of NME6 (NME/NM23 nucleoside diphosphate kinase 6) gene is resistant to acute L-glutamine deprivation condition in both types of used glioma cells (Figure 9).

Investigation of ZMAT3 (TP53 target zinc finger protein) gene shown that the expression of its mRNA is increased by L-glutamine deprivation condition in control as well as ERN1 knockdown glioma cells, but this effect was more significant in glioma cells with suppressed function of ERN1 signaling enzyme (Figure 10).

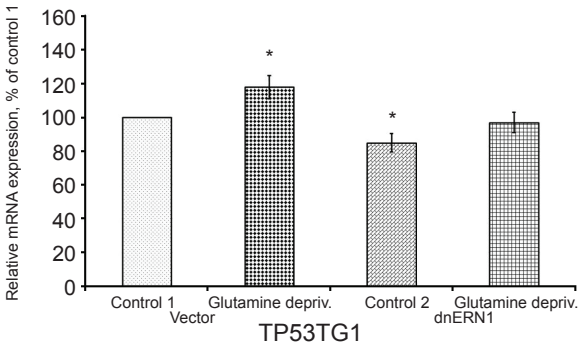

Fig. 8. Effect of L-glutamine deprivation on the expression of TP53 activated protein 1 (TP53TG1; TP53-inducible gene 1 protein) mRNA in glioma cell line U87 (Vector) and its subline with blockade of ERN1 (dnERN1). Values of TP53TG1 mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; *P < 0.05 as compared to control 1

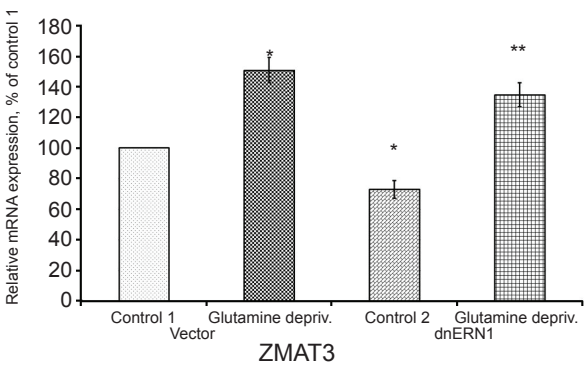

Fig. 10. Expression of ZMAT3 (zinc finger, Matrin-type 3; p53-activated gene 608 protein) mRNA in glioma cell line U87 (Vector) and its subline with a deficiency of the signaling enzyme ERN1 (dnERN1): effect of L-glutamine deprivation (16 hrs). Values of ZMAT3 mRNA expressions were normalized to beta-actin mRNA expression and represent as percent of control (100 %); n = 4; *P < 0.05 as compared to control 1; **P < 0.05 as compared to control 2

DISCUSSION

The TP53 protein coordinates diverse cellular functions through the regulation of the expression of target genes, thereby inducing cell cycle arrest and apoptosis [10, 29]. Despite the intensity of TP53 investigation the molecular mechanisms by which it coordinates diverse cellular functions remain enigmatic. More than 100 physical or genetic interactions with TP53 have been identified to date [29]. In this study we analyzed the expression of several important TP53-associated genes in U87 glioma cells in relation to acute L-glutamine deprivation condition as well as to blockade of ERN1, the major endoplasmic reticulum stress sensor and signaling enzyme, and shown that the blockade of ERN1 gene function in U87 glioma cells changes the expression of all studied TP53-related genes, but in different ways (Fig. 3). Our results clearly demonstrate that the expression of genes which enhance TP53 stability and function (RYBP and SESN1) is increased in glioma cells with ERN1 knockdown and that this increase of RYBP and SESN1 gene expressions may contribute to the suppression of glioma growth in cells without ERN1 functional activity via TP53-dependent growth regulatory pathway [4, 5, 30, 31]. Thus, up-regulation of RYBP, which inhibits ubiquitination and subsequent degradation of TP53, in glioma cells with suppressed ERN1 function (Fig. 4) plays a role in enhancing transcription of TP53 target genes. Moreover, RYBP interacts with MDM2 and decreases MDM2-mediated TP53 ubiquitination, stabilizing TP53 and increasing its activity and promotes apoptosis as well [12, 13]. Thus, increased expression of RYBP gene is associated with suppression of glioma growth [4, 5] and correlates with decreased expression in human cancer tissues [13].

Enhanced expression of TP53 regulated PA26 nuclear protein (SESN1) in glioma cells without ERN1 signaling (Fig. 5) also correlates to the suppression of tumor growth in these cells because this protein mediates TP53 inhibition of cell growth by activating AMP-activated protein kinase, which results in the inhibition of the mammalian target of rapamycin protein [32]. Thus, increased expression of SESN1 also may contribute in the suppression of glioma growth in cells with ERN1 knockdown by enhancing the TP53 transcriptional activity.

At the same time, the expression of NME6, TOPORS, and ZMAT3 genes is suppressed in glioma cells with ERN1 knockdown (Fig. 6, 9, 10) and this down-regulation of TP53-related genes may also contribute to the suppression of tumor growth which was shown for ERN1 knockdown glioma cells [4, 5]. NME6 is an inhibitor of TP53-induced apoptosis and its down-regulation should enhance TP53 apoptotic function. Moreover, nucleoside diphosphate kinases are evolutionarily conserved enzymes involved in many biological processes such as metastasis, proliferation, development, differentiation, vesicle transport, and apoptosis in vertebrates, but molecular mechanisms of these processes are still largely unknown [33, 34].

Topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase TOPORS also functions as an E3 SUMO1-protein ligase and is involved in cell growth, proliferation and apoptosis that regulate TP53 stability through ubiquitin-dependent degradation [35]. TOPORS induced TP53 sumoylation as well as a variety of other cellular proteins [36]. Thus, down-regulation of TOPORS in glioma cells by ERN1 knockdown may increase TP53 transcription activity which is inhibited by this factor and may contribute in the suppression of glioma growth in cells without ERN1 function [4, 5].

Blockade of ERN1 in glioma cells also leads to the decreased expression of ZMAT3 (TP53 target zinc finger protein) gene but molecular mechanisms of this relation is not yet clear. There is data that ZMAT3 participates in the TP53-dependent growth regulatory pathway by regulation of TP53 expression and translocation to the nucleus as well as RNA-mediated gene silencing [26, 28, 35]. At the same time, recently was shown that ZMAT3 as an mRNA stability-regulating protein prevents cellular senescence

[28] and its decrease possibly participate in suppression of ERN1 knockdown of glioma cells proliferation (Fig. 2). Moreover, the ZMAT3 protein can bind different types of double-stranded RNAs, including small interfering RNAs and microRNAs [26, 27] and this binding has a role in ZMAT3-mediated regulation of cell growth. It is possible that ZMAT3 protein also contributes to TP53-mediated apoptosis by regulation of TP53 expression and translocation to the nucleus and nucleolus [27, 37]. At the same time, ZMAT3 knockdown causes a dramatic inhibition of N-MYC expression and triggers differentiation in neuroblastoma cells [27].

We have also shown that L-glutamine deprivation affects the expression of most of the studied TP53-related genes and that these changes in gene expressions are depended upon ERN1 signaling enzyme function. It is interesting to note that acute L-glutamine deprivation induces the expression of genes which were up-regulated in glioma cells without ERN1 signaling (RYBP and SESN1) both in control glioma cells and ERN1 knockdown cells; however, blockade the ERN1 function enhances effect of L-glutamine deprivation on both gene expressions. At the same time, the expression of genes which were down-regulated in ERN1 knockdown glioma cells (TOPORS, TP53BP1, TP53TG1, and ZMAT3) is increased by L-glutamine deprivation condition in control glioma cells, but suppression of ERN1 signaling enzyme function modifies the expression of these genes. Thus, effect of L-glutamine deprivation on the expression of TP53BP1 and ZMAT3 genes was significantly higher in ERN1 knockdown cells and was eliminated on the expression of TP53TG1 and TOPORS genes.

Thus, most of studied TP53-related genes are responsible to L-glutamine deprivation condition in a different ways that can enhance or reduce the TP53 stability as well as functional activity. Moreover, the effect of ERN1 knockdown enhances the effect of L-glutamine deprivation condition on the expression of RYBP, TP53BP1, ZMAT3, and SESN1 genes, but eliminates enhanced its effect on TP53TG1 and TOPORS gene expressions. It is possible that L-glutamine deprivation condition may increase the effect of the blockade of ERN1 signaling enzyme on TP53 stability and transcriptional activity by changing the expression of RYBP and SESN1 genes and induces TP53-dependent cell growth repression and apoptosis. At the same time, the blockade of ERN1 signaling enzyme increases the effect of L-glutamine deprivation on the expression of TP53BP1 and ZMAT3 genes which have reversible effect on TP53 stability and its functional activity. Thus, endoplasmic reticulum stress as well as nutrient deprivation is necessary component of malignant tumor growth and cell survival; however, between the presence of nutrient deprivation and the regulation of cell death pathways exists a complex relationship [3, 38–40].

Results of this investigation clearly demonstrate that the expression of genes encoding TP53-related factors is mostly depended upon endoplasmic reticulum stress signaling as well as ischemic condition and mostly correlate with suppression of tumor growth in cells with ERN1 knockdown.

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ОСТРЫЙ ДЕФИЦИТ L-ГЛЮТАМИНА НАРУШАЕТ ЭКСПРЕССИЮ ГЕНОВ TP53-ЗАВИСИМЫХ ПРОТЕИНОВ В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87

Мы изучили эффект острого дефицита L-глютамина на экспрессию таких генов, зависимых от протеина опухолей p53 (TP53), как RYBP, TOPORS, TP53BP1, TP53TG1, SESN1, NME6, и ZMAT3 в клетках глиомы с выключенной активностью ERN1. Показано, что блокада функции гена ERN1 в клетках глиомы линии U87 усиливает экспрессию генов RYBP и SESN1, при этом интенсивность экспрессии генов TP53BP1, TP53TG1, TOPORS, NME6 и ZMAT3 снижается. Более того, уровень экспрессии генов RYBP, SESN1, TP53BP1 и ZMAT3 увеличивается в контрольных клетках глиомы при дефиците L-глютамина в среде, но выключение функции энзима ERN1 существенно усиливает этот эффект на экспрессию всех этих генов. В то же время выключение функции энзима ERN1 снимает зависимость экспрессии генов TP53TG1 и TOPORS от дефи-

цита L-глютамина. Результаты этой работы указывают на зависимость экспрессии большинства ассоциированных с TP53 генов от условий острого дефицита глютамина в среде, как и от ERN1, основной сигнальной системы стресса эндоплазматического ретикулума.
Ключевые слова: дефицит глютамина, стресс эндоплазматического ретикулума, RYBP, TP53BP1, TP53TG1, TOPORS, SESN1, ZMAT3, NME6, экспрессия генов.

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ГОСТРИЙ ДЕФІЦИТ L-ГЛУТАМІНУ ПОРУШУЄ ЕКСПРЕСІЮ ГЕНІВ TP53-ЗАЛЕЖНИХ ПРОТЕЇНІВ У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87

Ми дослідили ефект гострого дефіциту L-глутаміну на експресію таких генів, залежних від протеїну пухлин p53 (TP53), як RYBP, TOPORS, TP53BP1, TP53TG1, SESN1, NME6, та ZMAT3 у клітинах гліоми з виключеною активністю ERN1. Показано, що блокада функції гена ERN1 у клітинах гліоми лінії U87 посилює експресію генів RYBP та SESN1, при цьому інтенсивність експресії генів TP53BP1, TP53TG1, TOPORS, NME6 та ZMAT3 знижується. Більше того, рівень експресії генів RYBP, SESN1, TP53BP1 та ZMAT3 збільшується у контрольних клітинах гліоми за умов дефіциту L-глутаміну у середовищі, але виключення функції ензиму ERN1 істотно посилює цей ефект на експресію всіх цих генів. Водночас виключення функції ензиму ERN1 знімає залежність експресії генів TP53TG1 та TOPORS від дефіциту L-глутаміну. Результати цієї роботи вказують на залежність експресії більшості асоційованих з TP53 генів від умов гострого дефіциту глутаміну у середовищі, як і від ERN1, основної сигнальної системи стресу ендоплазматичного ретикулума.
Ключові слова: дефіцит глутаміну, стрес ендоплазматичного ретикулума, RYBP, TP53BP1, TP53TG1, TOPORS, SESN1, ZMAT3, NME6, експресія генів.

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