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IRE1 KNOCKDOWN MODIFIES THE EFFECT OF GLUTAMINE DEPRIVATION ON THE EXPRESSION OF A SUBSET OF PROTEASES IN U87 GLIOMA CELLS

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The aim of this research was to study the effect of glutamine deprivation on the expression of genes encoding for HTRA1/PRSS11, LONP1/PRSS15, and some cathepsins in U87 glioma cells in relation to inhibition of IRE1 (inositol requiring enzyme-1). It was shown that in control glioma cells (transfected by empty vector) glutamine deprivation up-regulated the expression of *LONP1, CTSD, CTSF, CTSO,* and *CTSS* genes, down-regulated *HTRA1*, *CTSC,* and *CTSK* gene expressions, and did not significantly change the expression of *CTSA, CTSB,* and *CTSL* genes. Inhibition of IRE1 signaling enzyme function in U87 glioma cells modified the effect of glutamine deprivation on the expression of *HTRA1, LONP1, CTSD, CTSL, CTSO,* and *CTSS* genes: removed the effect of glutamine deprivation on *HTRA1* and *CTSO* genes, introduces on *CTSL* gene, reduced — on *CTSD* gene, and enhanced — on *LONP1* and *CTSS* genes. Therefore, glutamine deprivation affect the expression level of most studied genes in relation to the functional activity of IRE1 enzyme, a central mediator of endoplasmic reticulum stress, which responsible for control of cell proliferation and tumor growth.

Key words: mRNA expression, HTRA1/PRSS11, LONP1/PRSS15, cathepsins, IRE1 knockdown, glutamine deprivation, U87 glioma cells.

The HtrA serine peptidase 1 (HTRA1) is a secreted enzyme that regulate the availability of insulin-like growth factors (IGFs) by cleaving IGF-binding proteins, inhibits signaling mediated by TGF-beta family members and thus has been suggested to be a regulator of cell proliferation [1–3]. HTRA1 overexpression exerts anti-tumor effect by blocking the NF- B signaling pathway [4]. The mitochondrial Lon peptidase 1 (LONP1), also known as serine protease 15 (PRSS15), is a mitochondrial matrix protein that belongs to the Lon family of ATP-dependent proteases and mediates the selective degradation of misfolded, unassembled or oxidatively damaged polypeptides as well as certain short-lived regulatory proteins in the mitochondrial matrix, but not aggregated proteins [5, 6]. It may also have a chaperone function in the assembly of protein complexes into inner membrane, and participate in the regulation of gene expression in mitochondria and maintenance of the integrity of the mitochondrial genome [7]. Lon peptidase 1 is not responsible for oncogenic transformation, but that is essential for proliferation and survival of cancer cells, because it is a key enzyme controlling mitochondrial bioenergetics in cancer [7, 8]. Therefore, this peptidase is an essential protein for life and that it also performs a critical function in tumorigenesis by regulating the bioenergetics of cancer cells as a central regulator of mitochondrial activity in oncogenesis [6]. Recently was shown that inhibition of Lon peptidase 1 by the triterpenoid, anticancer molecule, alters mitochondrial function and is associated to cell death in RKO human colon cancer cells [9]. Moreover, LONP1 expression is induced by various stimuli, including reactive oxygen species and hypoxia, and provides protection against cell stress [10]. Down-regulation of this enzyme is associated with organism ageing

and with cell senescence, while up-regulation is observed in cancer cells, and is correlated with a more aggressive phenotype of tumors [6]. Consequently, the mitochondrial Lon peptidase 1 is at the crossroads of oxidative stress, ageing and cancer. It was also shown that mitochondrial proteins, LONP1 and prohibitin, are over-expressed in HTRA2(−/−) mouse embryonic fibroblast cells and HTRA2 knock-down HEK293T cells, indicating that mitochondrial HTRA2 might be an upstream regulator of mitochondrial homeostasis [11].

A key role in cellular protein turnover has a group of lysosomal proteases, cathepsins, which play multiple roles in cancer and autophagy [12, 13]. Cathepsin A (CTSA), also known as chaperone protective protein and protective protein for beta-galactosidase, is a carboxypeptidase, which present at the cell surface, endoplasmic reticulum, nucleus and also secreted outside the cell. CTSA associates with these enzymes and exerts a protective function necessary for their stability and activity as well as involved in tumor progression and metastasis by degrading the extracellular matrix [13, 14]. It plays a significant role in the processing of endogenous bioactive peptides and is also involved in inhibition of chaperon-mediated autophagy [12]. In was recently shown that over-expression of CTSA associates with the cellular oxidative stress response [15].

Cysteine proteinase CTSB is also present at the cell surface, nucleus and mitochondrion, implicated in tumor invasion and metastasis as well as toll-like receptor (TLR) signaling pathway [16]. It was also shown that inhibition of cathepsin B activity by clioquinol-ruthenium complex impairs tumor cell invasion [17]. It is interesting to note that extracellular matrix remodeling by cell adhesion-related processes is critical for proliferation and tissue homeostasis [18]. Moreover, Huber et al. [19] shown that the UPAR (urokinase plasminogen activator receptor) interacts with CYR61 (cysteine-rich angiogenic inducer 61) and the YB-1 (Y-box-binding protein 1) in the triple-negative breast cancer and that both interactors significantly correlated with expression levels of cathepsin B and c-MET as well as the tumor grade. The expression level of CYR61 strongly correlated with cathepsin D level [19]. Cathepsin B also participates in autophagy, which mediates tumor suppression via cellular senescence [20].

Cysteine proteinase CTSC is associated with an enhanced degradation of glycosaminoglycans, proteoglycans, and glycoproteins, and results in their decreased tissue content. Its increased tissue activity is observed in many pathological conditions [11]. This cathepsin releases the glycosidases from complexes formed with cathepsin A, and reinstates their activity [11]. Furthermore, CTSB, CTSC, CTSD, and are increased in numerous tumors [21, 22]. It is interesting to note that matrix-metalloproteinase-9 is cleaved and activated by cathepsin K [23]. Moreover, almost identical substrate specificities were determined for cysteine cathepsins K, L and S [24].

The unfolded protein response/endoplasmic reticulum stress is responsible for enhanced cancer cell proliferation and knockdown of IRE1, a major signaling pathway of endoplasmic reticulum stress, by a dominant-negative construct of IRE1 (dnIRE) resulted in a significant anti-proliferative effect on glioma growth [25–27]. The rapid growth of solid tumors generates micro-environmental changes in association to nutrient deprivation, hypoxia, and acidosis, which promote neovascularisation, cell survival and proliferation [28–30]. IRE1 (inositol requiring enzyme-1) is a key regulator of cell life and death processes [28, 31]. Recently, we have shown that glutamine deprivation affects the expression of proliferation related genes in U87 glioma cells and that IRE1 knockdown modifies glutamine deprivation effects on these genes expression possibly contributing to suppression of glioma cells proliferation [32]. Previously, we have shown that most cathepsins as well as LONP1 are regulated by IRE1 signaling and hypoxia [33], but the precise mechanism of the exhibited by IRE1 knockdown is not clear yet.

Malignant gliomas are highly aggressive tumors with very poor prognosis and glutamine is important to development and a more agressive behaviour of these tumors [30, 34, 35]. However, mechanisms whereby cancer cells regulate glutamine catabolism remain largely unknown [32, 35, 36]. A better knowledge of tumor responses to glutamine deprivation condition is required to elaborate new therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms.

The aim of this study was investigation the effect of glutamine deprivation condition on the expression of HTRA1/PRSS11, LONP1/PRSS15, and a subset of cathepsins in glioma cells in relation to inhibition of IRE1, a major signaling enzyme of endoplasmic reticulum stress, with hopes of elucidating its mechanistic part in the development and progression of glioma and the contribution to unfolding protein response.

Materials and Methods

Cell Lines and Culture Conditions. In this study we used two sublines of U87 glioma cells, which are growing in high glutamine (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), streptomycin (0.1 mg/ml; Gibco) and penicillin (100 units/ml; Gibco) at 37 °C in a 5% CO_2 incubator. 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 (dnIRE1). This untreated subline of glioma cells (control glioma cells) was used as control 1 in the study of effects of glutamine deprivation on the expression level of PRSS15 and a subset of cathepsins mRNA. Second subline was obtained by selection of stable transfected clones with overexpression of dnIRE1 and has suppressed both protein kinase and endoribonuclease activities of this bifunctional sensing and signaling enzyme of endoplasmic reticulum stress. The expression level of all studied genes in these cells was compared with cells, transfected by vector (control 1). The subline, which overexpress dnIRE1, was also used as control 2 for investigation the effect of glutamine deprivation condition on the expression level of studied in cells with inhibited function of signaling enzyme IRE1. Clones were received by selection at 0.8 mg/ml geneticin (G418) and grown in the presence of this antibiotic at lower concentration (0.4 mg/ml). Glutamine deprivation condition were created by changing the complete DMEM medium into culture plates on the medium without glutamine (from Gibco) and plates were exposed to this condition for 16 h.

The suppression level of IRE1 both enzymatic activity in glioma cells that overexpress a dominant-negative construct of inositol requiring enzyme-1 was estimated previously [37] by determining the expression level of XBP1 alternative splice variant (XBP1s), a key transcription factor in IRE1 signaling, using cells treated by tunicamycin (0.01 mg/ml during 2 hrs). Efficiency of XBP1s inhibition was 95%. Moreover, the proliferation rate of glioma cells with mutated IRE1 is decreased in 2 fold [38]. Thus, the blockade of both kinase and endoribonuclease activity of signaling enzyme IRE1 has significant effect on proliferation rate of glioma cells.

RNA isolation. Total RNA was extracted from glioma cells using Trisol reagent (Invitrogen, USA) and chloroform as previously described [38]. The RNA was precipitated by equal volume of 2-propanol. 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. RNA concentration as well as spectral characteristics was measured using NanoDrop Spectrophotometer.

Reverse transcription and quantitative PCR analysis. QuaniTect Reverse Transcription Kit (QIAGEN, Hilden, Germany) was used for cDNA synthesis as described previously [38]. The expression level of HTRA1, LONP1, CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, and CTSS mRNA were measured in glioma cell line U87 and its subline (clone 1C5) by real-time quantitative polymerase chain reaction using "QuantStudio 5 Real-Time PCR System" (Applied Biosystems) and „RotorGene RG-3000" qPCR (Corbett Research, Germany) and Absolute qPCR SYBRGreen Mix (Thermo Fisher Scientific, ABgene House, Epsom, Surrey, UK). For amplification 40 cycles (94 °C — 20 s, 55 °C — 20 s and 72 °C — 20 s) were used. Polymerase chain reaction was performed in triplicate.

For quantitative polymerase chain reaction were used the pair of primers specific for each studied gene (Table). The primers were received from "Sigma-Aldrich" (St. Louis, MO, USA). The quality of amplification products was analyzed by melting curves and by electrophoresis using 2% agarose gel. An analysis of quantitative PCR was performed using special computer program "Differential Expression Calculator". The values of LONP1, CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, and CTSS mRNA expressions were normalized to the expression of beta-actin mRNA and represented as percent of control 1 (100%).

Statistical analysis. All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments. Statistical analysis was performed according to Student's *t*-test using Excel program as described previously [39].

Results and Discussion

To determine if glutamine deprivation regulates the genes of interest through the IRE1 branch of endoplasmic reticulum stress response, we investigated the effect

of glutamine deprivation condition on the expression of genes encoding HTRA1/PRSS11, LONP1/PRSS15, and a subset of cathepsins in U87 glioma cells in relation to knockdown of IRE1 signaling enzyme, which is a major component of the unfolded protein response/ endoplasmic reticulum stress. As shown in Fig. 1, the exposure of control glioma cells (transfected by empty vector) upon glutamine deprivation condition leads to up-regulation of LONP1 mRNA expression (+39%) as compared to cells growing with glutamine. The expression level of *CTSD, CTSF, CTSO,* and *CTSS* genes is also up-regulated at this experimental condition: +64, +34, +18, and +27%, correspondingly, as compared to control cells. At the same time, the exposure of control glioma cells to glutamine deprivation condition leads to down-regulation of *HTRA1*, *CTSC,* and *CTSK* gene expressions: −19, −26, and −15%, correspondingly (Fig. 1). Furthermore, the expression of *CTSA, CTSB,* and *CTSL* genes in control glioma cells was resistant to glutamine deprivation.

As shown in Fig. 2, inhibition of IRE1 signaling enzyme function in U87 glioma cells leads to significant up-regulation of *LONP1, CTSD, CTSF,* and *CTSS* gene expressions (+59, +38, +40, and +55%, correspondingly), as compared to control dnIRE1 expressed glioma cells. At the same time, the expression of *HTRA1* and *CTSO* genes does not change significantly upon glutamine deprivation in glioma cells without functional activity of signaling enzyme IRE1. Results, presented in Fig. 2, also demonstrate that exposure of glioma cells (transfected by dnIRE1) upon glutamine deprivation condition leads to down-regulation of CTSC and CTSK mRNA expression (−31 and −19%, correspondingly) as compared to cells growing with glutamine. Therefore, inhibition of IRE1 signaling enzyme function in U87 glioma cells by dnIRE1 introduces the sensitivity of *CTSL* gene expression to glutamine deprivation (+35%).

As shown in Fig. 3 and 4, inhibition of IRE1 signaling enzyme function in U87 glioma cells modifies the effect of glutamine deprivation on the expression of *HTRA1, LONP1, CTSD, CTSL, CTSO,* and *CTSS* genes and does not change significantly the sensitivity of *CTSA, CTSB, CTSF,* and *CTSK* gene expressions to glutamine deprivation condition. Thus, IRE1 knockdown in U87 glioma cells significantly

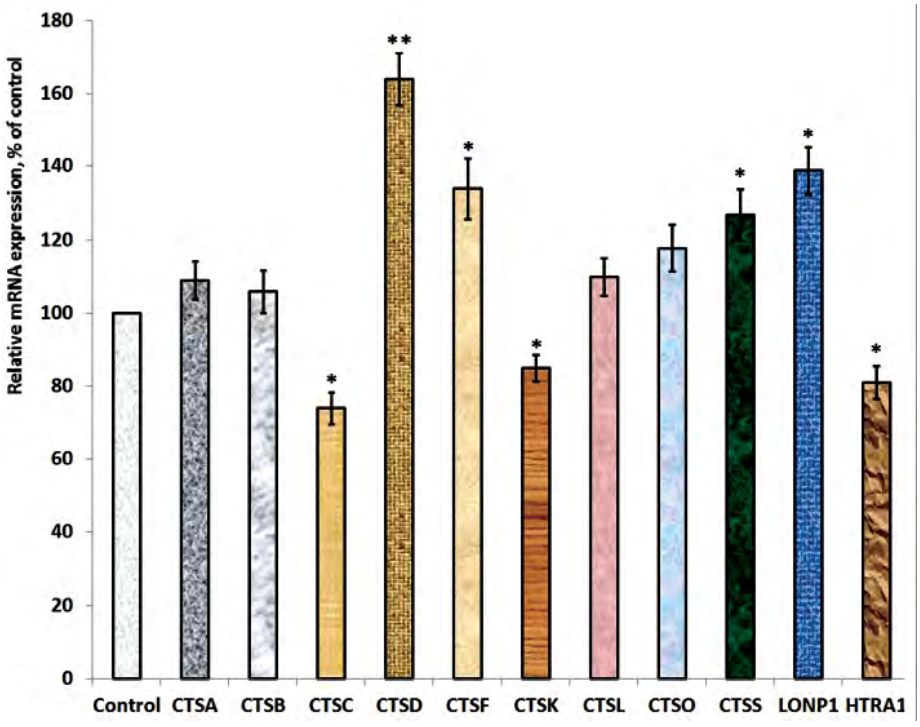

Fig. 1. **Effect of glutamine deprivation on the expression level of CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, CTSS, LONP1, and HTRA1 mRNA in control U87 glioma cells stable transfected with vector measured by qPCR. Values of these mRNA expressions were normalized to beta-actin mRNA and represented as percent of control (cells growing with glutamine; 100%);**
hereinafter: mean ± SEM; * — $P < 0.05$ versus control

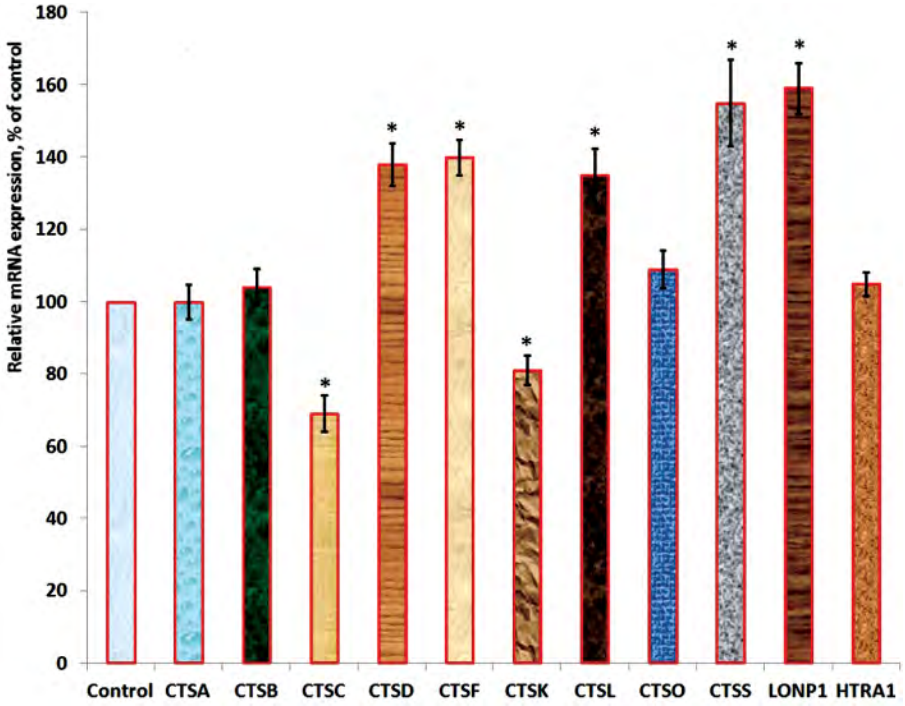

Fig. 2. **Effect of glutamine deprivation on the expression level of CTSA, CTSB, CTSC, CTSD, CTSF, CTSK, CTSL, CTSO, CTSS, LONP1, and HTRA1 mRNA in U87 glioma cells stable transfected with dnIRE1 measured by qPCR. Values of these mRNA expressions were normalized to beta-actin mRNA and represented as percent of control (dnIRE1 cells growing with glutamine; 100%)**

enhances effect of glutamine deprivation on the expression of *LONP1* and *CTSS* genes, but removes the sensitivity of *HTRA1* and *CTSO* genes to glutamine deprivation. The expression of CTSL mRNA was resistant to glutamine deprivation in control glioma cells with functionally active IRE1, but glutamine deprivation introduces sensitivity of this gene expression to glutamine deprivation (Fig. 4), indicating IRE1-dependent up-regulation of this gene expression by glutamine. At the same time, as shown in Fig. 3, the sensitivity of *CTSD* gene expression to glutamine deprivation is IRE1-dependent, because IRE1 knockdown significantly reduces the effect of glutamine deprivation on the expression of *CTSD* gene. Additionally, we found that the expression of genes encoding for cathepsin A and B are resistant to glutamine deprivation condition both in glioma cells containing dnIRE1 and cells with IRE1 knockdown.

Thus, this study has demonstrated that glutamine deprivation affects the expression of the majority of the genes encoding cathepsins as well as HTRA1/PRSS11 and LONP1/PRSS15 preferentially in the IRE1-dependent manner and that these genes potentially contribute to regulation of cell proliferation, apoptosis, and metastasis.

The endoplasmic reticulum stress is responsible for enhanced cancer cell proliferation and knockdown of IRE1, a major signaling pathway of endoplasmic reticulum stress, resulted in a significant anti-proliferative effect on glioma cell proliferation and tumor growth [35, 37, 38]. Our results demonstrate that IRE1 knockdown eliminates the suppressive effect of glutamine deprivation on the expression of *HTRA1* gene. The *HTRA1* gene overexpression exerts anti-tumor effect by blocking the NF-κB signaling pathway and regulates the availability of insulin-like growth factors (IGFs) by cleaving IGF-binding proteins, and thus has been suggested to be a regulator of cell proliferation [1–4]. Thus, our data correlates well with these results. It is known that LONP1 have variable functions [5, 6] and increased level of this gene transcript both in control and IRE1 knockdown glioma cells upon glutamine deprivation can be responsible for selective degradation of misfolded and certain short-lived regulatory proteins in the mitochondrial matrix, which should be induced by glutamine deprivation [26]. It is possible that the regulation of this gene expression by glutamine deprivation is mediated by IRE1 and blockade of this signaling enzyme function enhances the sensitivity of

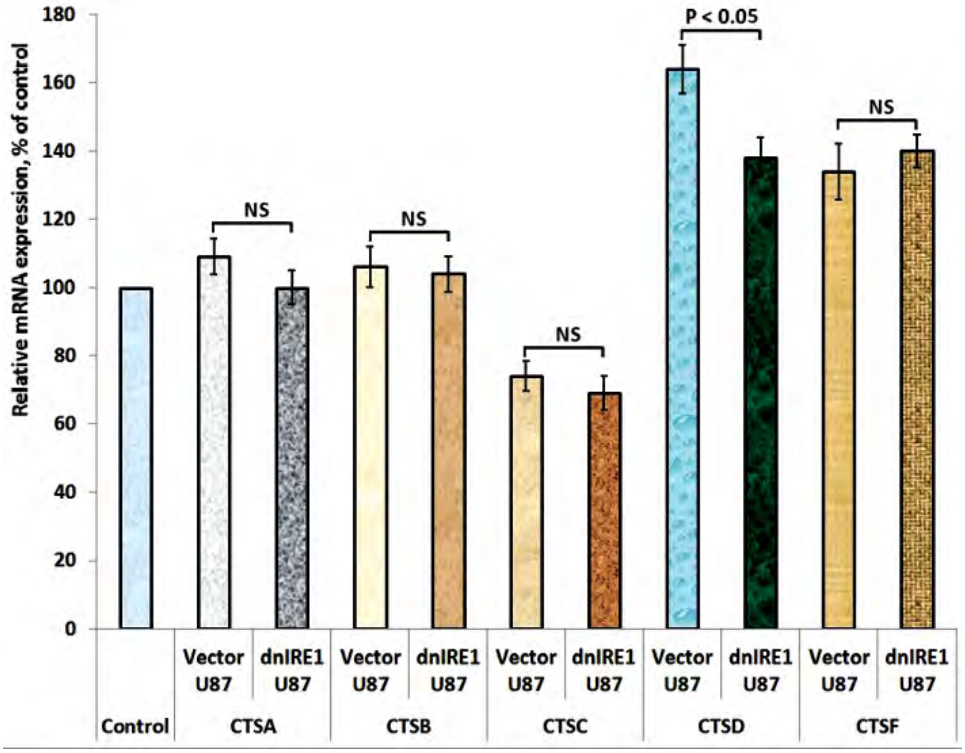

Fig. 3. **Relative effect of glutamine deprivation on the expression level of CTSA, CTSB, CTSC, CTSD, and CTSF genes in two types of glioma cells:**
control cells transfected by vector (Vector) and cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR. Values of these gene expressions were normalized to beta-actin and represented as percent of corresponding control (control for both cell types is accepted as 100%); hereinaffter: NS — no significant changes

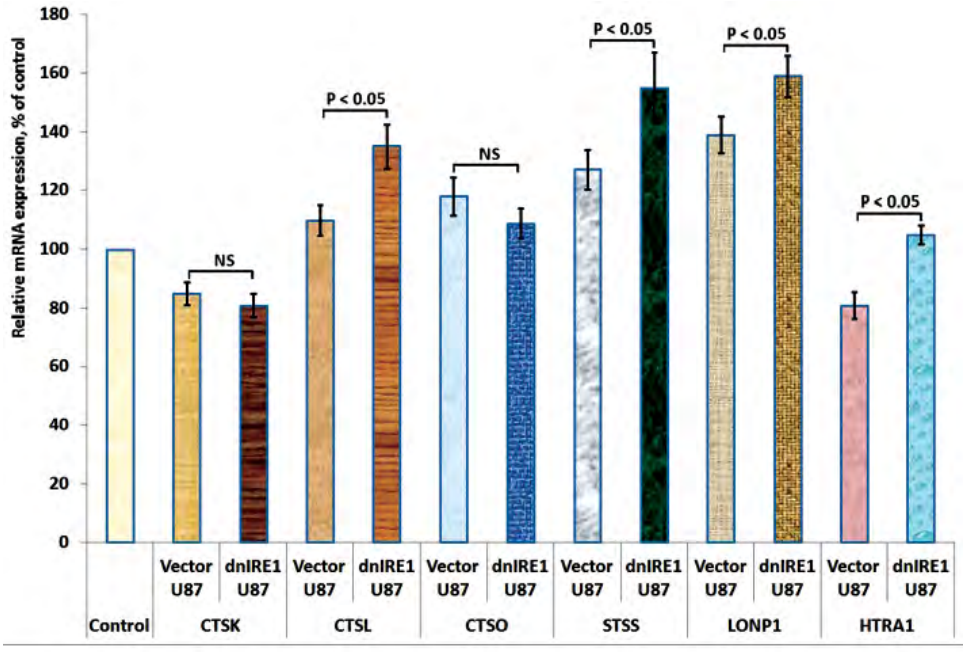

Fig. 4. **Relative effect of glutamine deprivation on the expression level of CTSK, CTSL, CTSO, CTSS, LONP1/PRSS15, and HTRA1/PRSS11 genes in two types of glioma cells:** control cells transfected by vector (Vector) and cells with a deficiency of the signaling enzyme IRE1 (dnIRE1) measured by quantitative PCR. Values of these gene expressions were normalized to beta-actin and represented as percent of corresponding control (control for both cell types is accepted as 100%)

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

Gene symbol	Gene name and EC number (Enzyme Commission number)	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
HTRA1 (PRSS11)	HtrA serine peptidase 1 (serine protease 11, IGF binding) EC_number="3.4.21.108"	F: 5′- tggaatctcctttgcaatcc R: 5′- acgctcctgagatcacgtct	1175–1194 1365–1346	NM_002775
LONP1 (PRSS15)	mitochondrial lon peptidase 1 (hLON ATP-dependent protease; serine protease 15) EC_number="3.4.21.53"	F: 5′- atctacctgagcgacatggg R: 5′- ttacggtgggtctgcttgat	1111–1130 1304–1285	NM_004793
CTSA	cathepsin A (chaperone protective protein; carboxypeptidase C) EC_number="3.4.16.5"	F: 5′- cagctgcttccacctacctc R: 5′- cttctggttgagggaatcca	1432–1451 1682–1663	NM_000308
CTSB	cathepsin B EC_number="3.4.22.1"	F: 5′- caagccaccccagagagtta R: 5′- tagaggccaccagaaaccag	360–379 680–661	NM_001908
CTSC	cathepsin C EC_number="3.4.14.1"	F: 5′- tcagaccccaatcctaagcc R: 5′- gcatggagaatcagtgcctg	949–968 1108–1089	NM_001814
CTSD	cathepsin D EC_number="3.4.23.5"	F: 5′- caagttcgatggcatcctgg R: 5′- cgggtgacattcaggtagga	712–731 930–911	NM_001909
CTSF	cathepsin F EC_number="3.4.22.41"	F: 5′- aggagctcttggactgtgac R: 5′- tagaccttggccttctctgc	1052–1071 1217–1198	NM_003793
CTSK	cathepsin K EC_number="3.4.22.38"	F: 5′- gctcaaggttctgctgctac R: 5′- tcttcactggtcatgtcccc	238–257 483–464	NM_000396
CTSL	cathepsin L EC_number="3.4.22.15"	F: 5′- acagcttcacaatggccatg R: 5′- aagcccaacaagaaccacac	562–581 717–698	NM_001912
CTSO	cathepsin O EC_number="3.4.22.42"	F: 5′- attatggctgcaatggaggc R: 5′- gggccaaaggtaagaagtgc	549–568 768–749	NM_001334
CTSS	cathepsin S EC_number="3.4.22.27"	F: 5′- aacaagggcatcgactcaga R: 5′- aagaaagaaggatgacgcgc	272–291 468–449	NM_004079
ACTB	beta-actin	F: 5′- ggacttcgagcaagagatgg R: 5′- agcactgtgttggcgtacag	747–766 980–961	NM_001101

the expression of *LONP1* gene to glutamine deprivation. Thus, the increased expression of the *LONP1* gene upon glutamine deprivation is agreed well with functional role of this protease [7, 8]. It is possible that the regulation of this gene expression by glutamine deprivation is mediated by other signaling pathways of endoplasmic reticulum stress like *ATF3*, *HOXC6*, and *FOXF1* genes [40]. Therefore, LONP1 protease is an essential enzyme for life and plays a critical function in tumorigenesis by regulating the bioenergetics of cancer cells as a central regulator of mitochondrial activity in oncogenesis. Furthermore, the expression of this mitochondrial protease is induced by various stimuli, including hypoxia and reactive oxygen species, and possibly provides protection against cell stress [6].

Glutamine deprivation is reduced the expression level of *CTSC* gene, which is a tissue-specific regulator of carcinogenesis [21], in glioma cells independently of IRE1 activity and possibly contributes in suppression of tumor growth. We have also shown that the expression of cathepsin D, which has multiple roles in cancer [19, 22], is also increased both in control and IRE1 knockdown glioma cells upon glutamine deprivation, but inhibition of IRE1 signaling enzyme in glioma cells decreases the sensitivity of this gene expression to glutamine

deprivation. It is possible that this decrease of sensitivity of *CTSD* gene expression to glutamine deprivation upon IRE1 inhibition can contribute to the suppression of proliferation of glioma cell without IRE1 function [37, 38]. Moreover, almost all cathepsins have specific functions and consequently diverse changes upon glutamine deprivation [41, 42].

In conclusion, our results demonstrate that the majority of the genes studied are both responsive to glutamine deprivation in IRE1 dependent manner and potentially contribute to regulation of cell proliferation, metastasis, and apoptosis through various signaling pathways and stress related transcription, but the mechanisms and functional significance of activation of their expression through IRE1 inhibition as well as glutamine deprivation are different and warrant further investigation. Thus, the changes observed in the studied genes expression partially agree with slower proliferation rate of glioma cells harboring dnIRE1, attesting to the fact that targeting the unfolded protein response is viable, perspective approach in the development of cancer therapeutics, because glutamine starvation by glutaminase inhibitor, transporter inhibitor, or glutamine depletion has shown to have significant anti-cancer effect in pre-clinical studies [28, 37, 43, 44].

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ПРИГНІЧЕННЯ IRE1 ЗМІНЮЄ ЕФЕКТ ДЕФІЦИТУ ГЛУТАМІНУ НА ЕКСПРЕСІЮ ГРУПИ ПРОТЕАЗ У КЛІТИНАХ ГЛІОМИ ЛІНІЇ U87

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Метою роботи було вивчити вплив дефіциту глутаміну на експресію генів, що кодують HTRA1/PRSS11, LONP1/PRSS15 та деякі катепсини у клітинах гліоми лінії U87 за умов пригнічення IRE1 (inositol requiring enzyme-1). Показано, що у контрольних (трансфікованих пустим вектором) клітинах гліоми дефіцит глутаміну посилював експресію генів *LONP1, CTSD, CTSF, CTSO* та *CTSS*, пригнічував експресію генів *HTRA1*, *CTSC* і *CTSK*, але істотно не впливав на експресію генів *CTSCA, CTSB* та *CTSL*. Пригнічення функції сигнального ензиму IRE1 у клітинах гліоми лінії U87 змінювало ефект дефіциту глутаміну на експресію генів *HTRA1, LONP1, CTSD, CTSL, CTSO* та *CTSS*: знімало ефект дефіциту глутаміну на гени *HTRA1* та *CTSO*, індукувало — на ген *CTSL*, зменшувало — на ген *CTSD* і посилювало — на гени *LONP1* та *CTSL*. Таким чином, дефіцит глутаміну змінював рівень експресії більшості досліджених генів залежно від функціональної активності ензиму IRE1, центрального медіатора стресу ендоплазматичного ретикулума, який відповідає за контроль проліферації клітин та росту пухлин.

Ключові слова: експресія мРНК, HTRA1/PRSS11, LONP1/PRSS15, катепсини, пригнічення IRE1, дефіцит глутаміну, клітини гліоми лінії U87.

УГНЕТЕНИЕ IRE1 ИЗМЕНЯЕТ ЭФФЕКТ ДЕФИЦИТА ГЛУТАМИНА НА ЭКСПРЕССИЮ ГРУППЫ ПРОТЕАЗ В КЛЕТКАХ ГЛИОМЫ ЛИНИИ U87

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Целью работы было изучить влияние дефицита глутамина на экспрессию генов, кодирующих HTRA1/PRSS11, LONP1/PRSS15 и некоторые катепсины в клетках глиомы линии U87 при угнетении IRE1 (inositol requiring enzyme-1). Показано, что в контрольных (трансфецированных пустым вектором) клетках глиомы дефицит глутамина усиливал экспрессию генов *LONP1, CTSD, CTSF, CTSO* и *CTSS*, угнетал экспрессию генов *HTRA1*, *CTSC* и *CTSK*, но существенно не влиял на экспрессию генов *CTSA, CTSB* и *CTSL*. Угнетение функции сигнального энзима IRE1 в клетках глиомы линии U87 изменяло эффект дефицита глутамина на экспрессию генов *HTRA1, LONP1, CTSD, CTSL, CTSO* и *CTSS*: снимало эффект дефицита глутамина на гены *HTRA1* и *CTSO*, индуцировало — на ген *CTSL*, уменьшало — на ген *CTSD* и усиливало — на гены *LONP1* и *CTSL*. Таким образом, дефицит глутамина изменял уровень экспрессии большинства изученных генов в зависимости от функциональной активности энзима IRE1, центрального медиатора стресса эндоплазматического ретикулума, отвечающего за контроль пролиферации клеток и роста опухолей.

Ключевые слова: экспрессия мРНК, HTRA1/PRSS11, LONP1/PRSS15, катепсини, угнетение IRE1, дефицит глутамина, клетки глиомы линии U87.