Dominant-Negative Constructs of IRE-1alpha as an Effective way to Suppression of Tumor Growth through the Inhibition of Cell Proliferation

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Abstract: Activation of cell proliferation and angiogenesis as well as the down-regulation of apoptosis are important for tumor growth through pathways of the unfolding protein response/endoplasmic reticulum stress, a fundamental phenomenon for secure protection of cells by maintaining the functional integrity of the endoplasmic reticulum. It is mediated by three sensor and signaling pathways: IRE-1α/ERN1 (inositol-requiring enzyme-1α/endoplasmic reticulum to nuclei 1), ATF6 (activating transcription factor 6), and PERK (double stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK). All three arms of the unfolded protein response are important for tumor cell survival and growth especially under hypoxic conditions, but the unfolded protein response signaling is mainly mediated through the ERN1 pathway.The inhibition of ERN1 by its dominant-negative constructs leads to a decrease of tumor growth through suppression of angiogenesis and cell proliferation as well as activation of apoptosis and tumor suppressors. Data concerning the molecular mechanisms of the effect a blockade of ERN1 signaling enzyme has on glioma growth is analyzed, including the expression of genes controlling angiogenesis, cell proliferation, cell cycle, and apoptosis. Moreover, the inhibition of ERN1 endoribonuclease only has more profound effect on the expression of most key regulatory genes as well as on cell proliferation than the blockade of both kinase and endonuclease activity of ERN1 in glioma cells. In conclusion, the inhibition ofERN1/IRE-1alpha coordinately regulated factors involved in tumor growth, lowering expression levels of pro-proliferative, pro-angiogenic and anti-apoptotic factors and enzymes and up-regulated the expression of anti-proliferative, anti-angiogenic and pro-apoptotic factors in a trend towards the level of these transcription factors in normal human astrocytes. These review attempts to summarize recent advances in the role of inhibition of ERN1 signalingby dominant/negative strategies in regulation of proliferation and apoptosis related genes and suppression of tumor growth, which will help to define the best therapeutic targets for the design of potent antitumor drug.

Keywords: Tumor growth, endoplasmic reticulum stress, inhibition of IRE-1α/ERN1, U87 glioma cells, angiogenesis, apoptosis, cell cycle, tumor suppressors.

INTRODUCTION

The endoplasmic reticulum is a dynamic intracellular organelle with exquisite sensitivity to alterations in homeostasis, and provides stringent quality control systems to ensure that only correctly folded proteins transit to the Golgi and unfolded or misfolded proteins are retained and ultimately degraded. The endoplasmic reticulum stress is a fundamental phenomenon for secure protection of cells by maintaining the functional integrity of the endoplasmic reticulum [1-3]. Malignant tumors use the endoplasmic reticulum stress response as well as hypoxia-induced signaling pathways to enhance tumor cells proliferation under stressful environmental conditions [4-6]. The rapid growth of solid tumors generates micro-environmental changes in association to nutrient deprivation, hypoxia, and acidosis, which induce cell proliferation and new blood vessels formation mainly through the activation of unfolding protein response/endoplasmic reticulum stress signalling pathways [4,7]. Moreover, the activation of these signalling pathways are important for tumor growth through the up-regulation of angiogenesis, cell proliferation and down-regulation of cell apoptosis [4,8].

UNFOLDING PROTEIN RESPONSE/ENDOPLASMIC RETICULUM STRESS SIGNALING

The unfolded protein response is mediated by at least three sensor and signaling pathways: IRE-1α/ERN1 (inositol-requiring enzyme-1α/endoplasmic reticulum to nuclei 1), ATF6 (activating transcription factor 6), and PERK (double stranded RNA activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) [3,9]. All three arms of the stress are integrated and important for tumor growth and cell survival especially under hypoxic and nutrient deprivation conditions; however, this stress signaling is mainly mediated through the IRE-1α/ERN1 pathway. Moreover, ERN1 pathway is the most evolutionary conversed and important sensor of the unfolded protein response to the accumulation of misfolded proteins and

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represents a key regulator of the life and death processes [4,5,10]. A better understanding of tumor responses to endoplasmic reticulum stress is required to elaborate therapeutical strategies of cell sensibilization, based on the blockade of survival mechanisms [4,11,12].

The IRE-1α/ERN1 enzyme is localized in the endoplasmic reticulum membrane and its N-terminus as sensor is localized in the lumen of endoplasmic reticulum and interact with chaperons, preferentially with HSPA5/BiP/GRP78 [13,14]. This chaperon functions as negative regulator of all sensing and signaling systems of endoplasmic reticulum stress, because it is associated with all three sensors in normal condition. TheIRE-1α/ERN1 enzyme is a bifunctional enzyme which has also cytoplasmic domain for two enzymatic activities: serine/threonine kinase and endoribonuclease [6,16]. The ERN1 protein kinase is activated upon induction of the endoplasmic reticulum stress and autophosphorylates ERN1 [17]. This results in the activation and dimerization of ERN1 in the endoplasmic reticulum membrane as well as in the activation of endoribonuclease. The main function of ERN1 endoribonuclease is alternative splicing of XBP1 pre-mRNA by excision of 26 bp fragment from the coding part. Resulting alternative splice variant of XBP1 encodes a bigger transcription factor with modified C-terminus. This splice variant of XBP1 is responsible for regulation of the expression of numerous genes encoded proteins for protein folding and degradation of unfolded proteins as well as affects broad aspects of cell fate and the metabolism of proteins, amino acids and lipids [18,19]. The activity of XBP1 splice variant is regulated by kinases and by interaction with other transcription factors [20-22]. The ERN1 endoribonuclease is also responsible for selective degradation of some mRNA upon endoplasmic reticulum stress conditions [23-26]. It is possible that this function of ERN1 endoribonuclease is very important in selective suppression of some signaling pathways. Thus, autophosphorylation of ERN1 by kinase is necessary for activation of ERN1 endoribonuclease; however, there is data that kinase inhibition by specific inhibitor activates endoribonuclease to confer cytoprotection against endoplasmic reticulum stress [27,28]. Therefore, the endoplasmic reticulum stress is a regulatory mechanism that allows cells to adapt to a series of metabolic, redox, and other environmental changes as well as directly influences life/death decisions at a cellular level.

During endoplasmic reticulum stress, homeostatic signaling through the unfolded protein response augments endoplasmic reticulum protein-folding capacity. If homeostasis is not restored, the unfolded protein response triggers apoptosis and ERN1 is a key component of this apoptotic switch. Under endoplasmic reticulum stress ERN1's endoribonuclease also causes endonucleolytic decay of chaperones, as early events culminating in apoptosis [12,28]. At the same time, the high level of chaperone expression in malignant tumor cells is considerably responsible for these cells surviving through suppression of apoptosis [29,30]. The bifunctional enzyme ERN1 has also an important additional function. Thus, recently was shown that peptides derived from this bifunctional enzyme can modulate ERN1 activity and protect cells from endoplasmic reticulum stress [31].

INHIBITION OF IRE-1α/ERN1 BY DOMINANT-NEGATIVE CONSTRUCTS SUPPRESSES GLIOMA GROWTH

Two dominant-negative constructs of IRE-1α/ERN1 was created for investigation of a role of ERN1 signaling in the control of cell proliferation and tumor growth: dn-ERN1, which has a sensor luminal part and a transmembrane domain without kinase and endoribonuclease domains, and dnr-ERN1, which has a mutation in endoribonuclease (Figure **1**).

The inhibition of IRE-1α/ERN1 in U87 glioma cells by its dominant-negative construct (dn-ERN1) has been shown to result in a significant anti-proliferative effect in glioma growth (Figure **2**) through suppression of angiogenesis and cell proliferation [11,33,34]. This is due to down-regulation of prevalent pro-angiogenic factors and up-regulation of anti-angiogenic genes, both *in vitro* and in the CAM (chorio-allantoic membrane) model, as well as in mice engrafted intracerebrally with U87 glioma cell clones. It was shown that A549/8 and U87 cells expressing a dominant-negative ERN1 transgene as well as ERN1-knockout mouse embryonic fibroblasts were unable to trigger VEGF-A up-regulation upon either oxygen or glucose deprivation [33-36]. This data therefore suggestan essential role for IRE-α-dependent signaling pathways inresponse to ischemia and identify this protein as a potential therapeutic target to control both the angiogenic switch and tumor development, because ERN1 is a common determinant linking hypoxia and unfolded protein responses to the up-regulation of vascular endothelial growth factor-A (VEGF-A) and other pro-proliferative factors [4,37].

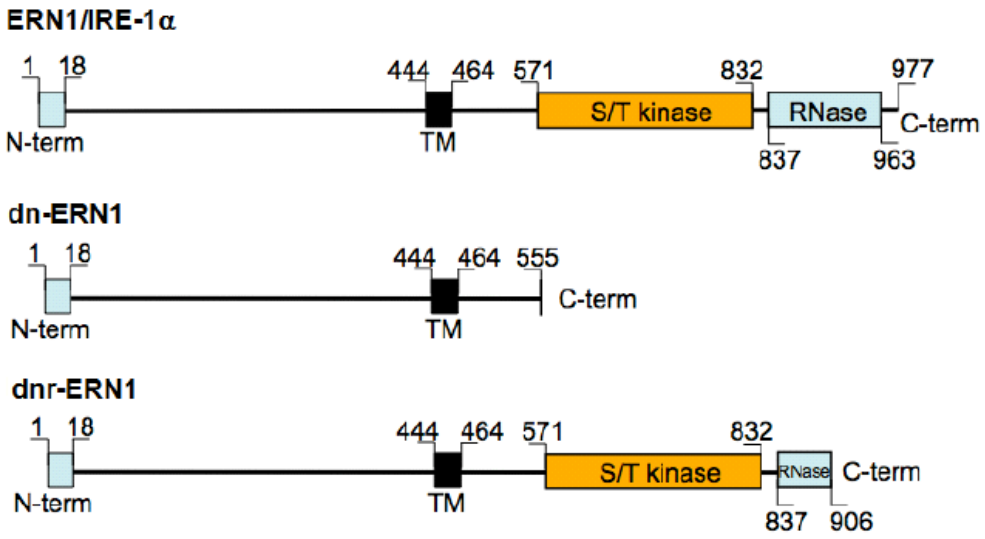

Figure 1: Schematic view of native ERN1/IRE-1α and its dominant-negative constructs: dn-ERN1 without kinase and endoribonuclease domains and dnr-ERN1, which expresses an IRE-1α protein truncated at its cytoplasmic C-terminal end in the RNase domain [11,32].

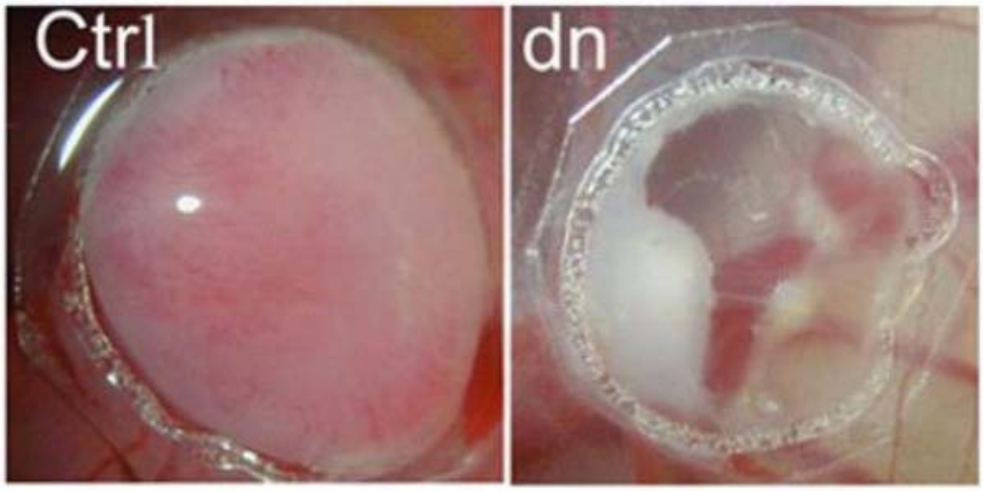

Figure 2: The tumor growth from IRE-1α-deficient (dn) versus control (Ctrl) U87 glioma cells deposited onto the chicken CAM for 4 days [11].

Therefore, in a human glioma model, inhibition of ERN1 by stable overexpression of dn-ERN1 correlated with down-regulation of other pro-angiogenic factors such as interleukins IL-1beta, IL-6, and IL-8 and significant up-regulation of anti-angiogenic factors such as SPARC, CTGF, HSPG2, decorin, thrombospondin-1, and several other extracellular matrix proteins functionally linked to mesenchymal differentiation as well as glioma invasiveness [34,38]. These changes were correlated with *in vivo* reduction of angiogenesis and blood perfusion, a decreased growth rate and blood vessel cooption both in the chick chorio-allantoic membrane assay and in the mouse orthotopic brain model [34]. Moreover, this phenotypic change is consistently associated with increased overall survival in glioma-implanted recipient mice. It is interesting to note that ectopic expression of IL-6 in ERN1-deficient tumors restored angiogenesis and neutralized vessel cooption but did not reverse the mesenchymal/infiltrative cell phenotype [34]. At the same time, an angiogenesis is a complex network and is regulated by hundreds of pro-angiogenic and anti-angiogenic factors. Thus, CD138-purified myeloma cells from 300 untreated patients do not show a significantly higher median number of expressed pro-angiogenic (45) or anti-angiogenic (31) genes, but almost all of these myeloma cells samples aberrantly express at least one of the angiogenic factors: HGF (hepatocyte growth factor), IL-15 (interleukin 15), ANG (angiogenin), FNFSF13/APRIL (tumor necrosis factor

(ligand) superfamily, member 13/a proliferation-inducing ligand), CTGF (connective tissue growth factor), also known as NOV2(nephroblastoma overexpressed 2) or TGFA (transforming growth factor, alpha) [39].

INHIBITION OF IRE-1α/ERN1 BY dn-ERN1 SUPPRESSES GLIOMA GROWTHTHROUGH DRAMATICALLY REDUCED EREG EXPRESSION

Recently was shown that epidermal growth factor (EGF) receptor ligand epiregulin (EREG) contribute to the development of malignant glioma in relation to the activity of the unfolded protein sensor IRE-1α through EGF receptor ErbB1/HER1 [11]. Thus, the high-expression rate of EREG in U87 cells was therefore linked to IRE-1α, because its inhibition by dn-ERN1 dramatically reduced EREG expression both in cell culture and in human xenograft tumor models (Figure **3**) as well as suppressed glioma cell proliferation (Figure **4**). Moreover, a stimulatory autocrine loop mediated by EREG was evidenced by the decrease in cell proliferation using specific blocking antibodies directed against either ErbB1 (cetuximab) or EREG itself [11].

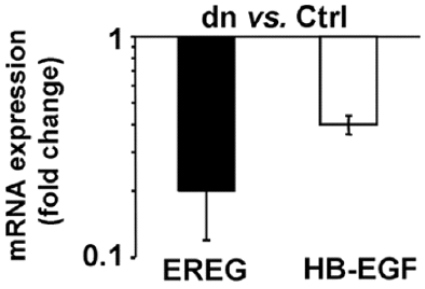

Figure 3: Expression of *EREG* and *HB-EGF* genes in IRE-1α-deficient (dn) versus control (Ctrl) U87 glioma cells [11].

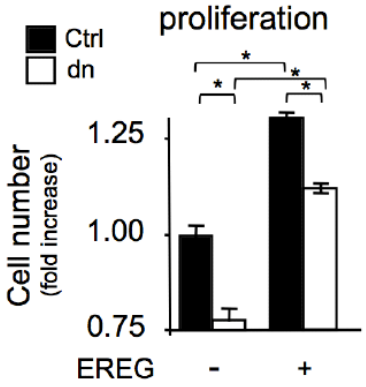

Figure 4: Effects of EREG on IRE-1α-deficient (dn) versus control (Ctrl) U87 glioma cell proliferation. In the proliferation assay, cells were grown for four days [11]. The total cell number was reported as fold-increase of the standard value (1.00) obtained withU87Ctrl cells in the absence of EREG; *-$p < 0.05$.

In addition, IRE1-mediated production of EREG did not depend on IRE-1α RNase domain, as neither the selective dominant-negative invalidation of the RNase activity by dnr-ERN1 (IRE-1α kinase active) nor the siRNA-mediated knockdown of XBP1 had significant effect on EREG expression [11]. Finally, chemical inhibition of c-Jun N-terminal kinases (JNK) by the SP600125 compound reduced the ability of U87 cells to express EREG, demonstrating a link between the growth factor production and JNK activation under the dependence of IRE-1α. It is interesting to note that EGF receptor also suppresses the maturation of specific tumour-suppressor-like miRNAs in response to hypoxic stress through phosphorylation of argonaute 2 [40].

INHIBITION OF IRE-1α/ERN1 AND CELL CYCLE REGULATION

Furthermore, the IRE-1α/ERN1 arm of unfolded protein response controls cell cycle gene expressions and inhibition of IRE-1α by dn-ERN1 also significantly affects the expression of numerous genes, which participate in cell cycle regulation and cell proliferation [41-48]. Thus, a blockade of the endoplasmic reticulum stress sensor IRE-1α by dn-ERN1 changes the expression of numerous cyclin genes in U87 glioma cells: down-regulates the expression of cyclin D1, which forms a complex with, and functions as a regulatory subunit of cyclin-dependent kinases 4 or 6, whose activity is required for cell cycle G1/S transition and may contribute to tumorigenesis, and up-regulates the expression of cyclin G2, which appears to be a negative cell-cycle regulator in some cancers [42,43,49]. It is important to note that the expression of both growth arrest-specific genes (GAS1 and GAS6) is strongly up-regulated in glioma cells without IRE-1 kinase and ribonuclease activities (cells overexpressed dn-ERN1) and down-regulated in hypoxia (Figure **5**) [42]. Thus, suppressive effect of IRE-1α by dn-ERN1 on cell proliferation and tumor growth [11,34] possibly mediated by down-regulation of the expression of the pro-proliferative cyclin D1 and up-regulation of a negative cell-cycle regulator cyclin G2 as well as growth arrest-specific genes GAS1 and GAS6.

Furthermore, an inhibition of the ERN1 enzyme function by dn-ERN1 also affects the expression of POLO-like kinase gene family in U87 glioma cells: down-regulates PLK1 and up-regulates PLK2 and PLK4 [44]. This changes possibly mediated by ERN1 kinase, because the inhibition of ERN1 endoribonuclease by dnr-ERN1 does not change the

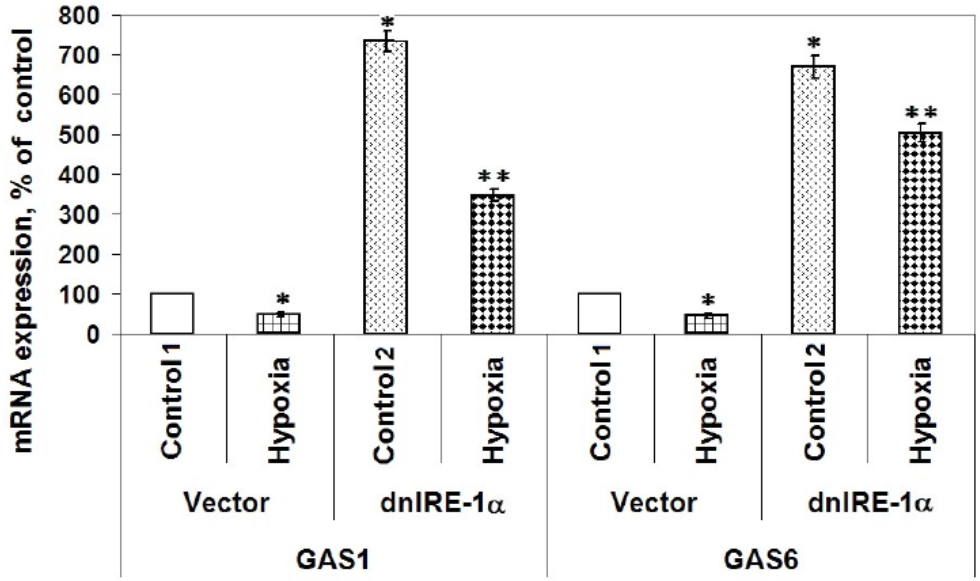

Figure 5: Expression of growth arrest-specific genes *GAS1* and *GAS6* in glioma cell line U87 and its subline with IRE-1-deficiency measured by qPCR: effect of hypoxia (3 % oxygen – 16 hours); * - P < 0.05 versus control 1 (Vector); ** - P < 0.05 versus control 2 (dnIRE-1α) [42].

expression of these genes in U87 glioma cells. Moreover, knockdown of ERN1 by dn-ERN1 inU87 glioma cells modifies the hypoxic regulation of POLO-like kinase gene expressions [44]. POLO-like kinases play an important role in cell cycle regulation and participate in tumorigenesis [50]. PLK1 is highly expressed in a broad spectrum of human tumors, strongly promotes progression of the cell cycle and is responsible for aggressive proliferation of tumor cells. Thus, down-regulation of PLK1 gene expression in glioma cells without ERN1 signaling enzyme function possibly contributes to suppression of these cell proliferation and glioma growth [11,34]. This data correlates to results Harris *et al*. [51] that POLO-like kinase 1 inhibition suppresses medulloblastoma cell growth.

ANTI-PROLIFERATIVE EFFECT OF IRE-1α INHIBITION THROUGH UP-REGULATION OF TUMOR SUPPRESSOR GENES

It is possible that anti-proliferative effect of ERN1/IRE-1α blockade is also associated with specific changes in the expression of retinoblastoma and retinoblastoma-related genes as well as circadian genes [46,47]. Thus, the knockdown of IRE-1α by dn-ERN1 leads to up-regulation of the expression of retinoblastoma and retinoblastoma-like 1 (Figure **6**) as well as most retinoblastoma related genes: *EID1, JARID1B, E2F1, E2F3, RBAP48* and *CTIP*, which possibly plays an important role in suppression of glioma cell proliferation [46]. At the same time, hypoxia decreases the expression levels of retinoblastoma-like 1 and most retinoblastoma-related genes (*E2F3, RBAP46, RBAP48* and *CTIP*), but ERN1 knockdown mainly modulates hypoxic regulation [46]. Furthermore, the inhibition of IRE-1α by dn-ERN1 affects the expression most circadian genes in different ways: up-regulates the expression of *PER1, PER3,* and *CLOCK* genes and down-regulates the *CRY1, PER2*, and *ARNTL* genes [47]. Moreover, hypoxia also has different effects on the expression levels of circadian genes and these effects are dependent on ERN1 signaling enzyme function [47]. The expression and function of most circadian genes are controlled by casein kinases and blockade of IRE-1α by dn-ERN1

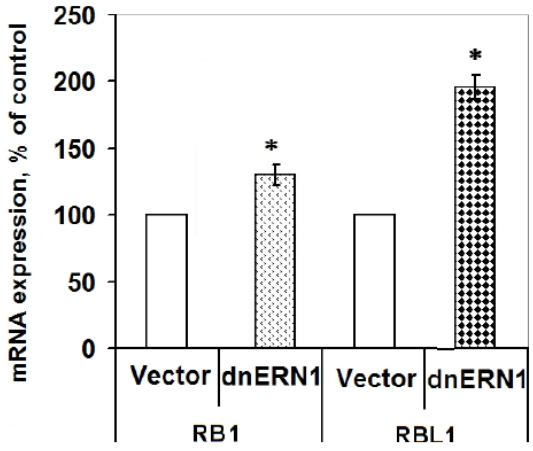

Figure 6: Expression of *RB1* and *RBL1* genes in control glioma cell line U87 (Vector) and its subline with IRE-1-deficiency (dnERN1) measured by qPCR; * - P < 0.05 versus control (Vector) [46].

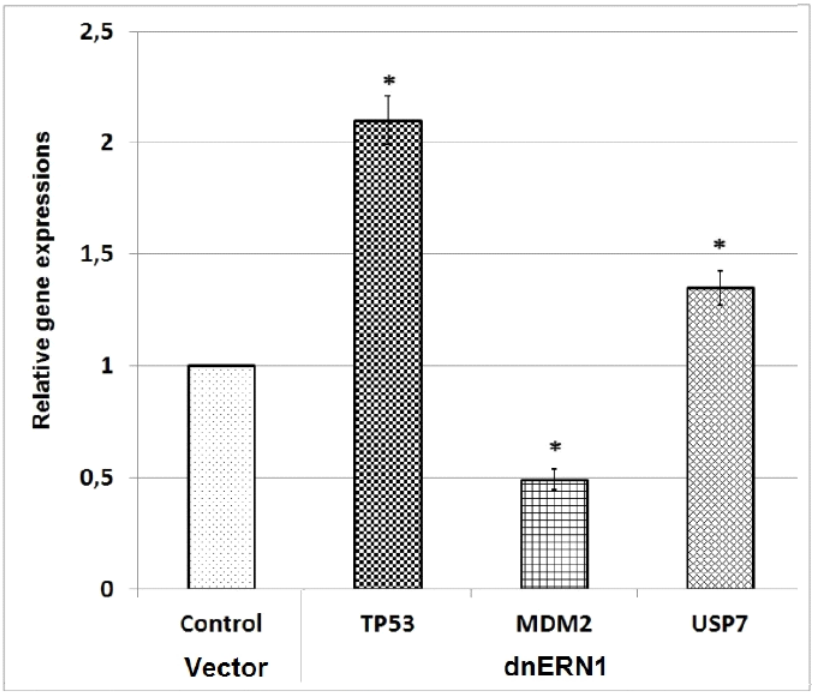

Figure 7: Expression of *TP53, MDM2,* and *USP7* genes in control U87glioma cell and its subline with IRE-1-deficiency (dnERN1) measured by qPCR; * - P < 0.05 versus control [52].

also affects the expression of different casein kinase genes in diverse ways [48]. Hypoxia induces or suppresses the expression of most casein kinase genes mainly in ERN1-knockdown cells only.

It is important to note that a blockade of glioma cell proliferation by inhibiting the ERN1 signaling enzyme with dn-ERN1 has is also associated with overexpression of TP53 and specific changes in the expression of TP53-related genes, which control apoptosis (Figure **7**) [52,53]. TP53 has numerous functions in the cells, including repression of POLO-like kinase-1 [58]. Endoplasmic reticulum stress is tightly linked to cell survival and death mainly through TP53 (tumor protein 53) pathway [54-57]. It was shown that blockade of ERN1 gene function in U87 glioma cells induces the expression of USP7 (ubiquitin specific peptidase 7) gene, but decreased the expression of MDM2 (TP53 E3 ubiquitin protein ligase homolog) gene [52]. Both enzymes are related to control of cell proliferation and apoptosis, because USP7 deubiquitinates TP53 and MDM2 and strongly stabilizes TP53 even in the presence of excess MDM2, and also induces TP53-dependent cell growth repression and apoptosis [59]. Thus, an enhanced expression of TP53 gene in ERN1 knockdown glioma cells correlates with decreased level of ubiquitin ligase MDM2 and increased expression level of USP7 which deubiquitinates TP53 and MDM2 and induces TP53-dependent cell growth repression and apoptosis. At the same time, the expression levels of *TP53, MDM2,* and *USP7* genes do not change significantly in glioma cells with suppression by dnr-ERN1 of endoribonuclease activity only [52]. It is possible that changes in the expression of *TP53, MDM2*, and *USP7* genes in glioma cells with ERN1 knockdown are responsible upon blockade of ERN1 kinase activity. Moreover, MDM2 promotes proteasome-dependent ubiquitin-independent degradation of retinoblastoma RB1 protein. Thus, the expression of genes encoding TP53 and related to TP53 enzymes (MDM2 and USP7) depends upon the endoplasmic reticulum stress signaling and correlates with suppression of glioma growth under ERN1 knockdown.

Stability as well as activity of TP53 depends upon different factors such as *TOPORS* (topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase), *RYBP/DADAF* (RING1 and YY1-binding protein/DAD-associated factor), *TP53BP1* (TP53 binding protein 1), *TP53BP2, NME6* (TP53 binding protein 1), *SESN1* (TP53 binding protein 1), and *ZMAT3* (zinc finger, Matrin-type 3). There is also data that the expression of *TOPORS, TP53BP1, NME6,* and *ZMAT3* is down-regulated in glioma cells expressing dominant-negative ERN1 [53]. At the same time, inhibition of ERN1 function in U87 glioma cells resulted in increased expression of *RYBP/DADAF, TP53BP2,*

and *SESN1*genes. Increased expression of RYBP/DADAF inhibits ubiquitination and subsequent degradation of TP53, and thereby plays a role in regulating transcription of TP53 target genes, interacts with MDM2 and decreases MDM2-mediated TP53 ubiquitination, stabilizing TP53 and increasing its activity as well as promotes apoptosis [60,61]. TP53 binding proteins modulate TP53 function, suppress tumor growth, and promote susceptibility to apoptosis, but their activity depends upon different factors [62,63]. NME6 participates in oncogenesis and inhibits TP53-induced apoptosis. SESN1and ZMAT3 are TP53 target genes which have a role in the TP53-dependent growth regulatory pathway. Thus, inhibition of ERN1 branch of endoplasmic reticulum stress response correlates with induction of p53 signaling and slower tumor growth [11,34].

In conclusion, the inhibition of ERN1/IRE-1alpha coordinately regulated factors involved in tumor growth, lowering expression levels of pro-proliferative, pro-angiogenic and anti-apoptotic factors and enzymes and up-regulated the expression of anti-proliferative, anti-angiogenic and pro-apoptotic factors. These investigations will help to define the best therapeutic targets for the design of specific inhibitors that could act as potent antitumor drug.

REFERENCES

[1] Bravo R, Parra V, Gatica D, *et al.* Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration. Int Rev Cell Mol Biol 2013; 301: 215-90. http://dx.doi.org/10.1016/B978-0-12-407704-1.00005-1

[2] Kaufman RJ, Back SH, Song B, Han J, Hassler J. The unfolded protein response is required to maintain the integrity of the endoplasmic reticulum prevent oxidative stress and preserve differentiation in beta-cells. Diabetes obesity & metabolism 2010; 12(Suppl 2): 99-107. http://dx.doi.org/10.1111/j.1463-1326.2010.01281.x

[3] Schröder M. Endoplasmic reticulum stress responses. Cell Mol Life Sci 2008 65: 862-94. http://dx.doi.org/10.1007/s00018-007-7383-5

[4] Moenner M, Pluquet O, Bouchecareilh M, Chevet E. Integrated endoplasmic reticulum stress responses in cancer. Cancer Res 2007; 67: 10631-4. http://dx.doi.org/10.1158/0008-5472.CAN-07-1705

[5] Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. J Cell Biol 2012; 197: 857-67. http://dx.doi.org/10.1083/jcb.201110131

[6] Chakrabarti A1, Chen AW, Varner JD. A review of the mammalian unfolded protein response. Biotechnol Bioeng 2011; 108(12): 2777-93. http://dx.doi.org/10.1002/bit.23282

[7] Wu J, Kaufman RJ. From acute ER stress to physiological roles of the Unfolded Protein Response. Cell Death Differ 2006; 13(3): 374-84. http://dx.doi.org/10.1038/sj.cdd.4401840

[8] Woehlbier U, Hetz C. Modulating stress responses by the UPRosome: a matter of life and death. Trends Biochem Sci 2011; 36(6): 329-37. http://dx.doi.org/10.1016/j.tibs.2011.03.001

[9] Lee SK, Kim YS. Phosphorylation of eIF2α attenuates statin-induced apoptosis by inhibiting the stabilization and translocation of p53 to the mitochondria. Int J Oncol 2013; 42(3): 810-6.

[10] Marciniak SJ, Ron D. Endoplasmic reticulum stress signaling in disease. Physiol Rev 2006; 86(4): 1133-49. http://dx.doi.org/10.1152/physrev.00015.2006

[11] Auf G, Jabouille A, Delugin M, *et al.* High epiregulin expression in human U87 glioma cells relies on IRE1alpha and promotes autocrine growth through EGF receptor. BMC Cancer 2013; 13: 597. http://dx.doi.org/10.1186/1471-2407-13-597

[12] Yuzefovych LV, Musiyenko SI, Wilson GL, Rachek LI. Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice. PLoS One 2013; 8(1): e54059. http://dx.doi.org/10.1371/journal.pone.0054059

[13] Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D. Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2000; 2(6): 326-32. http://dx.doi.org/10.1038/35014014

[14] Backer MV, Backer JM, Chinnaiyan P. Targeting the unfolded protein response in cancer therapy. Methods Enzymol 2011; 491: 37-56. http://dx.doi.org/10.1016/B978-0-12-385928-0.00003-1

[15] Schröder M, Kaufman RJ. The mammalian unfolded protein response. Annu Rev Biochem 2005; 74: 739-89. http://dx.doi.org/10.1146/annurev.biochem.73.011303.074134

[16] Shen X, Zhang K, Kaufman RJ. The unfolded protein response--a stress signaling pathway of the endoplasmic reticulum. J Chem Neuroanat 2004; 28(1-2): 79-92. http://dx.doi.org/10.1016/j.jchemneu.2004.02.006

[17] Korennykh AV, Egea PF, Korostelev AA, *et al.* The unfolded protein response signals through high-order assembly of Ire1. Nature 2009; 457(7230): 687-93. http://dx.doi.org/10.1038/nature07661

[18] Acosta-Alvear D, Zhou Y, Blais A, *et al.* XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks. Molecular Cell 2007; 27: 53-66. http://dx.doi.org/10.1016/j.molcel.2007.06.011

[19] Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007l; 8(7): 519-29. http://dx.doi.org/10.1038/nrm2199

[20] Lee J, Sun C, Zhou Y, *et al.* p38 MAPK-mediated regulation of Xbp1s is crucial for glucose homeostasis. Nat Med 2011; 17(10): 1251-60. http://dx.doi.org/10.1038/nm.2449

[21] Park SW, Zhou Y, Lee J, Lu A, Sun C, Chung J, Ueki K, Ozcan U. The regulatory subunits of PI3K, p85alpha and p85beta, interact with XBP-1 and increase its nuclear translocation. Nat Med 2010; 16(4): 429-37. http://dx.doi.org/10.1038/nm.2099

[22] Zhou Y, Lee J, Reno CM, *et al.* Regulation of glucose homeostasis through a XBP-1-FoxO1 interaction. Nat Med 2011; 17(3): 356-65. http://dx.doi.org/10.1038/nm.2293

[23] Hollien J, Lin JH, Li H, *et al.* Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. J Cell Biol 2009; 186: 323-31. http://dx.doi.org/10.1083/jcb.200903014

[24] Aragón T, van Anken E, Pincus D, *et al.* Messenger RNA targeting to endoplasmic reticulum stress signalling sites. Nature 2009; 457: 736–40. http://dx.doi.org/10.1038/nature07641

[25] Pluquet O, Dejeans N, Bouchecareilh M, *et al.* Posttranscriptional regulation of PER1 underlies the oncogenic function of IREα. Cancer Res 2013; 73: 4732–43. http://dx.doi.org/10.1158/0008-5472.CAN-12-3989

[26] Oikawa D, Tokuda M, Iwawaki T. Site-specific cleavage of CD59 mRNA by endoplasmic reticulum-localized ribonuclease, IRE1. Biochem Biophys Res Commun 2007; 360(1): 122-7. http://dx.doi.org/10.1016/j.bbrc.2007.06.020

[27] Han D, Upton JP, Hagen A, Callahan J, Oakes SA, Papa FR. A kinase inhibitor activates the IRE1alpha RNase to confer cytoprotection against ER stress. Biochem Biophys Res Commun 2008; 365(4): 777-83. http://dx.doi.org/10.1016/j.bbrc.2007.11.040

[28] Han D, Lerner AG, VandeWalle L, *et al.* IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. Cell 2009; 138(3): 562-75. http://dx.doi.org/10.1016/j.cell.2009.07.017

[29] Backer MV, Backer JM, Chinnaiyan P. Targeting the unfolded protein response in cancer therapy. Methods Enzymol 2011; 491: 37-56. http://dx.doi.org/10.1016/B978-0-12-385928-0.00003-1

[30] Lee AS. GRP78 induction in cancer: therapeutic and prognostic implications. Cancer Res 2007; 67(8): 3496-9. http://dx.doi.org/10.1158/0008-5472.CAN-07-0325

[31] Bouchecareilh M, Higa A, Fribourg S, Moenner M, Chevet E. Peptides derived from the bifunctional kinase/RNase enzyme IRE1α modulate IRE1α activity and protect cells from endoplasmic reticulum stress. FASEB J 2011; 25(9): 3115-29. http://dx.doi.org/10.1096/fj.11-182931

[32] Nguyen DT, Kebache S, Fazel A, *et al.* Nck-dependent activation of extracellular signal-regulated kinase-1 andregulation of cell survival during endoplasmic reticulumstress. Mol Biol Cell 2004; 15: 4248-60. http://dx.doi.org/10.1091/mbc.E03-11-0851

[33] Drogat B, Auguste P, Nguyen DT, *et al.* IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. Cancer Res 2007; 67: 6700-7. http://dx.doi.org/10.1158/0008-5472.CAN-06-3235

[34] Auf G, Jabouille A, Guerit S, *et al.* Inositol-requiring enzyme 1alpha is a key regulator of angiogenesis and invasion in malignant glioma. Proc Natl Acad Sci USA 2010; 107: 15553–8. http://dx.doi.org/10.1073/pnas.0914072107

[35] Minchenko DO, Kubajchuk KI, Ratushna OO, Komisarenko SV, Minchenko OH. The effect of hypoxia and ischemic conditionon the expression of VEGF genes in glioma U87 cells is dependent from ERN1 knockdown. Adv Biol Chem 2012; 2: 198-206.

[36] Pereira ER, Liao N, Neale GA, Hendershot LM. Transcriptional and post-transcriptional regulation of proangiogenic factors by the unfolded protein response. PLoS One 2010; 5(9): e12521. http://dx.doi.org/10.1371/journal.pone.0012521

[37] Zhang K, Kaufman RJ. The unfolded protein response: a stress signaling pathway critical for health and disease. Neurology 2006; 66 (Suppl 1): S102-9. http://dx.doi.org/10.1212/01.wnl.0000192306.98198.ec

[38] Kubaichuk KI, Minchenko DO, Danilovskyi SV, Kuznetsova AY, Jasim AR, Minchenko OH. Hypoxic regulation of the expression of anti-angiogenic genes in U87 glioma cells with ERN1 signaling enzyme loss of function. Studia Biologica 2012; 6(3): 15-28.

[39] Hose D, Moreaux J, Meissner T, *et al.* Induction of angiogenesis by normal and malignant plasma cells. Blood. 2009; 114(1): 128-43. http://dx.doi.org/10.1182/blood-2008-10-184226

[40] Shen J, Xia W, Khotskaya YB, *et al.* EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2. Nature 2013; 497(7449): 383-7. http://dx.doi.org/10.1038/nature12080

[41] Thorpe JA1, Schwarze SR. IRE1alpha controls cyclin A1 expression and promotes cell proliferation through XBP-1. Cell Stress Chaperones 2010; 15(5): 497-508. http://dx.doi.org/10.1007/s12192-009-0163-4

[42] Minchenko D, Hubenya O, Terletsky B, Kuznetsova A, Moenner M, Minchenko O. Blockade of the endoplasmic reticulum stress sensor inositol requiring enzyme-1 changes the expression of cyclin and growth arrest-specific genes in glioma cells. Annales Universitatis Mariae Curie-Sklodowska. 2010; 23(3): 179-84.

[43] Minchenko DO, Hubenya OV, Terletsky BM, Moenner M, Minchenko OH. Effect of hypoxia, glutamine and glucosede privation on the expression of cyclin and cyclin-dependent kinase genes in glioma cell line U87 and its subline with suppressed activity of signaling enzyme endoplasmic reticulum-nuclei-1. Ukr Biokhim Zh 2011; 83(1): 18-29.

[44] Bakalets T, Minchenko D, Danilovskyi S, Minchenko O. Expression of genes of the protein kinase PLK family in U87 gliomacells with suppressed function of endoplasmic reticulum stress signaling enzyme ERN1. Visnyk Taras Shevchenko Kyiv National Univ. Biology 2013; 63: 7-13.

[45] Minchenko DO, Kharkova AP, Hubenia OV, Minchenko OH. Insulin receptor, IRS1, IRS2, INSIG1, INSIG2, RRAD, and BAIAP2 gene expression singlioma U87 cells with ERN1 loss of function: effect of hypoxia and glutamineorglucosede privation. Endocrine Regulations 2013; 47: 15-26. http://dx.doi.org/10.4149/endo_2013_01_15

[46] Minchenko DO, Karbovskyi LL, Danilovskyi SV, Moenner M, Minchenko OH. Effect of hypoxia and glutamine or glucose deprivation on the expression of retinoblastoma and retinoblastoma-related genes in ERN1 knockdown glioma U87 cell line. Am J MolBiol 2012; 2: 21-31.

[47] Karbovskyi LL, Minchenko DO, Danylovskyi SV, Moenner M, Minchenko OH. Endoplasmic reticulum–nuclei signaling enzyme-1 knockdown modulates effect of hypoxia and ischemia on the expression of circadian genes in glioma cells. Studia Biologica 2011; 5(2): 37-50.

[48] Minchenko DO, Karbovskyi LL, Danilovskyi SV, Kharkova AP, Minchenko OH. Expression of casein kinase genesingliomacellline U87: effect of hypoxia and glucoseorglutamined eprivation. Nat Sci 2012; 4: 38-46.

[49] Aggarwal P, Vaites LP, Kim JK, *et al.* Nuclear cyclin D1/CDK4 kinase regulates CUL4 expression and triggers neoplastic growth via activation of the PRMT5 methyltransferase. Cancer Cell 2010; 18(4): 329-40. http://dx.doi.org/10.1016/j.ccr.2010.08.012

[50] Francescangeli F, Patrizii M, Signore M, *et al.* Proliferation state and polo-like kinase1 dependence of tumorigenic colon cancer cells. Stem Cells 2012; 30: 1819-1830.

[51] Harris PS, Venkataraman S, Alimova I, Birks DK, Donson AM, Knipstein J, Dubuc A, Taylor MD, Handler MH, Foreman NK, Vibhakar R. Polo-like kinase 1 (PLK1) inhibition suppresses cell growth and enhances radiation sensitivity in medulloblastoma cells. BMC Cancer 2012; 12: 80.

[52] Danilovskyi SV, Minchenko DO, Karbovskyi LL, Moliavko OS, Kovalevska OV, Minchenko OH. ERN1 knockdown modifies the hypoxicregulation of TP53, MDM2, USP7 and PERP gene expressions in U87 gliomacells. Ukr Biochem J 2014; 86(4): 90-102.

[53] Minchenko DO, Danilovskyi SV, Kryvdiuk IV, Bakalets TV, Lypova NM, Karbovskyi LL, Minchenko OH. Inhibition of

ERN1 modifies the hypoxicregulation of the expression of TP53-related genesin U87 gliomacells. Endoplasmic Reticulum Stressin Diseases 2014; 1: 18-26.

[54] Malhotra JD, Kaufman RJ. ER stress and its functional link to mitochondria: role in cell survival and death. Cold Spring Harb Perspect Biol 2011; 3: a004424. http://dx.doi.org/10.1101/cshperspect.a004424

[55] Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. Annu Rev Biochem 2008; 77: 557-82. http://dx.doi.org/10.1146/annurev.biochem.77.060806.091238

[56] Kruse JP, Gu W. Modes of p53 regulation. Cell 2009; 137: 609-22. http://dx.doi.org/10.1016/j.cell.2009.04.050

[57] Brown CJ, Lain S, Verma CS, Fersht AR, Lane DP. Awakening guardian angels: drugging the p53 pathway. Nat Rev Cancer 2009; 9: 862-73. http://dx.doi.org/10.1038/nrc2763

[58] McKenzie L, King S, Marcar L, *et al.* p53-dependent repression of polo-like kinase-1 (PLK1). Cell Cycle 2010; 9: 4200-12. http://dx.doi.org/10.4161/cc.9.20.13532

[59] Nicholson B, Suresh Kumar KG. The multifaceted roles of USP7: new therapeutic opportunities. Cell Biochem Biophys 2011; 60: 61–8. http://dx.doi.org/10.1007/s12013-011-9185-5

[60] Chen D, Zhang J, Li M, Rayburn ER, Wang H, Zhang R. RYBP stabilizes p53 by modulating MDM2. EMBO Rep 2009; 10: 166-72. http://dx.doi.org/10.1038/embor.2008.231

[61] Novak RL, Phillips AC. Adenoviral-mediated Rybp expression promotes tumor cell-specific apoptosis. Cancer Gene Ther 2008; 15: 713-22. http://dx.doi.org/10.1038/cgt.2008.25

[62] Hong S, Li X, Zhao Y, Yang Q, Kong B. TP53BP1 suppresses tumor growth and promotes susceptibility to apoptosis of ovarian cancer cells through modulation of the Akt pathway. Oncol Rep 2012; 27: 1251-7.

[63] Li X, Xu B, Moran MS, *et al.* 53BP1 functions as a tumor suppressor in breast cancer via the inhibition of NF-κB through miR-146a. Carcinogenesis 2012; 33: 2593-600. http://dx.doi.org/10.1093/carcin/bgs298

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