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Insulin receptor substrate 1 gene expression is strongly up-regulated by HSPB8 silencing in U87 glioma cells

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Objective. The aim of the present investigation was to study the expression of genes encoding IRS1 (insulin receptor substrate 1) and some other functionally active proteins in U87 glioma cells under silencing of polyfunctional chaperone HSPB8 for evaluation of the possible significance of this protein in intergenic interactions.

Methods. Silencing of HSPB8 mRNA was introduced by HSPB8 specific siRNA. The expression level of *HSPB8*, *IRS1*, *HK2*, *GLO1*, *HOMER3*, *MYL9*, *NAMPT*, *PER2*, *PERP*, *GADD45A*, and *DEK* genes was studied in U87 glioma cells by quantitative polymerase chain reaction.

Results. It was shown that silencing of HSPB8 mRNA by specific to HSPB8 siRNA led to a strong down-regulation of this mRNA and significant modification of the expression of IRS1 and many other genes in glioma cells: strong up-regulated of *HOMER3*, *GLO1*, and *PERP* and down-regulated of *MYL9*, *NAMPT*, *PER2*, *GADD45A*, and *DEK* gene expressions. At the same time, no significant changes were detected in the expression of *HK2* gene in glioma cells treated by siRNA, specific to HSPB8. Moreover, the silencing of HSPB8 mRNA enhanced the glioma cells proliferation rate.

Conclusions. Results of this investigation demonstrated that silencing of HSPB8 mRNA affected the expression of *IRS1* gene as well as many other genes encoding tumor growth related proteins. It is possible that the dysregulation of most of the studied genes in glioma cells after silencing of HSPB8 is reflected by a complex of intergenic interactions and that this polyfunctional chaperone is an important factor for the stability of genome function and regulatory mechanisms contributing to the tumorigenesis control.

Key words: silencing HSPB8, mRNA expression, IRS1, HOMER3, PERP, CLO1, MYL9, PER2, GADD45A, U87 glioma cells

The heat shock 22 kDa protein 8 (HSPB8), also known as E2-induced gene 1 protein, alpha-crystallin C chain, and protein kinase H11, belongs to the superfamily of small heat-shock proteins containing a conservative alpha-crystallin domain at the C-terminal part of the molecule. It is induced by estrogen in estrogen receptor-positive breast cancer cells and involved in the regulation of cell proliferation, apoptosis, and carcinogenesis (Modem et al. 2011; Acunzo et al. 2012; Cui et al. 2012; Li et al. 2014; Suzuki et al. 2015; Varlet et al. 2017; Piccolella et al. 2017; Shen et al. 2018). HSPB8 knockdown induces expression of a multifunctional protein SAM68 (Src-associated protein in mitosis, 68 kDa) and stimulates the proliferation of the glioblastoma cells (Modem et al. 2011). There are also data indicating that HSPB8

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could promote the proliferation and inhibit the apoptosis of gastric cancer cells by activating ERK-CREB signaling, since there is a strong positive correlation between HSPB8 expression level and MAPK and CREB signaling pathways (Shen et al. 2018). At the same time, there are data indicating that overexpression of HSPB8 exhibits antileukemia effect (Cui et al. 2012). Antitumor effect of HSPB8 expression has been also shown by Smith et al. (2012), during studying the growth of genetically diverse melanoma tumors. It is possible that there is a tissue/tumor specific functional significance of HSPB8 expression changes.

Furthermore, it has recently been shown that HSPB8 is an impotent component of the triad (HRI, EIF2A, and HSPB8), which represents a novel cytosolic unfolded protein response essential for the optimal innate immune signaling by large molecular platforms, functionally homologous to the PERK/eIF2α/HSPA5 axis of the endoplasmic reticulum UPR (Abdel-Nour et al. 2019). These results agree well with previously published data that protein HSPB8 participates in protein folding and induces autophagy via the EIF2A pathway (Carra et al. 2010; Crippa et al. 2010a, b). It has also been shown that HSPB8 binds BAG3 to promote spatial sequestration of ubiquitinated proteins (Guilbert et al. 2018). Moreover, there are data indicating that during heat shock recovery, NF-kappaB activates selective removal of misfolded or aggregated proteins by controlling the expression of BAG3 and HSPB8 and by modulating the level of the BAG3-HspB8 complex (Nivon et al. 2012). It is interesting to note that silencing of HSPB8 markedly decreased the mitotic levels of BAG3 in HeLa cells, supporting its crucial role in BAG3 mitotic functions and exhibited increased incidence of nuclear abnormalities that result from failed cytokinesis as well as is associated with abnormal accumulation of F-actin at the intercellular bridge (Varlet et al. 2017). These results support the role of the HSPB8-BAG3 chaperone complex in quality control of actin-based structure dynamics.

The underlying molecular mechanisms of heat shock 22 kDa protein 8 in physiological and pathological processes are not fully understood yet. The aim of the present investigation was to study the effect of polyfunctional chaperone HSPB8 silencing on the expression of genes encoding insulin receptor substrate 1 and some other proliferation related proteins in U87 glioma cells for evaluation of the possible significance of this protein in the intergenic interactions, in genome reprogramming. Among them: hexokinase 2 (HK2), glyoxalase 1 (GLO1), period circadian regulator 2 (PER2), growth arrest and DNA-damage-inducible, alpha (GADD45A), homer scaffolding protein 3 (HOMER3), nicotinamide phosphoribosyltransferase (NAMPT), myosin regulatory light chain 9 subunit (MYL9), TP53 apoptosis effector (PERP) and DEK proto-oncogene (DEK). These polyfunctional proteins play an important role in the regulation of cell proliferation and surviving (Shen et al. 2016; Chiavarina et al. 2017; Hacker et al. 2018; Qu et al. 2018; Xiong et al. 2018; Zhou et al. 2018; Chen et al. 2014, 2019; Xie et al. 2019). Thus, protein IRS1, which can interact with insulin receptor and IGF receptors, is stress responsible phosphorylated by insulin receptor tyrosine kinase. It mediates effects of insulin and insulin-like growth factors on various cellular processes and preferentially exhibits pro-oncogenic properties (Nishiyama and Wands 1992; Minchenko et al. 2013; Kim et al. 2018; Li et al. 2018; Xu et al. 2018; Yoneyama et al. 2018; Sun et al. 2019; Wu et al. 2019).

Chen et al. (2014, 2019) have shown that hexokinase 2 has pro-oncogenic properties and its overexpression promotes the proliferation and survival of laryngeal squamous cell carcinoma and that long non-coding RNA PVT1 promotes tumor progression by regulating the miR-143/HK2 axis in gallbladder cancer. Furthermore, hepatocellular carcinoma patients with increased HK2 expression showed poor overall survival and inhibition of this enzyme synergistically augments the anti-tumor efficacy of sorafenib (Yoo et al. 2019). Glyoxalase 1, a methylglyoxal degradation enzyme, is implicated in the progression of human malignancies and inhibition of GLOI induces anticancer effects, significantly reduces tumor-associated properties such as migration and proliferation in different cancer cells (Guo et al. 2016; Hutschenreuther et al. 2016). At the same time, no functional alterations where found by overexpression of GLO1 in HEK 293 cells (Hutschenreuther et al. 2016). Furthermore, Chiavarina et al. (2017) have detected a low GLO1 activity in high stage tumors and they concluded that GLO1 depletion in colorectal cancer cells promoted tumor growth *in vivo*. Polyfunctional adipokine NAMPT is involved in many important biological processes with anti-diabetic properties. It is up-regulated in glioma as well as other cancers, promotes proliferation and inhibits p53-dependent apoptosis and its inhibition by specific inhibitors decreases cancer growth, induces apoptosis, and sensitizes tumor cells to temozolomide (Bong et al. 2016; Feng et al. 2016; Alaee et al. 2017; Xu et al. 2017; Zhao et al. 2017; Hesari et al. 2018; Liang et al. 2018; Meram et al. 2019). Furthermore, NAMPT is involved in the

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

Gene symbol	Gene name	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
HSPB8 (H11)	Heat shock 22kDa protein 8; protein kinase H11; E2-induced gene 1 protein	F: 5'-ggaggtgtctggcaaacatg R: 5'-tggcatctcaggtacaggtg	739–758 980–961	NM_014365
1RS1	Insulin receptor substrate 1	F: 5'-agtcccagcaccaacagaac R: 5'-tcatccgaggagatgaaacc	1094–1113 1341–1322	NM_005544
HK2	Hexokinase 2	F: 5'-tctatgccatccctgaggac R: 5'-tctctgccttccactccact	786–805 1005–986	NM_000189
GLO1	Glyoxalase I; lactoyl glutathione lyase	F: 5'-gcgtagtgtgggtgactcct R: 5'-tctctgccttccactccact	61–80 240–221	NM_006708
HOMER3	Homer homolog 3 (Drosophila); homer scaffolding protein 3	F: 5'-gcaatgtgtaccgcatcatc R: 5'-cttctcggcaaactgtgtca	383–402 564–545	NM_004838
NAMPT (PBEF)	Nicotinamide phosphoribosyltransferase; pre-B-cell colony-enhancing factor; visfatin	F: 5'-tcttcacggtggaaaacaca R: 5'-gctcctatgccagcagtctc	700–719 931–912	NM_005746
MYL9 (MYRL2)	Myosin, light chain 9, regulatory; myosin regulatory light chain 2	F: 5'-accccacagacgaatacctg R: 5'-ccggtacatctcgtccactt	260–279 501–482	NM_006097
PERP	PERP, TP53 apoptosis effector	F: 5'-tgtggcttcatcatcctggt R: 5'-atgaagggtgaaggtctggg	430–449 603–584	NM_022121
DEK	DEK proto-oncogene	F: 5'-agtctcatcgtggaaggcaa R: 5'-cctggcctgttgtaaagcag	306–325 508–489	NM_003472
PER2	Period circadian regulator 2	F: 5'-cacacagaaggaggagcaga R: 5'-gatccggtgctctcagatga	2480–2499 2719–2700	NM_022817
GADD45A (DDIT1)	Growth arrest and DNA-damage-inducible alpha (DNA damage-inducible transcript-1)	F: 5'-acgaggacgacgacagagat R: 5'-tcccggcaaaaacaaataag	479–498 740–721	NM_001924
ACTB	beta-actin	F: 5'-ggacttcgagcaagagatgg R: 5'-agcactgtgttggcgtacag	747–766 980–961	NM_001101

regulation of key basic cellular functions such as cell signaling, transcription, translation, and metabolism (Abu Aboud et al. 2016; Cheleschi et al. 2018; Lee et al. 2018; Nielsen et al. 2018; Shi et al. 2018).

The proteins encoding by *PER2*, *HOMER3*, *GADD45A*, and *DEK* genes, also involved in cancer cell proliferation and tumor growth, are dysregulated in cancer cells (Li et al. 2013; Shen et al. 2016; Hacker et al. 2018; Xiang et al. 2018; Xiong et al. 2018; Hu et al. 2019; Rahman et al. 2019). Protein PERP controls apoptosis, the marked increase in PERP expression induces apoptosis but loss of PERP protein promotes resistance to apoptosis as well as tumorigenesis (Beaudry et al. 2010; Awais et al. 2016; Zhou et al. 2018). Myosin regulatory subunit plays an important role in the regulation of various processes and high level of MYL9 expression associates with poor prognosis in patients with glioblastoma and esophageal squamous cell carcinoma (Wang et al. 2017; Kruthika et al. 2019). At the same time, there are data indicating that decreased expression of MYL9 in stroma may predict malignant progression of prostate cancer (Huang et al. 2014).

Here, we provide experimental evidence that silencing of HSPB8 mRNA resulted in increased glioma cell proliferation and affected expression of *IRS1* and many other genes encoding polyfunctional tumor growth related proteins, which possibly reflected a complex mechanism of intergenic interactions and an important role of chaperone HSPB8 in the control of genome functional stability and tumorigenesis.

Materials and methods

Cell line and culture conditions. 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 (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (FBS; Equitech-Bio, Inc., USA), penicillin (100 units/ml; Gibco) and

streptomycin (0.1 mg/ml; Gibco) at 37 °C in incubator with 5% CO_2.

Silencing of HSPB8 mRNA was introduced by specific to HSPB8 siRNA (human alpha C-crystallin-specific siRNA, sc-72422, Santa Cruz Biotechnology, Inc., Dallas, TX, U.S.A.). Control siRNA-A (sc-37007, Santa Cruz Biotechnology, Inc.) was used for transfection of control glioma cells. Transfection was performed for 48 h using lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocol, with siRNA at a final concentration of 100 nM.

RNA isolation. Total RNA was extracted from glioma cells using the Trizol reagent according to manufacturer's protocol (Invitrogen). 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 and spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. The effect of HSPB8 silencing on the expression levels of IRS1, HK2, GLO1, HOMER3, NAMPT, MYL9, PER2, GADD45A, PERP, and DEK mRNAs as well as ACTB (beta-actin) mRNA was measured in control U87 glioma cells and cells with a deficiency of HSPB8, introduced by specific to HSPB8 siRNA. Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse transcription and for quantitative polymerase chain reaction – SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) as well as qPCR "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). Polymerase chain reaction was performed in triplicate. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The pair of primers specific for each studied gene was received from Sigma-Aldrich (St. Louis, MO, U.S.A.) and used for quantitative polymerase chain reaction (Table 1). Quantitative PCR analysis was performed using a special computer program "Differential expression calculator".

Statistical analysis. Statistical analysis was performed Excel program and OriginPro 7.5 software as described previously (Bochkov et al. 2006; Minchenko et al. 2015). Comparison of two means was performed by the use of two-tailed Student's t-test. The value $p<0.05$ was considered significant in all cases. The values of *IRS1*, *HK2*, *GLO1*, *HOMER3*, *NAMPT*, *MYL9*, *PER2*, *GADD45A*, *PERP*, and *DEK* gene 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. The amplified DNA fragments were also analyzed on a 2% agarose gel and that visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

Results

To investigate a possible role of polyfunctional chaperone HSPB8 in the control of gene expressions, functional stability of genome and tumorigenesis through intergenic interactions, which underlying molecular mechanisms of this protein both in physiological and pathological processes, the effect of HSPB8 silencing on the expression of genes encoding IRS1 and some other important polyfunctional proteins in U87 glioma cells we studied.

As shown in Figure 1A, the expression level of HSPB8 mRNA in U87 glioma cells, transfected by siRNA, specific to HSPB8, is strongly down-regulated (–76%) in comparison with control glioma cells, transfected by control siRNA, 48 hours after transfection. Furthermore, silencing of HSPB8 has smaller but statistically significant effect (+22%) on proliferation rate of these glioma cells in comparison with control glioma cells, transfected by control siRNA (Figure 1B). We next investigated the effect of HSPB8 silencing on the expression of gene encoding insulin receptor substrate 1, which can interact with insulin receptor and insulin-like growth factor receptors and mediate the control of various cellular processes by insulin as well as insulin-like growth factors, in glioma cells. As shown in Figure 2, the expression level of IRS1 mRNA is strongly up-regulated (+389%) in glioma cells after silencing of HSPB8 in comparison with cells, transfected by control siRNA. At the same time, no significant changes were observed in the expression level of hexokinase 2 mRNA in glioma cells with depleted level of HSPB8 (Figure 2).

Strong up-regulation of the expression level was also shown for gene encoding glyoxalase 1 (+252%) in glioma cells treated by specific to HSPB8 siRNA in comparison with control cells (Figure 3). However, the expression level of gene encoding period circadian regulator 2 in glioma cells with deficiency in HSPB8, introduced by specific to this chaperone siRNA, is down-regulated (–37%) in comparison with cells transfected by control siRNA (Figure 3). Furthermore, as shown in Figure 4, silencing of HSPB8 in glioma cells significantly up-regulates (+136%) the expression level of gene encoding homer scaffolding

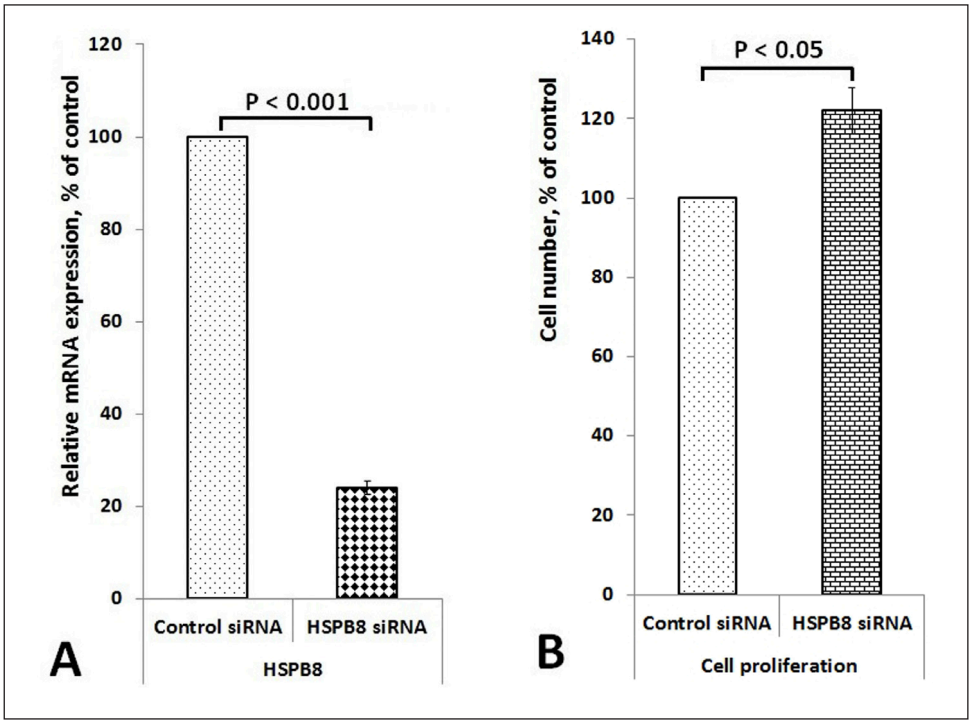

Figure 1. A) The expression level of heat shock protein B8 (HSPB8) mRNA in glioma cells treated by siRNA HSPB8 as compared to cells transfected by control siRNA. The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n=4. **B**) Proliferation rate of glioma cells treated by specific to HSPB8 siRNA as compared to cells treated by control siRNA.

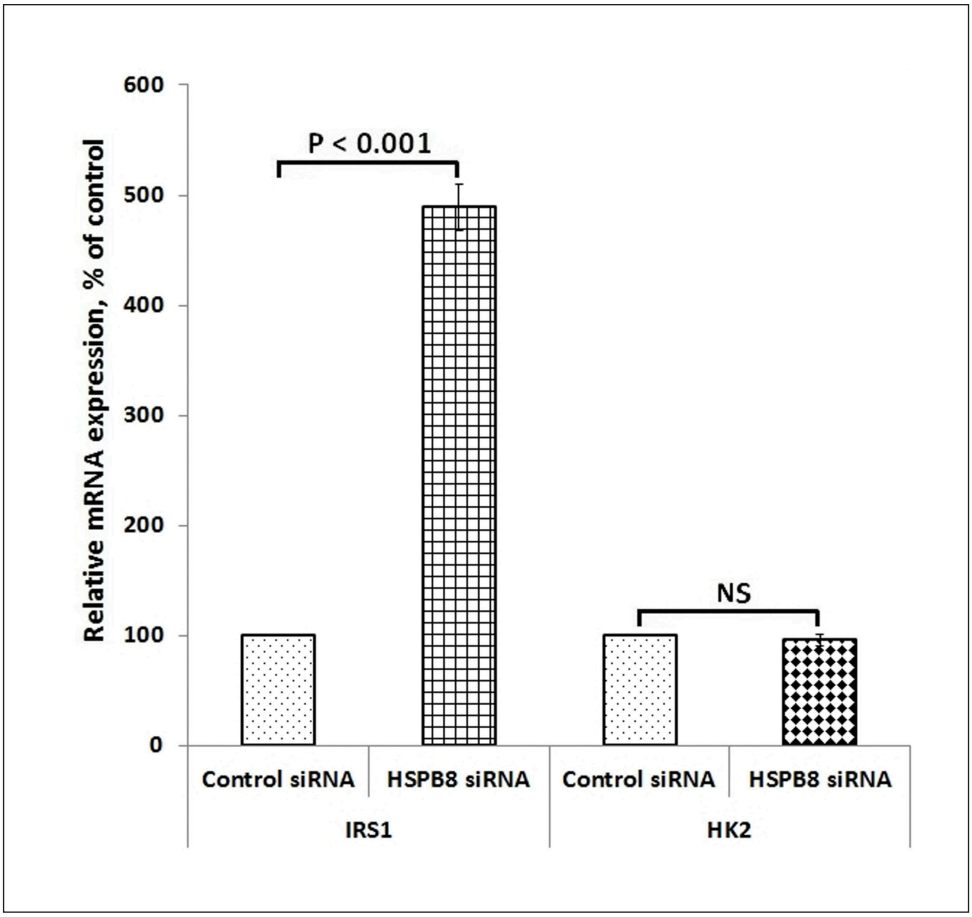

Figure 2. Effect of HSPB8 silencing on the expression of insulin receptor substrate 1 (IRS1) and hexokinase 2 (HK2) mRNAs in glioma cells transfected by specific to HSPB8 siRNA and control siRNA. The values of these mRNA expressions were normalized to beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n=4.

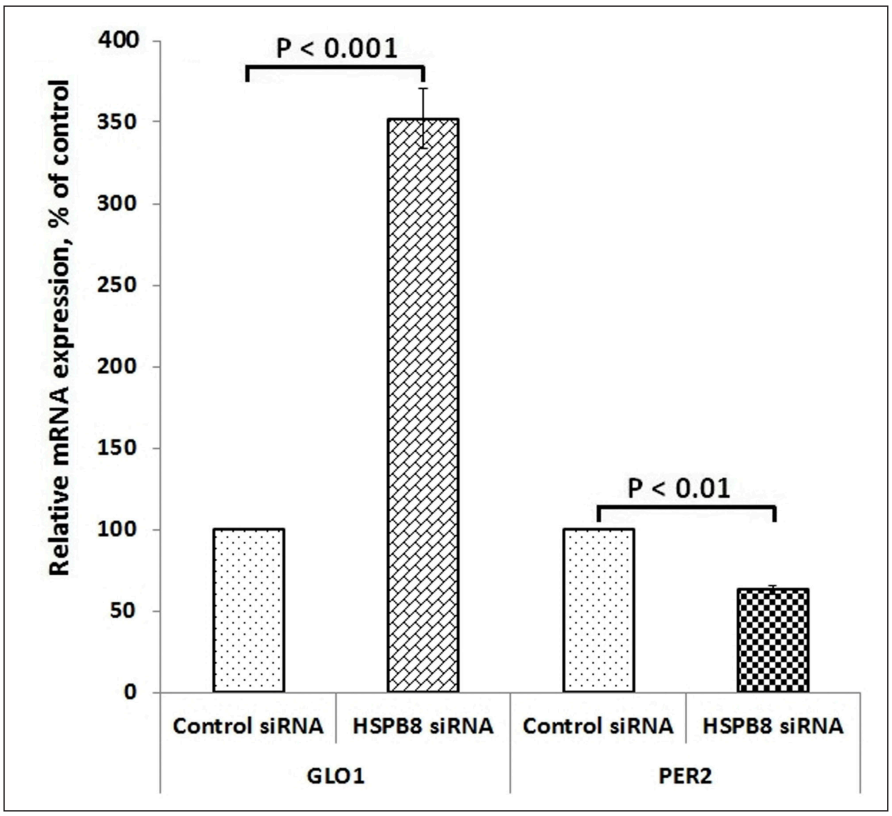

Figure 3. The expression level of glyoxalase 1 (GLO1) and period circadian regulator 2 (PER2) mRNAs in glioma cells transfected by siRNA HSPB8 as compared to cells treated by control siRNA. The values of GLO1 and PER2 mRNA expressions were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n=4.

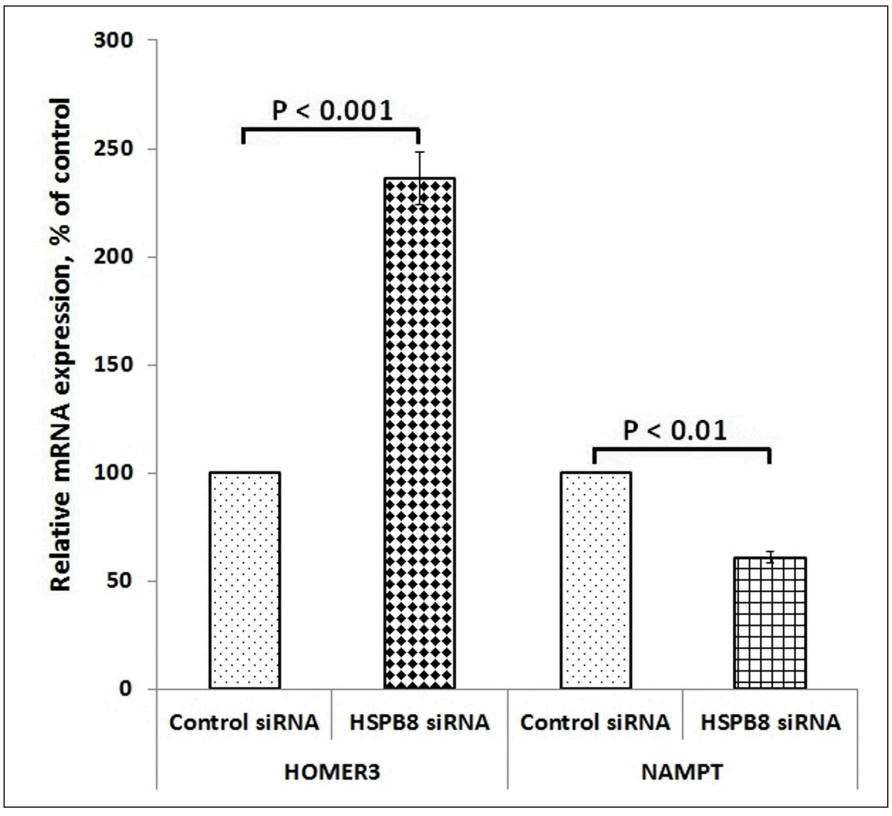

Figure 4. The expression level of homer scaffolding protein 3 (HOMER3) and nicotinamide phosphoribosyltransferase (NAMPT) mRNAs in glioma cells treated by siRNA HSPB8 as compared to cells transfected by control siRNA. The values of these mRNA expressions were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n=4.

protein 3 (HOMER3), but down-regulates *NAMPT* (nicotinamide phosphoribosyltransferase) gene as compared to control glioma cells. Next, we studied the expression of *GADD45A* and *MYL9* genes. As shown in Figure 5, the expression level of both genes is down-regulated in glioma cells treated by specific to HSPB8 siRNA in comparison with control cells, being more significant for *MYL9* gene (–47%). Investigation of *PERP* and *DEK* gene expressions in glioma cells with depleted level of HSPB8 shown significant up-regulation (+54%) of the expression level of TP53 apoptosis effector gene and down-regulation (–15%) of DEK proto-oncogene gene as compared to control glioma cells (Figure 6).

Consequently, silencing of HSPB8, introduced by specific to this chaperone siRNA, leads to variable changes in the expression level of genes encoding very important polyfunctional proteins that play a key role in the regulation of cell proliferation, surviving, apoptosis, and biological clock (Figure 7).

Discussion

In this work, we studied the expression of genes encoding insulin receptor substrate 1, glyoxalase I, and several other genes, which have relation to the regulation of cell proliferation, surviving, apoptosis, circadian rhythm, and metabolism, in relation to silencing of HSPB8, introduced by specific to this chaperone siRNA. It is important for the evaluation of possible significance of HSPB8 in the control of glioma growth through intergenic interactions as well as for the functional stability of genome. There are data indicating that the expression of *HSPB8* gene is involved in the regulation of cell proliferation, apoptosis, and carcinogenesis (Modem et al. 2011; Shen et al. 2018; Wu et al. 2019). Therefore, HSPB8 knockdown induces the expression of a multifunctional protein SAM68 (Src-associated protein in mitosis, 68 kDa) and stimulates proliferation of glioblastoma cells (Modem et al. 2011). Our results concerning up-regulation of proliferation rate of glioma cells treated by specific to HSPB8 siRNA agree well with Dr. Modem's et al. (2011) results. At the same time, there are data indicating that HSPB8 could promote the proliferation and inhibit the apoptosis of gastric cancer cells by activating ERK-CREB signaling (Shen et al. 2018). It is possible that there is a tumor specific functional significance of this chaperone.

As shown in Figure 7, HSPB8 silencing leads to a strong up-regulation of the expression of gene encoding insulin receptor substrate 1, which can

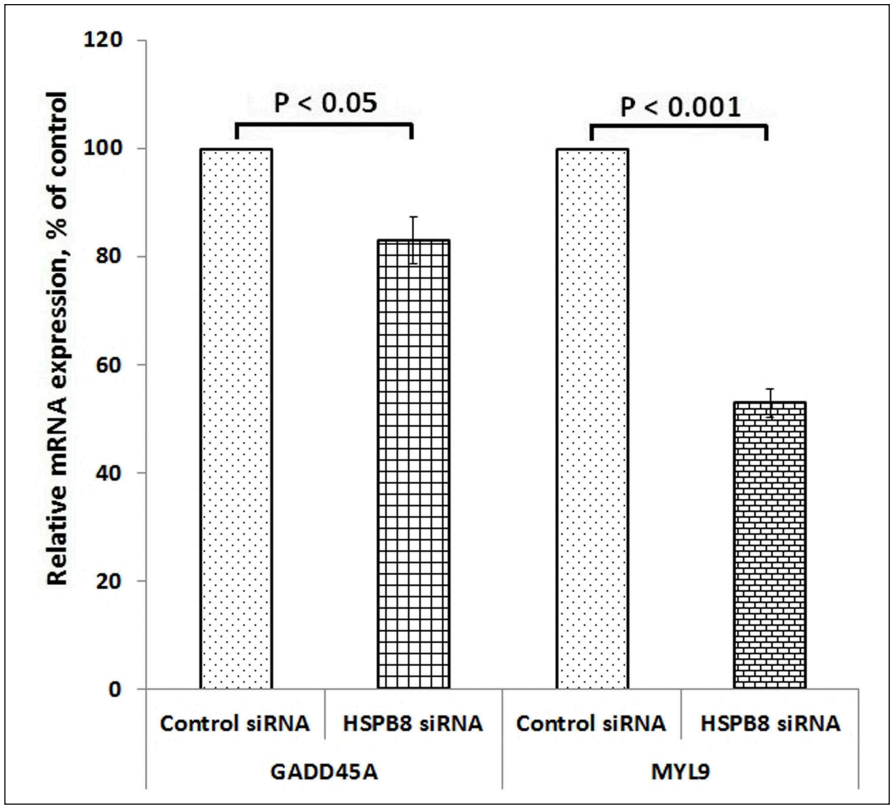

Figure 5. Effect of HSPB8 mRNA silencing on the expression of growth arrest and DNA-damage-inducible alpha (GADD45A) and myosin, light chain 9, regulatory (MYL9) mRNAs in glioma cells transfected by siRNA HSPB8 and control siRNA. The values of GADD45A and MYL9 mRNA expressions were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n=4.

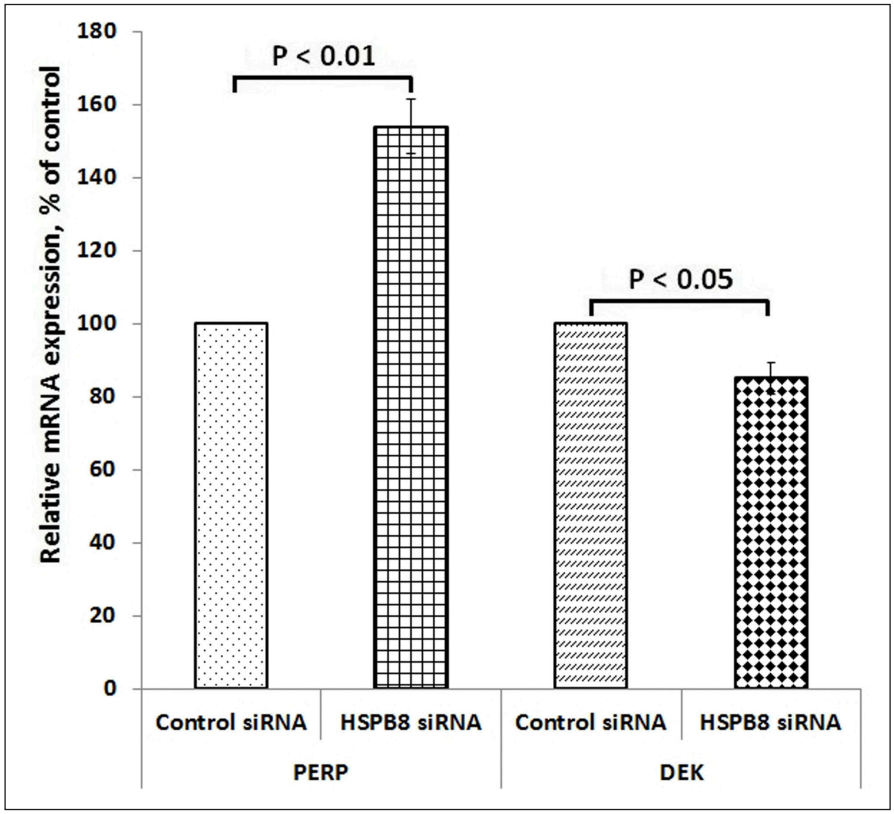

Figure 6. Effect of HSPB8 mRNA silencing on the expression of PERP, TP53 apoptosis effector (PERP) and DEK proto-oncogene (DEK) mRNAs in glioma cells transfected by siRNA HSPB8 and control siRNA. The values of these mRNA expressions were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n=4.

mediate the control of various cellular processes by insulin as well as insulin-like growth factors. This data correlated with increased proliferation rate of glioma cells with depleted level of HSPB8 and agree well with pro-proliferative and pro-oncogenic functional role of IRS1 (Nishiyama and Wands 1992; Sun et al. 2019; Wu et al. 2019). In glioma cells with depleted level of HSPB8, we also observed significant induction of glyoxalase 1, a methylglyoxal degradation enzyme, which is implicated in the progression of human malignancies. Thus, our results with up-regulation of GLO1 agree well with tumor-associated properties of this enzyme such as migration and proliferation observed in different cancer cells (Guo et al. 2016; Hutschenreuther et al. 2016).

We have also shown a significant up-regulation of the expression for two other genes, *HOMER3* and *PERP*, in glioma cells with depleted level of HSPB8 by mRNA silencing. There are data indicating that HOMER3 is a cancer-associated gene and may play a tumorigenic role in the esophageal squamous cell carcinoma (Shen et al. 2016). These data agree with our results, but there are also data available that loss of HOMER3 is associated with acute myeloid leukemia (Li et al. 2013). It is possible that these differences in functional significance of changes in HOMER3 expression level are due to the features of different tumors. At the same time, upregulation of PERP in cells treated by HSPB8 silencing does not agree with functional significance of this protein, because PERP has anti-proliferative properties, it induces apoptosis (Beaudry et al. 2010; Awais et al. 2016; Zhou et al. 2018). Furthermore, our results showed that silencing of HSPB8 leads to a down-regulation of *MYL9*, *PER2*, and *GADD45A* gene expressions in glioma cells and these data agree with anti-tumor properties of proteins encoding by these genes (Cui et al. 2017; Hacker et al. 2018; Xiang et al. 2018). At the same time, there are data indicating that decreased expression of MYL9 in stroma predicts malignant progression of prostate cancer (Huang et al. 2014). Results of our investigation also showed that silencing of HSPB8 down-regulates of *DEK* gene expression in glioma cells and increases cell proliferation, which does not agree with the functional significance of protein, encoding by this gene, because DEK induces apoptosis and it has anti-proliferative properties (Hacker et al. 2018; You et al. 2018).

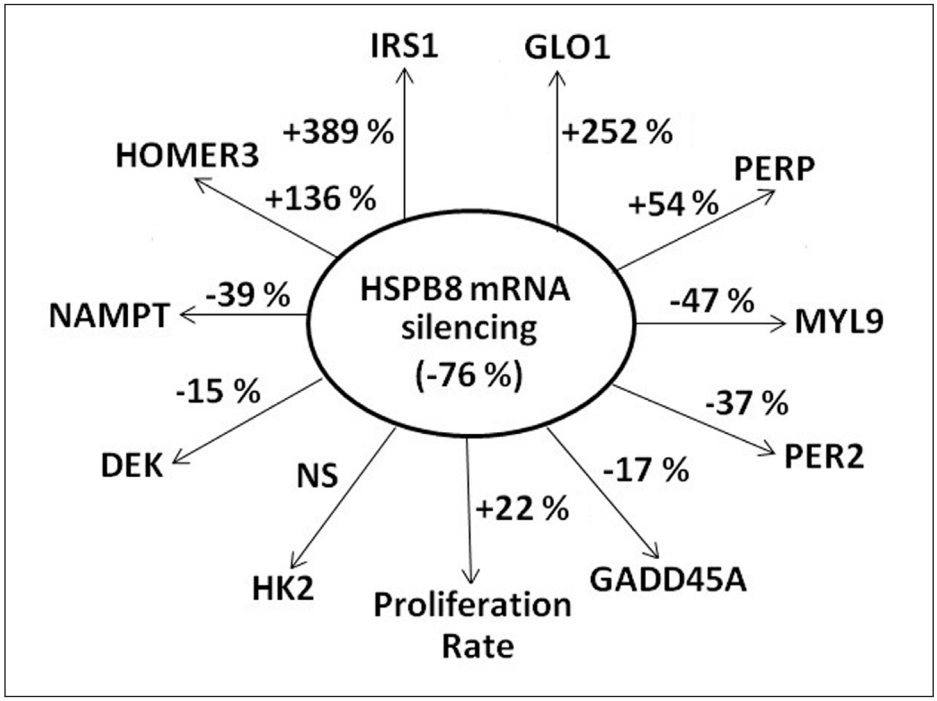

Figure 7. Schematic demonstration of the expression profile of *IRS1*, *HK2*, *GLO1*, *HOMER3*, *NAMPT*, *MYL9*, *PER2*, *GADD45A*, *PERP*, and *DEK* genes as well as cell proliferation rate in glioma cells after HSPB8 mRNA silencing by specific to this mRNA siRNA as compared to control cells transfected by control siRNA; NS – no significant changes.

Next, we showed that silencing of chaperone HSPB8 leads to a down-regulation of *NAMPT* gene expression in glioma cells (Figure 7). These data do not agree well with the pro-oncogenic properties of protein encoded by this gene (Chen et al. 2014, 2019; Yoo et al. 2019). There are data indicating that the expression of *NAMPT* gene is up-regulated in gliomas and that overexpression of this adipokine induces glioma and other cancers growth and that inhibition of NAMPT by specific inhibitors suppresses tumor growth, and enhances apoptosis (Abu Aboud et al. 2016; Alaee et al. 2017; Xu et al. 2017; Zhao et al. 2017; Hesari et al. 2018). Furthermore, silencing of NAMPT decreased the proliferation rate of both glioma and RPMI 8226 cells (Bong et al. 2016; Minchenko et al. 2020). Furthermore, Xu et al. (2017) have shown that inhibition of nicotinamide phosphoribosyltransferase by NAMPT inhibitor FK866 decreases the cell growth and enhances susceptibility to oxidative stress in the FK866-treated 293T cells through down-regulation of 325 proteins that are involved in diverse cellular processes including nucleobase-containing compound metabolic process, protein metabolic process, antioxidant and DNA repair processes.

It is important to note that silencing HSPB8 and NAMPT has opposite effect on cell proliferation rate but similar effect on the expression of *IRS1*, *PER2* and *HK2* genes (Minchenko et al. 2020). Thus, the results of this study and our earlier data (Minchenko et al. 2013, 2015, 2016a, 2016b, 2017, 2020) as well as results of Xu et al. (2017) clearly demonstrated that suppression of one protein quantity or function independently from method (by mRNA silencing, chemical inhibitor or knockdown) introduce changes in the genome function, disturbs genome functional stability, leads to numerous changes in the expression of genes directly or indirectly initiated by the excepted gene and reflects a complex intergenic interactions.

This study provides unique insights into the molecular mechanisms regulating the expression of genes encoding IRS1, HK2, GLO1, HOMER3, NAMPT, MYL9, PER2, GADD45A, PERP, and DEK proteins in glioma cells in response to silencing of chaperone HSPB8 and their correlation with increased cell proliferation rate, attesting to the fact that this polyfunctional protein is a necessary component of the malignant tumor growth and cell survival as well as

gene interaction mechanisms. It is possible that there is functional gene network and that knockdown one gene disrupts genome stability and leads to numerous changes in the expression of genes directly or indirectly initiated by the excluded gene, in particular HSPB8. However, the detailed molecular mechanisms of this regulation have not been clearly defined yet and warrant further investigation.

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