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Silencing effect of lentiviral vectors encoding shRNA of Herp on endoplasmic reticulum stress and inflammatory responses in RAW 264.7 macrophages

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ABSTRACT. Herp, a mammalian protein with a ubiquitin-like domain, can be strongly upregulated by endoplasmic reticulum (ER) stress during ER-associated protein degradation. However, the other cellular functions of Herp remain unclear. We explored the effect of Herp on ER stress and inflammatory responses in RAW 264.7 macrophages that had been exposed to tunicamycin or thapsigargin. We successfully constructed recombinant lentiviral vectors for Herp short-hairpin RNA (shRNA) expression to better understand the contribution made by Herp to other signaling pathways. Western blotting revealed that the recombinant Herp lentiviral shRNA vector significantly inhibited the expression of the Herp protein in the thapsigargin-treated RAW 264.7 macrophages. The reverse transcription quantitative

polymerase chain reaction results showed that knockdown Herp inhibited the expression of ER stress-related genes during exposure to tunicamycin or thapsigargin. In RAW 264.7 macrophages, knockdown Herp markedly attenuated the expression of inflammatory cytokines when exposed to tunicamycin; however, it strongly enhanced the expression of inflammatory cytokines when exposed to thapsigargin. We concluded that Herp lentiviral shRNA vectors had been successfully constructed; knockdown Herp inhibited ER stress and had a different effect on inflammatory responses in RAW 264.7 macrophages depending on whether they were exposed to tunicamycin or thapsigargin.

Key words: Herp; Endoplasmic reticulum stress; Inflammatory responses; RAW 264.7 macrophages

INTRODUCTION

RNA interference (RNAi) silences specific gene sequences triggering the degradation of target mRNA transcripts. Its exquisite sequence specificity and high silencing efficiency make RNAi a powerful tool for analyzing gene function (Hannon and Rossi, 2004). Lentiviral vectors encoding short-hairpin RNA (shRNA) have been widely used recently in fundamental biology, functional genomics, and gene therapy research. Lentiviral vectors encoding shRNA can be effectively transduced into dividing and non-dividing cells, and stably integrated into the chromosomes of target cells (Naldini et al., 1996; Naldini, 2011). Additionally, they are characterized by reduced genotoxicity, efficient pseudotyping, and the ability to carry large transgenes. The most up-to-date lentiviral packaging system used for research has been named “the third-generation self-inactivating lentiviral vector”; it is safer and yields higher titers than conventional systems, and comprises a transducing vector and three packaging plasmids to co-transfect the HEK 293T packaging cell line (Dull et al., 1998). Major applications of the third generation system include generating transgenic animals, studying gene functions, modeling genetic diseases, and disease therapy (Nguyen et al., 2009; Li et al., 2012; An et al., 2014; Chandrashekran et al., 2014).

The endoplasmic reticulum (ER) stress response is induced by physiological and/or pathological stress signals, such as would be caused by a disturbed ER microenvironment, altered calcium homeostasis, or accumulated misfolded or unfolded proteins in the ER. The ER stress response attenuates protein synthesis and eliminates misfolded or unfolded proteins via the activation of ER chaperones, foldases, and components of ER-associated protein degradation (ERAD) and autophagy (Cawley et al., 2011). A variety of evidence supports a relationship between ER stress response and inflammatory response (Zhang and Kaufman, 2008). Exogenous pathogens and endogenous metabolic factors can not only elicit an ER stress response, but also cause an inflammatory response. For example, thapsigargin enhances lipopolysaccharide (LPS)-induced inducible nitric oxide synthase (iNOS) mRNA expression (Ohta et al., 2011). However, Ho et al. (2012) demonstrated that tunicamycin attenuates LPS-stimulated expression of the iNOS gene. Thus, the interaction between ER stress and inflammation responses is still not fully understood and may depend on the cell type, the nature of the inflammatory mediator, and/or the type of ER stress inducer.

The homocysteine-responsive ER-resident protein (Herp), localized in the ER membrane, is expressed during ER stress response (Kokame et al., 2000; van Laar et al., 2000). Herp plays an

important role in the ERAD pathway by recruiting ubiquitin (Okuda-Shimizu and Hendershot, 2007; Kim et al., 2008; Kny et al., 2011). The cellular functions of Herp are gradually being elucidated. In addition to its role in the ERAD pathway, Herp inhibits apoptosis triggered by ER stress (Chan et al., 2004; Hori et al., 2004). Herp stabilizes ER Ca^{2+} homeostasis and maintains mitochondrial function in neuronal cells during ER stress (Chan et al., 2004). It has been associated with the pathogenesis of type 2 diabetes, neurodegeneration, sarcopenia, and atherosclerosis (Sai et al., 2003; Nogalska et al., 2006; Chigurupati et al., 2009; Slodzinski et al., 2009; Yan et al., 2012; Shinozaki et al., 2013). Recently, it has been reported that knockdown Herp prevents cell death by upregulating autophagy under glucose deprivation and attenuates the ER stress-induced inflammatory response in the presence of tunicamycin (Quiroga et al., 2013; Shinozaki et al., 2013). To date, many functions of Herp have been discovered. Herp not only acts as a regulator of ER stress, but also plays an important role in the tunicamycin-induced inflammatory response. However, it is uncertain whether Herp has other roles in ER stress and inflammatory reactions under other stress. In the present study, we aimed to construct a recombinant Herp shRNA lentiviral vector and determine whether Herp has different roles in ER stress response and inflammatory response under other stresses.

MATERIAL AND METHODS

Reagents

Tunicamycin and thapsigargin were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide immediately before use. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from the Gibco Company (USA). A Total Protein Extraction Kit and a BCA Protein Assay Kit were purchased from Nanjing KeyGen Biotech Co., Ltd (Nanjing, Jiangsu, China). TRIzol, a PrimeScript™ RT Reagent Kit, and a SYBR Premix Ex Taq II Kit were purchased from TaKaRa Chemical Co. (Dalian, China). Anti-β-actin antibody was obtained from Beijing CWBIO Co., Ltd. (Beijing, China). Anti-Herp antibody was obtained from Santa Cruz Biotechnology (USA).

Cell culture

HEK 293T cells and RAW 264.7 macrophages were cultured in DMEM with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin solution in a humidified incubator at 37°C with 5% CO_2. When the cells reached 70-80% confluence, they were treated, collected, and processed for further experiments.

Construction and identification of Herp shRNA lentiviral vectors

We designed three RNAi sequences according to GenBank data for Herp (NM_022331.1). The target sequences began with a G dinucleotide and included 21 nucleotides that were not homologous to other coding sequences according to the BLAST algorithm. The shRNA candidate sequences are shown in Table 1. The sense and antisense primers contained *Eco*RI and *Bam*HI restriction sites at the 5'- and 3'-ends, respectively, and each contained sense siRNA sequences, a 6-bp loop sequence, an antisense siRNA sequence, and an RNA polymerase III terminator sequence. The U6 RNAi cassette fragment from pSilencer 2.1-U6 hygro (AM5760; Life

Technologies, Carlsbad, CA, USA) was amplified and cloned into pCD513B-1 (SBI, Mountain View, CA, USA) to generate a pCD513B-U6 lentiviral vector (An et al., 2014). The primers were annealed and inserted into the U6 RNA polymerase III promoter downstream. The recombinant plasmids, called pCD513B-U6-Herp-shRNA (shHerp), were identified by polymerase chain reaction (PCR) and transformed into DH5α *Escherichia coli*. The primers for the PCR were as follows: forward, 5'-TTCTTGGGTAGTTTGCAGTT-3'; and reverse, 5'-CGGAGCCAGTACACGACA-3'. Positive clones were sent for DNA sequencing. The negative control, called pCD513B-U6-shRNA-NC (shNC), was constructed in our laboratory (Table 2) (Chen et al., 2014).

Table 1. Short-hairpin interfering RNA (shRNA) inserts.

siRNA	Sequence (loop in bold letters) (5' to 3')
shHerp-1	GATCCGTTCAGAACTTCCCGGATGAT**CTCGAG**ATCATCCGGGAAGTTCTGAACTTTTTG
	AATTCAAAAAGTTCAGAACTTCCCGGATGAT**CTCGAG**ATCATCCGGGAAGTTCTGAACG
shHerp-2	GATCCGTTATTCTGAAGAGCTTTAA**CTCGAG**TTAAAGCTCTTCAGAATAACGTTTTTG
	AATTCAAAAACGTTATTCTGAAGAGCTTTAA**CTCGAG**TTAAAGCTCTTCAGAATAACGG
shHerp-3	GATCCGAGCAGCCGGACAACTCTAAT**CTCGAG**ATTAGAGTTGTCCGGCTGCTCTTTTTG
	AATTCAAAAAGAGCAGCCGGACAACTCTAAT**CTCGAG**ATTAGAGTTGTCCGGCTGCTCG

Table 2. Features of recombinant lentiviral vectors.

Viruses	Vectors	Features	Inserts	Resource
CD513B-1	pCD513B-U6	Promoter CMV replaced with U6	U6 RNAi fragment	Storage in laboratory (Chen et al., 2014)
CD513B-U6	pCD513B-U6-Herp-shRNA-1	Herp shRNA-1 sequence inserted	Herp-shRNA-1	This study
CD513B-U6	pCD513B-U6-Herp-shRNA-2	Herp shRNA-2 sequence inserted	Herp-shRNA-2	This study
CD513B-U6	pCD513B-U6-Herp-shRNA-3	Herp shRNA-3 sequence inserted	Herp-shRNA-3	This study
CD513B-U6	pCD513B-U6-NC-shRNA	Negative shRNA sequence inserted	NC-shRNA	Storage in laboratory (Chen et al., 2014)

Transduction of RAW 264.7 macrophage cells with Herp shRNA lentivirus

Virus packaging and virus titer determination were performed as described previously (Chen et al., 2014). The packaging plasmids (pGag/Pol, pRev, and pVSV-G) and pCD513B-U6-Herp-shRNA were co-transfected into HEK 293T. Lentivirus-containing supernatants were collected and stored at -80°C. RAW 264.7 macrophages were cultured in DMEM supplemented with 10% FBS, and were transduced with the shHerp or shNC lentivirus (multiplicity of infection = 20), containing 8 μg/mL Polybrene. Following transduction for 48 h, stably infected colonies of shHerp and shNC were selected using 5 μg/mL puromycin.

Western blotting

After treatment, the RAW 264.7 macrophages were washed with cold PBS and lysed using radioimmunoprecipitation (RIPA) buffer on ice for 30 min. Protein concentration was determined by the bicinchoninic acid assay. Equal volumes of total cellular protein were electrophoresed on 12% sodium dodecyl sulfate polyacrylamide electrophoresis gel and electrotransferred to polyvinylidene fluoride membranes. After blocking with 10% non-fat milk, the membranes were incubated overnight at 4°C with an anti-Herp antibody and an anti-β-actin mouse monoclonal antibody. The next day, the membranes were incubated with secondary antibody conjugated to horseradish peroxidase (1:5000; Zhongshan Golden Bridge Biotechnology, Nanjing, China) at room temperature for 1 h. Finally,

immunoreactive bands were visualized using a gel imaging system (Tannon Biotech, Shanghai, China) and digitized with the Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using TRIzol according to the manufacturer instructions. The complementary DNAs (cDNAs) were synthesized using a PrimeScript™ RT Reagent Kit (Dalian, China). RT-qPCR was performed with Bio-Rad iQ5 and Bio-Rad iQ5 Optical System softwares (iQ5, Bio-Rad Laboratories, Inc., Hercules, CA) using the SYBR Premix Ex Taq II Kit according to the manufacturer instructions. The sequences of the specific primers used are list in Table 3. These reactions were repeated thrice for every sample as technical replicates. Gene mRNA quantifications were performed using the $2^{-\Delta\Delta Ct}$ method, and the amount of transcript in each sample was normalized using the β-actin gene as an internal control to correct the differences in the cDNA used.

Table 3. Primer sequences used for reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Gene-encoded protein	GenBank accession No.	Forward (5'-3')	Reverse (5'-3')	Product (bp)
β-actin	NM_007393	GCAAGCAGGAGTACGATGAG	CCATGCCAATGTT GTCTCTT	148
Herp	NM_022331.1	GCAGTTGGAGTGTGAGTCG	TCTGTGGATTCAGCACCCTTT	229
GRP78	NM_001163434.1	AGAAACTCCGGCGTGAGGTAGA	TTCCTGGACAGGCTTCATGGTAG	176
CHOP	NM_007837.3	AGCTGGAAGCCTGGTATGAGGA	AGCTAGGGACGCAGGGTCAA	134
ATF4	X61507	CTCTTGACCACGTTGGATGAC	CAACTTCACTGCCTAGCTCTAAA	226
BCL-2	NM_009741.4	CTACCGTCGTGACTTCGCA	TACCCAGCCTCCGTTATCC	268
BAX	NM_007527.3	TTTCATCCAGGATCGAGCAGG	GCAAAGTAGAAGAGGGCAACCAC	264
XBP-1	NM_001271730	TGAGTCCGCAGCAGGTG	GACAGGGTCCAACTTGT	130
IL-α	NM_010554	TCTATGATGCAAGCTATGGCTCA	CGGCTCTCCTTGAAGGTGA	109
IL-β	NM_008361	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG	116
IL-6	NM_031168	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG	131
TNF-α	NM_031693	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG	89

Statistical analysis

The experimental results are reported as means ± SE of triplicate experiments. Data were analyzed with one-way analysis of variance, followed by the Fisher least significant difference test (Fisher LSD) and the independent-sample *t*-test using the SPSS (Statistical Package for the Social Sciences) software (Version 13.0; SPSS, Inc., Chicago, IL). Differences were considered to be significant when $P < 0.05$.

RESULTS

Construction of Herp recombinant lentiviral vectors

The recombinant plasmids were identified by PCR. The positive vectors were named pCD513B-U6-Herp-shRNA-1, -2, and -3 (Table 2). The PCR products were checked by 2.0% agarose gel electrophoresis. The shRNA insert was 360 bp and the empty plasmid was 300 bp (Figure 1). The result of DNA sequencing further confirmed that the inserted fragments were consistent with our target sequences.

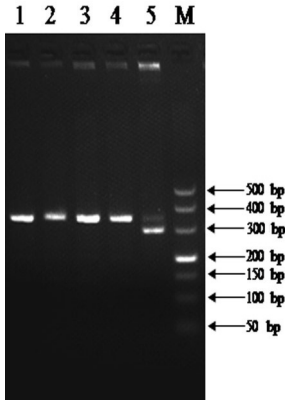

Figure 1. Construction of Herp shRNA recombinant lentiviral vectors. Agarose gel electrophoresis Herp shRNA sequences were cloned into pCD513B-U6. *Lanes 1*, *2*, *3*, and *4* = pCD513B-U6-Herp-shRNA-1, -2, -3, and pCD513B-U6-NC-shRNA, respectively; *lane 5* = pCD513B-U6; *lane M* = DL500 DNA marker.

Effect of Herp-shRNA on the expression of Herp in the thapsigargin-treated RAW 264.7 macrophages

To identify the interference effect of Herp shRNA, the RAW 264.7 macrophages were transduced with the pCD513B-U6-Herp-shRNA lentivirus (Figure 2A). Western blotting showed that shHerp-2 and -3 significantly reduced the expression of Herp protein compared with the shNC group during thapsigargin-induced ER stress, and the expression of the Herp gene was downregulated by more than 50 and 80%, respectively (Figure 2B and C).

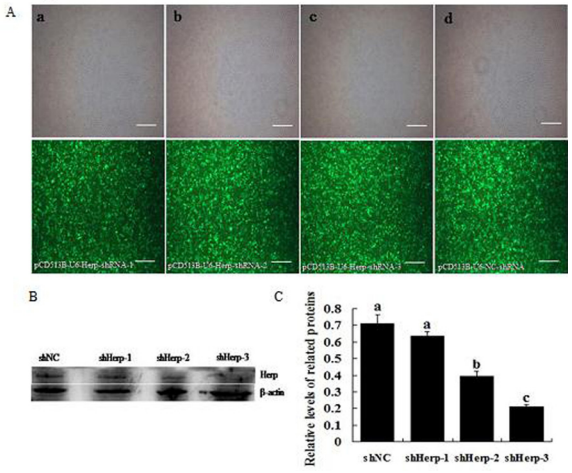

Figure 2. Effect of inhibition of Herp expression in the RAW 264.7 macrophages by transduction with Herp-shRNA lentiviruses. **A.** Fluorescence images of RAW 264.7 macrophages transduced with lentiviruses for 48 h. Green fluorescent protein (GFP) expression was observed under light (top panels) or fluorescence microscopy (bottom panels). a-d: pCD513B-U6-Herp-shRNA-1, -2, -3, and NC-shRNA, respectively. Scale bars, 50 µm. **B.** and **C.** Western blotting analysis of Herp protein expression levels in the RAW 264.7 macrophages transduced for 48 h and treated with 400 nM thapsigargin for 6 h. Analyses of band intensity on films are reported as the relative ratio of Herp to β-actin. Statistical analysis is shown in the bar graphs. Data are reported as means ± SE. Bars with different letters are significantly different ($P < 0.05$).

Effect of knockdown Herp on unfolded protein response (UPR) during ER stress in the RAW 264.7 macrophages

To determine the effect of knockdown Herp on the UPR during ER stress, we performed RT-qPCR to determine the expression of ER stress-related proteins at the mRNA level. Stable knockdown shHerp and shNC RAW 264.7 macrophages were treated with tunicamycin (1 μg/mL) and thapsigargin (400 nM) for 6 h. The RT-qPCR results showed that the tunicamycin and thapsigargin treatments both induced the expression of ER stress response genes that encode GRP78, CHOP, ATF4, BCL-2, BAX, XBP-1, and Herp (Figure 3A-G). Except for the genes that encode GRP78 and BCL-2, the expression of the genes was strongly induced during exposure to tunicamycin compared with thapsigargin. Knockdown Herp decreased the amount of Herp mRNA by 80% in RAW264.7 macrophages treated with tunicamycin and thapsigargin, while the shNC group did not affect the amount of Herp mRNA (Figure 3A). The induction of GRP78, CHOP, ATF4, BAX, and XBP-1 was significantly suppressed in the shHerp macrophages treated with tunicamycin (Figure 3B-D, F, and G). However, there were no significant differences between the shHerp and shNC cells in terms of the ER stress-induced BCL-2 mRNA expression during exposure to tunicamycin (Figure 3E). Although the expression levels of GRP78, CHOP, and BCL-2 were significantly reduced in the thapsigargin-treated shHerp group, the expression of BAX increased (Figure 3F). There were no significant differences between the expression levels of ATF4 and XBP-1 in the thapsigargin-treated shHerp and shNC groups.

Effect of knockdown Herp on inflammatory responses in the RAW 264.7 macrophages

To evaluate the effect of knockdown Herp on the expression of inflammatory cytokines under ER stress, the shHerp and shNC RAW 264.7 macrophages were treated with tunicamycin (1 μg/mL) or thapsigargin (400 nM) for 6 h. The RT-qPCR results showed that tunicamycin and thapsigargin treatment both induced the expression of inflammatory cytokines IL-1α, IL-1β, IL-6, and TNF-α (Figure 4A-D). Except for IL-6, the expression of the genes was strongly induced with thapsigargin compared with tunicamycin. The induction of IL-1α, IL-1β, and IL-6 was significantly suppressed in the shHerp group treated with tunicamycin (Figure 4A-C). However, there were no significant differences between the shHerp and shNC groups in terms of the ER stress-induced TNF-α mRNA expression when treated with tunicamycin (Figure 4D). Although the expression levels of IL-6 and TNF-α did not show significant differences between the shHerp and shNC thapsigargin-treated groups (Figure 4C and D), the expression levels of IL-1α and IL-1β were significantly increased (Figure 4A and B).

DISCUSSION

The RAW 264.7 macrophage, an immortalized macrophage clone isolated from BALB/c mice, is a valuable model for the study of innate immunity (Raschke et al., 1978). To study Herp-mediated macrophage function, we used the third generation lentiviral packaging system to construct Herp shRNA recombinant lentiviral vectors and a stably knocked-down Herp gene in the RAW 264.7 macrophages. The study of cellular functions broadly utilizes the expression of modified genes in target cells. However, the delivery of recombinant genetic constructs into macrophages is

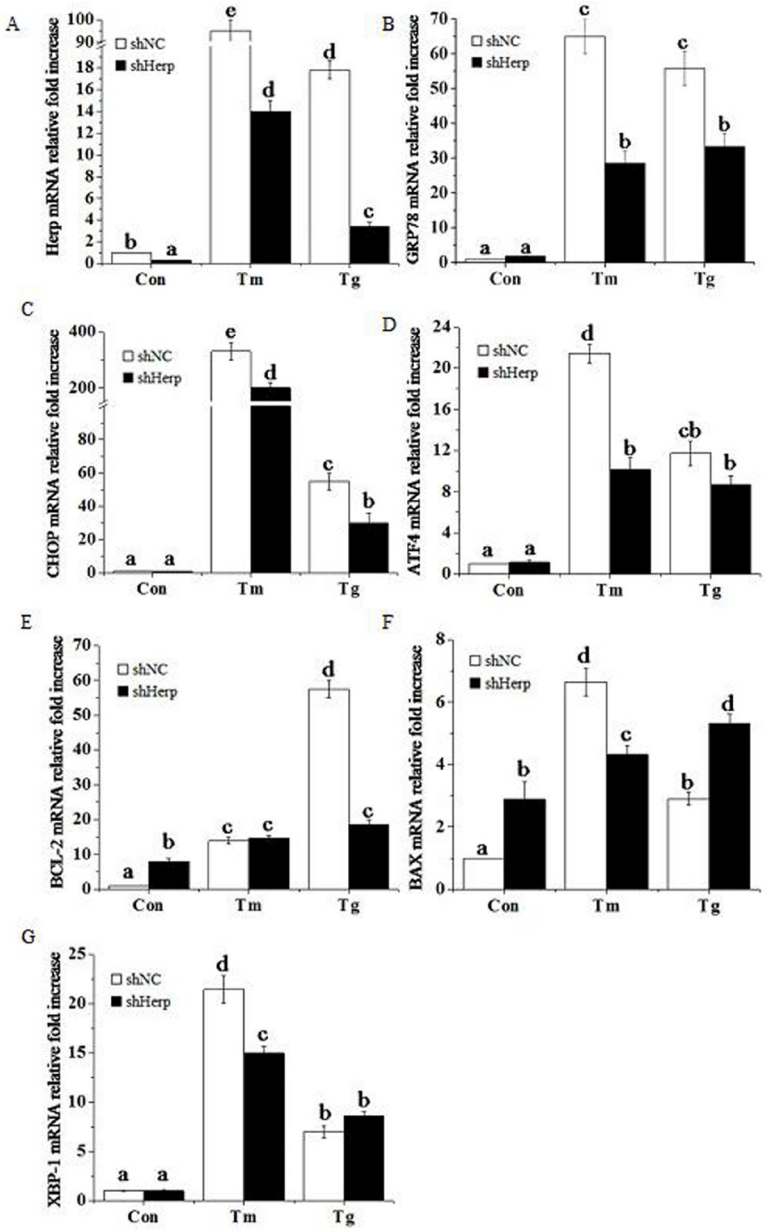

Figure 3. Effect of knockdown Herp on expression of endoplasmic reticulum (ER) stress-related proteins in the RAW 264.7 macrophages. Stable knockdown shHerp and shNC RAW 264.7 macrophages were treated with tunicamycin (Tm, 1 μg/mL) or thapsigargin (Tg, 400 nM) for 6 h. The mRNA expression levels of ER stress-related genes were analyzed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). **A.** Herp; **B.** GRP78; **C.** CHOP; **D.** ATF4; **E.** BCL-2; **F.** BAX; **G.** XBP-1. Statistical analysis is shown in the bar graphs. The mRNA levels were normalized to the level of β-actin mRNA. The mRNA levels are reported as the relative ratio to the mRNA level in tunicamycin- or thapsigargin-treated macrophages. Data are reported as means ± SE. Bars with different letters are significantly different ($P < 0.05$). Con = control.

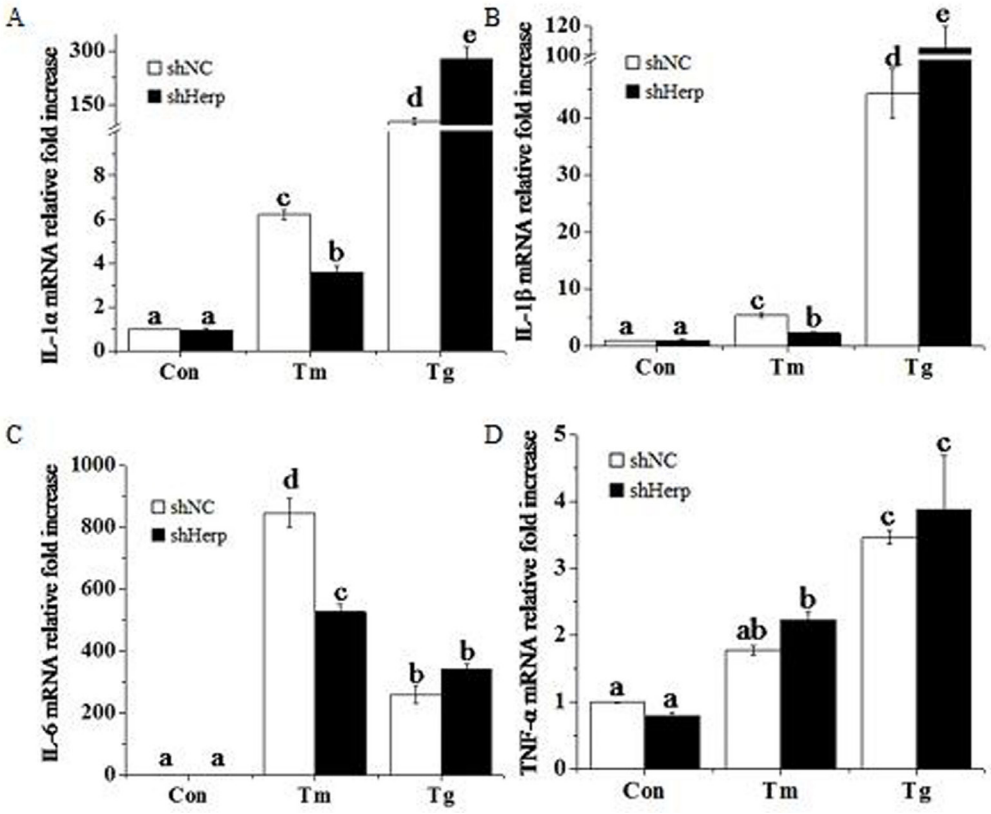

Figure 4. Effect of knockdown Herp on expression of inflammatory cytokines in the RAW 264.7 macrophages. Stable knockdown shHerp and shNC RAW 264.7 macrophages were treated with tunicamycin (Tm, 1 μg/mL) or thapsigargin (Tg, 400 nM) for 6 h. The mRNA expression levels of inflammatory cytokines were analyzed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). **A.** IL-1α; **B.** IL-1β; **C.** IL-6; **D.** TNF-α. Statistical analysis is shown in the bar graphs. The levels of mRNA were normalized to the level of β-actin mRNA. The mRNA levels are reported as the relative ratio to the mRNA level in tunicamycin- or thapsigargin-treated macrophages. Data are reported as means ± SE. Bars with different letters are significantly different ($P < 0.05$). Con = control.

difficult. Although the RAW 264.7 macrophages can be efficiently transfected by plasmid DNA via electroporation, electroporation can induce macrophage cell death (Krysko et al., 2006; Liu et al., 2012). Recombinant vectors based on adenoviruses and lentiviruses have been used to deliver modified genes into macrophages much more efficiently (Stein and Falck-Pedersen, 2012; Tong et al., 2014). However, adenoviruses are not ideal vectors because of their immunogenicity and the transient nature of their gene expression. Lentiviral vectors have a number of advantages for research: not only do they carry larger transgenes and integrate them into the chromosomes of target cells, both dividing and non-dividing, with a relatively high transduction efficiency, but they are also condensed into high titers and express genes long-term without causing an immune response (Blesch, 2004). Therefore, we chose lentiviral vectors to transduce Herp into the macrophages. Our results showed that Herp-shRNA-3 effectively inhibited the expression of Herp in the RAW 264.7 macrophages, hence this construct was chosen for further experiments.

Previous studies have proved that ER stress induces the expression of Herp (Kokame et al., 2000; Hori et al., 2004). Furthermore, Herp also affects the expression of ER stress-related genes. Shinozaki et al. (2013) proved that Herp depletion suppresses GRP78 and CHOP during tunicamycin-induced ER stress. In this context, our study strengthened the concept that Herp, a protein encoded by an ER stress-induced gene, functions in the RAW 264.7 macrophages. Our results showed that knockdown Herp had no effect on the ER stress-related gene transcripts in cells growing normally under non-stressful conditions. However, knockdown Herp inhibited the expression of GRP78, ATF4, and CHOP once ER stress was activated by tunicamycin or thapsigargin in this case. However, the further roles of Herp in the regulation of ER stress require investigation.

As well as responding to ER stress, other functions of Herp have been speculated in other tissues. Herp stabilizes ER Ca^{2+} homeostasis and maintains mitochondrial function in neuronal cells (Chan et al., 2004). Knockout Herp modulates glucose tolerance in the liver, suppresses ER stress-induced inflammation, and attenuates the development of atherosclerosis (Eura et al., 2012; Shinozaki et al., 2013). Herp operates as a relevant factor in the defense against glucose starvation by modulating autophagy levels (Quiroga et al., 2013). Thus, Herp has various functions in different tissues. To determine the effect of Herp in future research, knockout or knockdown Herp is very important. Consistent with previous reports (Shinozaki et al., 2013), inflammatory cytokines, such as IL-1α, IL-1β, and IL-6, were significantly suppressed in the shHerp group during exposure to tunicamycin. However, we also found that IL-1β and IL-1α were significantly increased in the shHerp group during thapsigargin exposure. IL-6 and TNF-α did not show significant differences between the shHerp and shNC groups during thapsigargin exposure. There were significant and differently modulated inflammatory responses during thapsigargin exposure compared with tunicamycin exposure. The precise mechanisms underlying the regulation of inflammatory cytokine production under different ER stress conditions due to Herp knockdown are unclear.

Several possible mechanisms based on the functions of Herp have been reported by other research groups. Tunicamycin is an antibiotic that inhibits glycosylation, thereby affecting the maturation and transport of most secreted proteins that cause ER stress (Criscuolo and Krag, 1982; Elbein, 1984). The suppression of inflammatory cytokines by Herp deficiency may involve UPR-mediated transcription factor nuclear factor-κB (NF-κB) activation. The mRNA expression levels of molecules downstream of the UPR pathway, such as ATF4 and XPB-1, were significantly attenuated by Herp deficiency in the tunicamycin-treated macrophages. The attenuated UPR-mediated reduction in the level of inhibitors of NF-κB (IκB) results in decreased NF-κB activation (Hotamisligil, 2010). Thapsigargin is a non-competitive inhibitor of sarco/endoplasmic reticulum Ca^{2+}-ATPase, and its presence causes depletion of the calcium store in the ER leading to ER stress responses. Knockdown Herp can suppress thapsigargin-induced ER stress responses and accelerate the release of calcium from the ER. The calcium released from the ER further increases reactive oxygen species (ROS) production and protein misfolding (Görlach et al., 2006). Further calcium release, ROS production, and protein misfolding lead to stronger inflammatory responses (Malhotra and Kaufman, 2007). However, further studies on the molecular mechanism by which Herp regulates inflammatory responses are clearly needed.

In conclusion, we constructed recombinant lentiviral vectors for Herp shRNA expression and selected stable expression in knockdown Herp RAW 264.7 macrophages. The vector significantly decreased the expression of Herp and suppressed the expression of ER stress-related genes. However, different ER stress inducers had different effects on inflammatory responses.

Conflicts of interest

The authors declare no conflict of interest.

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