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HSF1 promotes the viability of islet β-cells *via* upregulating SIRPα expression

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Abstract. – OBJECTIVE: Increasing evidence has shown that HSF1 is involved in glycemia regulation, and SIRPα plays a pivotal role in islet β-cell viability. However, it is still unknown whether SIRPα is associated with HSF1 in regulating the cell viability and cell death of islet β-cells.

MATERIALS AND METHODS: Western blot and qPCR were applied to determine protein and mRNA levels of HSF1 and SIRPα. Cell viability and death were investigated by cell counting kit-8 and trypan blue exclusion assay. Meanwhile, cell apoptosis was analyzed by detecting caspase3 activity. Moreover, luciferase reporter assay was applied to explore the mechanism by which HSF1 transcriptionally upregulated SIRPα expression.

RESULTS: Our study reveals that HSF1 expression was lower in islets from T1DM compared to normal mice. We found that overexpression of HSF1 decreased the apoptosis of islet β-cell lines. Moreover, we demonstrated that overexpression of HSF1 decreased the apoptosis of islet β-cells through increasing the expression of SIRPα. In terms of mechanism, luciferase reporter assays showed that HSF1 upregulated SIRPα expression by activating its gene promoter region. The binding site (-1809 to -1795) was required for HSF1-induced increase of SIRPα gene promoter activity.

CONCLUSIONS: These results indicate that the low expression of HSF1/SIRPα may be one of the mechanisms of islet β-cell death and targeting HSF1/SIRPα may be a novel strategy for the treatment of T1DM.

Key Words:
HSF1, SIRPα, T1DM, Transcriptional regulation, Apoptosis.

Abbreviations

HSF1 = Heat shock transcription factor 1, SIRPα = Signal-regulatory protein α, T1DM = Type 1 diabetes mellitus, CCK-8 = Cell Counting Kit-8, DMEM = Dulbecco's Modified Eagle Medium, qPCR = Quantitative real-time polymerase chain reaction, RIPA = Radioimmunoprecipitation assay, GAPDH = Glyceraldehyde 3-phosphate dehydrogenase, PBS = Phosphate buffered saline, Act D = Actinomycin D, EP300 = Histone acetyltransferase E1A binding protein P300, HSPs = Heat shock proteins.

Introduction

Type 1 diabetes mellitus (T1DM), a common chronic disease, has become one of the most leading causes of death all over the world[1-3]. In recent years, its incidence is on the rise[4,5]. T1DM is caused by absolute insulin deficiency due to islet dysfunction[6]. Islet β-cells are endocrine cells, accounting for about 70% of the total number of islet cells, mainly located in the central part of the islet, which can secrete insulin[7,8]. The apoptosis of islet β-cells is one of the most important mechanisms of T1DM[9]. Therefore, investigating the potential mechanism of inhibiting apoptosis is of great significance to understand the pathogenesis of T1DM and develop new strategies to treat T1DM.

Heat shock transcription factor 1 (HSF1) is a member of the heat shock transcription factor family and plays an important role in regulating heat shock response in mammalian cells[10,11]. Studies have shown that HSF1 participates in glycemia regulation[12-15]. In fact, hepatic overexpression of HSF1 activates Akt Pathways to ameliorate hyperglycemia in high-fat diet mice[12], suggesting that HSF1 is associated with better control of glycemic. A recent study has reported that restoring HSF1 activity in β-cells prevents glucolipotoxicity-induced endoplasmic reticulum stress and apoptosis, representing a novel strategy

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for the maintenance of a functional β-cell mass[16]. These studies suggest that the mechanism of HSF1 regulating glycemia may be related to its regulation of islet β-cell survival.

Signal-regulatory protein α (SIRPα)[17], a transmembrane protein, plays a key role in regulating macrophage phagocytosis. Scholars[18-20] have reported that SIRPα is related to the occurrence of DM. SIRPα promotes insulin secretion from β-cells and thereby protects against DM[18]. Recently, research has showed that knockdown of SIRPα significantly enhances β-cell death, and increasing the expression of SIRPα in β-cells promotes cell viability[21], suggesting that the expression of SIRPα was positively correlated with the survival of islet β-cells. However, to date, it is still unknown whether SIRPα is associated with HSF1 in regulating the cell viability and cell death of islet β-cells.

In the present study, we, for the first time, demonstrated that overexpression of HSF1 decreased the apoptosis of islet β-cell lines. Moreover, we found that HSF1 decreased the apoptosis of islet β-cells via upregulating SIRPα expression. In terms of mechanism, HSF1 increased SIRPα expression by activating its gene promoter region. Collectively, these findings suggest that the low expression of HSF1/SIRPα may be one of the important mechanisms of islet β-cell death and targeting HSF1/SIRPα may be a novel strategy for T1DM treatment.

Materials and Methods

Cell Lines and Animals

MIN-6 and NIT-1 mouse islet β-cell lines were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). These two cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (Sigma-Aldrich) and 100 μM β-mer-captoethanol at 37°C in 5% CO_2 incubator. The type 1 diabetic mice and normal control male C57BL/6J mice were from Beijing Huafukang Bioscience (Beijing, China).

Quantitative Real-time PCR (qPCR)

The Total RNA from MIN-6 or NIT-1 cells was extracted with TRIzol reagent (Comwin Biotechnology, Beijing, China). Then, the first-strand cDNA was synthesized by PrimeScript RT master mix (TaKaRa, Dalian, China). Subsequently, qPCR was performed with SYBR select master mix (Applied Biosystems, Foster City, CA, USA) in a 20-μl volume following the manufacturer's instruction, taking β-Actin as a control. The reactions were performed using the following parameters: 95° C for 5 min followed by 40 cycles of 95° C for 15 s and 60° C for 30 s. Relative mRNA levels of SIRPα and HSF1 were calculated with the $2^{-\Delta\Delta Ct}$ method. The sequences of primers were listed in **Supplementary Table I**.

Western Blot Analysis

Total protein from MIN-6 or NIT-1 cells was extracted with RIPA (Beyotime, Shanghai, China). Then the concentration was measured by the BCA Protein Assay Kit (Beyotime). Next, Western blot was performed as described[22]. Briefly, the total proteins (50 μg/well) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked using 5% non-fat milk for 1 h, and then incubated with antibodies over night at 4°C. After washed with TBST (Boster, China), blots were incubated with respective second antibody for 1 h at room temperature. Immunoblots were visualized using the ECL chemiluminescent detection system. The antibodies anti-HSF1 and anti-SIRPα were from Cell Signaling Technology (CST, Danvers, MA, USA); anti-GAPDH was from Proteintech Group (Chicago, IL, USA).

Transfection Assay

After grown to 70% confluence, MIN-6 and NIT-1 cells were transfected with siRNAs or expression plasmids with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. After 24 h, the other corresponding experiments were carried out. The sequence of siRNA for SIRPα was listed in **Supplementary Table II**.

Cell Viability Assay

Cell viability was detected using Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan). MIN-6 and NIT-1 cells were transfected with siRNAs or expression plasmids for 24 h, and then mixed with 10 μl of CCK-8 reagent per well and incubated for another 1 h at 37 °C. The absorbance of formaldehyde dyes produced by cellular dehydrogenase activity was measured at 450 nm using a microplate reader (Molecular De-

vices, Sunnyvale, CA, USA). The optical density values of each well represented the cell viability of MIN-6 and NIT-1 cells.

Trypan Blue Exclusion Assay

MIN-6 and NIT-1 cells were transfected with siRNAs or expression plasmids. After 24 h, the trypan blue exclusion assay was carried out following the manufacturer's instruction. Firstly, the cells were incubated with the trypan blue solution (Beyotime) for 3 min. Secondly, the cells were photographed under an optical microscope. The cell death rate (%) = number of dead cells/number of total cells (including all living and dead cells) × 100%.

Analysis of Caspase3 Activity

Caspase3 activity was measured by colorimetric assay kit (MBL International Corporation, Nagoya, Japan) following the manufacturer's instruction. After corresponding treatment, MIN-6 and NIT-1 cells were washed in ice-cold phosphate buffered saline (PBS), and the proteins were then extracted. Subsequently, cell lysates (20 μl) were added to the buffer containing p-nitroaniline (pNA)-conjugated substrate for caspase3 (Ac-DEVD-pNA) to obtain 100 μl total reaction volume. After incubated at 37°C, the released pNA concentrations were calculated according to the absorbance values at 405 nm. The caspase3 activities of control groups were set as 100%.

Plasmid Construction

The expression plasmids pCMV-HSF1 and pcDNA3.1-SIRPα were from Chongqing Lab Cell Biotechnology Co. Ltd (Chongqing, China). The reporter plasmids containing different promoter regions of SIRPα gene (pF1 -2988 to +180, pF2 -1755 to +180, pF3 -1646 to +180 and pF4 -214 to +180) and the pF1 Mut plasmid (-1809 AGATACTTCTTTAAG -1795, the underlined nucleotides were mutated) were from Chongqing Lab Cell Biotechnology Co. Ltd (Chongqing, China).

Luciferase Reporter Assay

After grown to 70% confluence, MIN-6 and NIT-1 cells were co-transfected with the luciferase reporters, pRL-TK plasmid and siRNA (or expression plasmid). After 24 h, the luciferase activity was detected by the dual-luciferase reporter system (Promega, Madison, WI, USA) following the manufacturer's instruction. Each experimental group was performed three times in triplicate, and the ratio of firefly luciferase activity to Renilla luciferase activity was taken as the result. Data were shown as relative luciferase activity over the corresponding control.

Statistical Analysis

Unless otherwise specified, all results were shown as mean ± SD. Comparisons between two groups were determined by two-tailed unpaired *t*-test. Comparisons among three or more groups were analyzed using one-way analysis of variance (ANOVA). The Tukey post-hoc was used to validate ANOVA for pairwise comparisons. $p < 0.05$ was considered statistically significant.

Results

Overexpression of HSF1 Decreased the Apoptosis of Islet β-Cells

Firstly, we isolated the islets from type 1 diabetic mice and normal control male C57BL/6J mice and determined the protein level of HSF1 in these tissues. As shown in Figure 1A, the protein level of HSF1 was lower in islets from type 1 diabetic mice compared to those from normal control C57BL/6J mice. These results indicated that HSF1 may play a role in T1DM. Since the death of islet β-cells is one of the most important mechanisms of T1DM, we speculate that HSF1 may be related to the death of islet β-cells. Therefore, two mouse β-cell lines (MIN-6 and NIT-1) were used to explore the role of HSF1 in islet β-cell death. Subsequently, HSF1 was overexpressed with pCMV-HSF1 plasmid in MIN-6 and NIT-1 cells. The overexpression efficiency was shown in Figure 1B and 1C. Moreover, Figure 1D-1F showed that overexpression of HSF1 in MIN-6 and NIT-1 cells increased the cell viability (Figure 1D), decreased the cell death (Figure 1E) and reduced the activity of caspase3 (Figure 1F). These data indicated that HSF1 decreased the apoptosis of islet β-cells.

HSF1 Upregulated the Expression of SIRPα

To explore the molecular mechanism of HSF1 in reducing apoptosis of islet β-cells, the following experiments were carried out. Research has shown that knockdown of SIRPα significantly enhances β-cell death. Subsequently, SIRPα was overexpressed in MIN-6 and NIT-1 cells. The overexpression efficiency was shown in Figures 2A and 2B. Moreover, Figure 2C and 2D showed that overexpression of SIRPα increased the cell

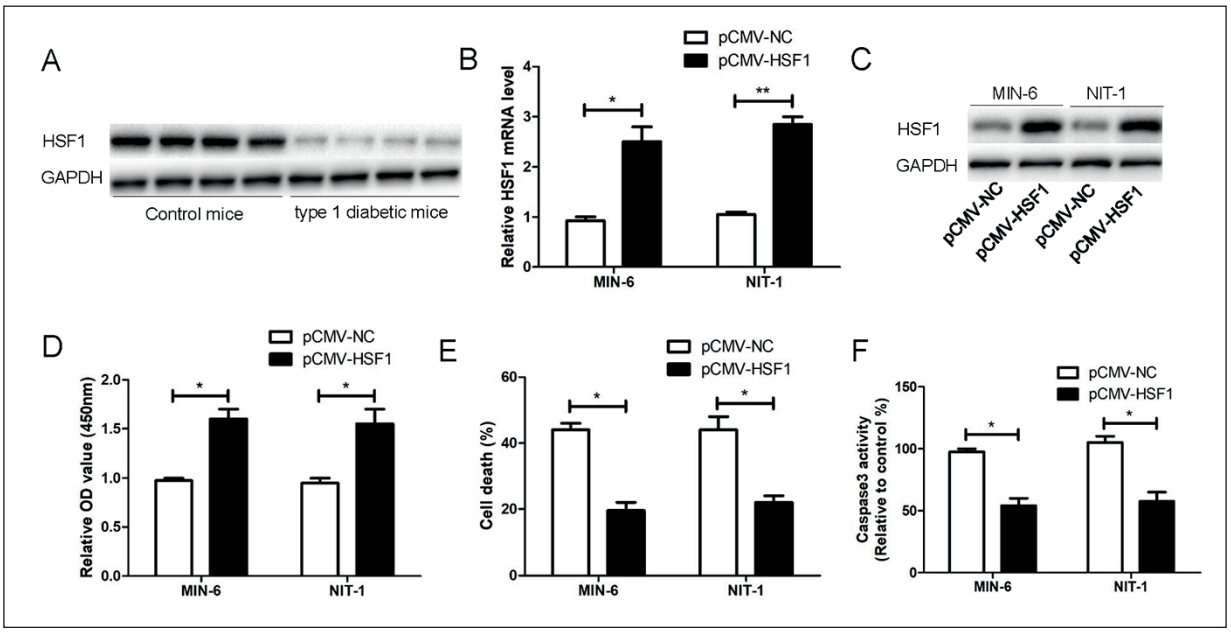

Figure 1. Overexpression of HSF1 decreased the apoptosis of islet β-cells. **A,** Western blot analysis of HSF1 protein level from the islets of type 1 diabetic mice and normal control male C57BL/6J mice (n = 4 per group). **B-F,** MIN-6 and NIT-1 cells were transfected with pCMV-HSF1 or pCMV-NC for 24 h. Subsequently, the mRNA level of HSF1 was detected by qPCR (**B**), the protein level of HSF1 was determined by Western blot (**C**), the optical density values at 450 nm were measured by CCK-8 (**D**), the dead cells were counted after trypan blue exclusion assay (**E**), and the caspase3 activity was measured to reflect apoptosis (**F**). The OD value at 405 nm in caspase3 activity analysis was normalized against that of the control, the control group was defined as 100%. pCMV-HSF1: HSF1 expression vector. **p* <0.05, ***p* <0.01.

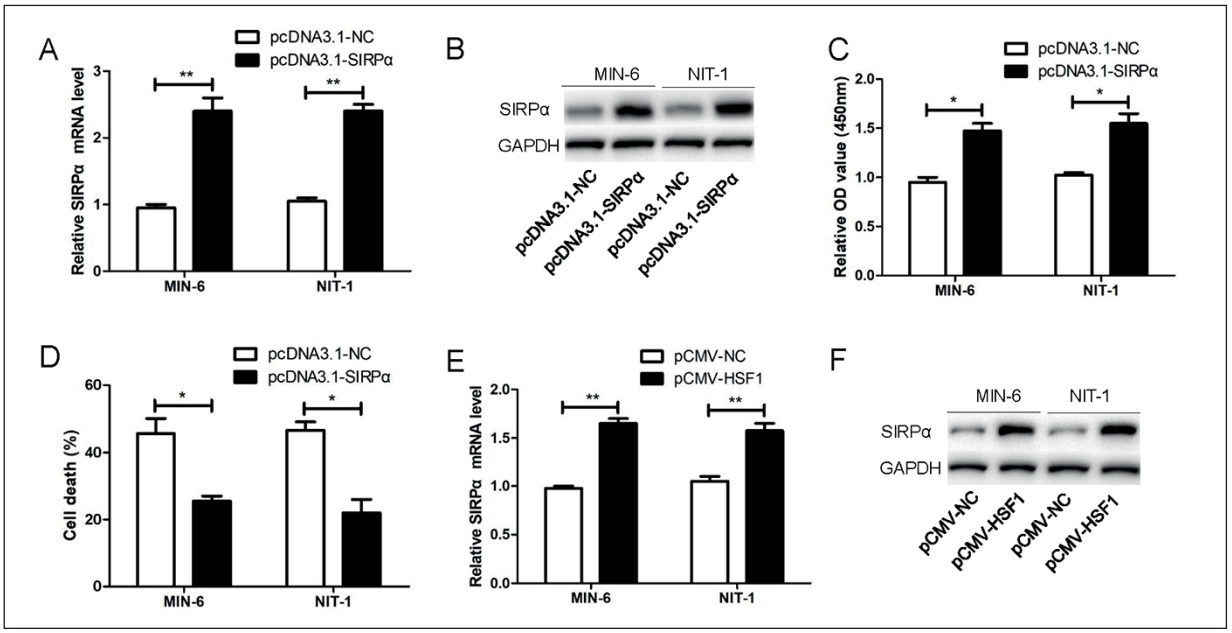

Figure 2. HSF1 upregulated the expression of SIRPα. **A-D,** MIN-6 and NIT-1 cells were transfected with pcDNA3.1-SIRPα or pcDNA3.1-NC for 24 h. Subsequently, the mRNA level of SIRPα was detected by qPCR (**A**), the protein level of SIRPα was determined by Western blot (**B**), the optical density values at 450 nm were measured by CCK-8 (**C**), the dead cells were counted after trypan blue exclusion assay (**D**). **E-F,** MIN-6 and NIT-1 cells were transfected with pCMV-HSF1 or pCMV-NC for 24 h. Then the mRNA level of SIRPα was detected by qPCR (**E**), the protein level of SIRPα was determined by Western blot (**F**). pcDNA3.1-SIRPα: SIRPα expression vector. **p* <0.05, ***p* <0.01.

viability (Figure 2C) and decreased the cell death (Figure 2D) in MIN-6 and NIT-1 cells. Bioinformatics predicts that there may be binding sites of transcription factor HSF1 in SIRPα promoter region. Therefore, we determined the mRNA and protein levels of SIRPα after overexpression of HSF1. The results showed that overexpression of HSF1 upregulated the mRNA (Figure 2E) and protein (Figure 2F) levels of SIRPα.

HSF1 Attenuating the Apoptosis of Islet β-Cells via Upregulating SIRPα

The above results have showed that HSF1 reduced the apoptosis of islet β-cells and upregulated the expression of SIRPα. The next experiments are to explore whether the effect of HSF1 on islet β-cell apoptosis was associated with SIRPα. Afterwords, SIRPα was silenced with siRNA in MIN-6 and NIT-1 cells. The silencing efficiency of siRNA for SIRPα was shown in Figure 3A. Furthermore, as shown in Figure 3B, overexpression of HSF1 increased the cell viability, while silencing of SIRPα attenuated the cell viability of MIN-6 and NIT-1 cells. Besides, upon silencing of SIRPα, overexpression of HSF1 did not further enhance the cell viability of MIN-6 and NIT-1 cells (Figure 3B). Meanwhile, overexpression of HSF1 decreased the cell death and caspase3 activity, while silencing of SIRPα increased the cell death and caspase3 activity in MIN-6 and NIT-1 cells (Figure 3C and 3D). Upon silencing of SIRPα, overexpression of HSF1 did not further reduce the cell death and caspase3 activity in MIN-6 and NIT-1 cells (Figure 3C and 3D). These results showed that HSF1 attenuates the apoptosis of islet β-cells by upregulating the expression of SIRPα.

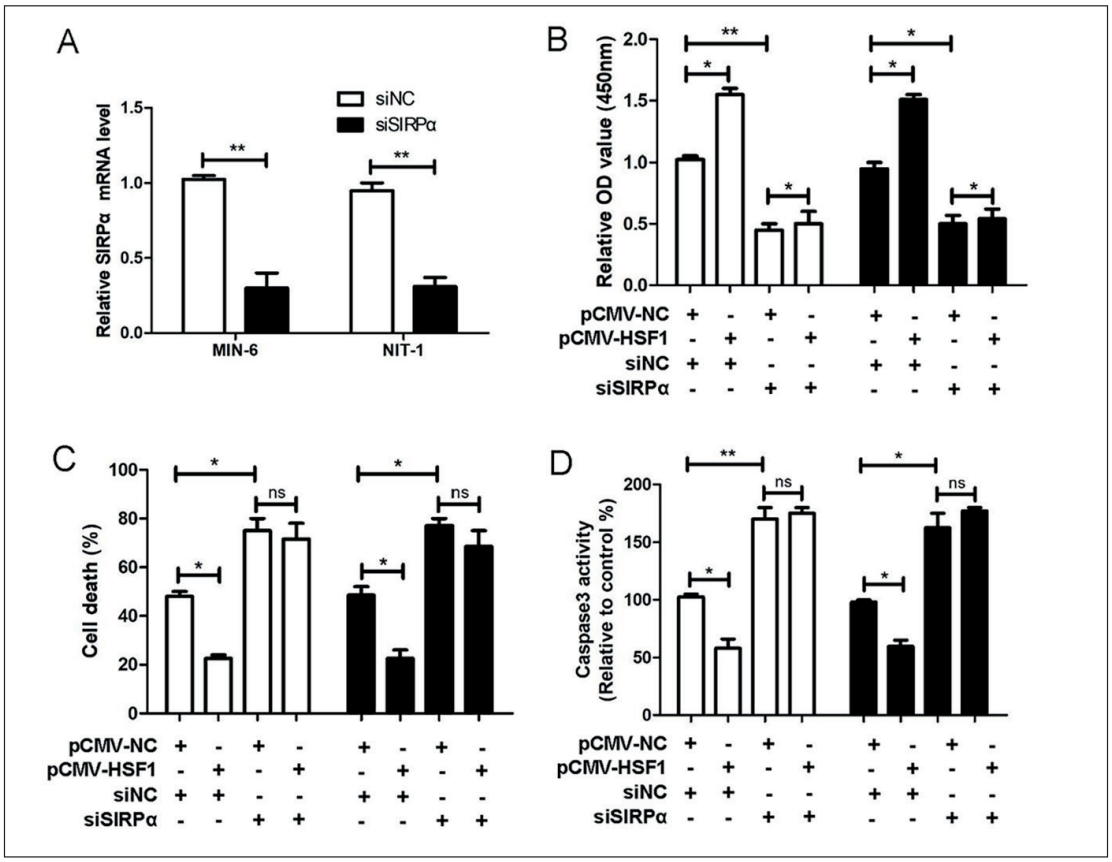

Figure 3. HSF1 attenuating the apoptosis of islet β-cells via upregulating SIRPα. **A,** MIN-6 and NIT-1 cells were transfected with siSIRPα or siNC for 24 h. Next, the mRNA level of SIRPα was detected by qPCR. **B-D,** After transfected with pCMV-HSF1 or pCMV-NC in the presence or absence of siSIRPα for 24 h, the optical density values at 450 nm were measured by CCK-8 (**B**), the dead cells were counted after trypan blue exclusion assay (**C**), and the caspase3 activity was measured to reflect apoptosis (**D**). The OD value at 405 nm in caspase3 activity analysis was normalized against that of the control, the control group was defined as 100%. pCMV-HSF1: HSF1 expression vector, siSIRPα: siRNA for SIRPα, siNC: negative control siRNA, *p <0.05, **p <0.01, ns: no significance.

HSF1 Increased SIRPα Expression by Activating Its Gene Promoter Region

To explore the mechanism by which HSF1 elevated SIRPα mRNA level, actinomycin D (Act D, a transcription inhibitor) assays were performed. Figure 4A and 4B showed that the overexpression of HSF1 in MIN-6 and NIT-1 cells had no influence on the degradation speed of SIRPα mRNA in the presence of Act D, suggesting that HSF1 did not affect SIRPα mRNA stability. Later, four luciferase reporter plasmids containing different promoter regions of SIRPα gene were constructed, which were named as pF1 (-2988 to +180), pF2 (-1755 to +180), pF3 (-1646 to +180) and pF4 (-214 to +180) (Figure 4C). The luciferase reporter assays showed that overexpression of HSF1 in MIN-6 and NIT-1 cells significantly increased the activity of pF1 rather than other three luciferase reporters (pF2, pF3 and pF4) (Figure 4D and 4E). These results suggested that a HSF1 binding site may be within the sequence (-2988 to -1755) of SIRPα promoter region, and the binding site should be (-1809 to -1795) according to the online prediction. Furthermore, overexpression of HSF1 in MIN-6 and NIT-1 cells did not affect the activity of pF1 Mut (containing mutations in the sequence -1809 to -1795) (Figure 4F and 4G). These data suggested that HSF1 increased SIRPα expression by activating its gene promoter region, and the potential binding site (-1809 to -1795) was required for HSF1-induced increase of SIRPα gene promoter activity.

Discussion

SIRPα is a transmembrane protein. Its extracellular region contains three Ig-like domains and its cytoplasmic region contains four tyrosine phosphorylation sites[23]. SIRPα is particularly abundant in myeloid cells such as macrophages, and regulates macrophage phagocytosis by binding with CD34, which is a member of the Ig superfamily of proteins[24]. It has been reported that SIRPα is also expressed in islet β-cells, and involved in regulating islet β-cell death[21]. Knockdown of SIRPα significantly enhances β-cell death and increasing the expression of SIRPα in islet β-cells promotes cell viability[21]. In our present study, we found

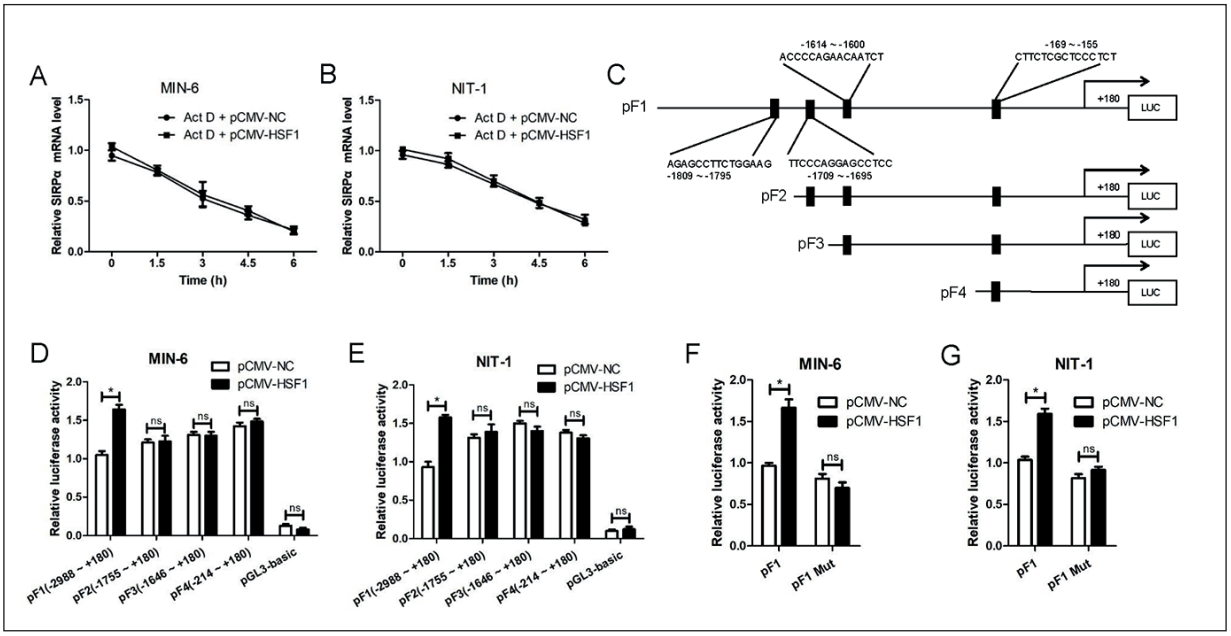

Figure 4. HSF1 increased SIRPα expression by activating its gene promoter region. **A-B,** MIN-6 (**A**) and NIT-1 (**B**) cells were transfected with pCMV-HSF1 or pCMV-NC for 24 h, followed by the treatment with actinomycin D (Act D, 5 mg/ml) for the indicated time. Then the level of SIRPα mRNA was detected by qPCR. **C,** The four putative HSF1 binding sites in human SIRPα promoter region were predicted using the online JASPAR database, and the corresponding luciferase reporters were named as pF1 to pF4. **D-G,** MIN-6 (**D, F**) and NIT-1 (**E, G**) cells were co-transfected with the luciferase reporters (**D, E**) [or pF1 Mut (**F, G**)] and pRL-TK in the presence of pCMV-HSF1 (or pCMV-NC) for 24 h. The luciferase activity was measured with the dual-luciferase reporter system. The firefly luciferase activity was normalized against the renilla luciferase activity. Luc: luciferase, pCMV-HSF1: HSF1 expression vector, pF1 Mut: pF1 mutant, **p* <0.05, ns: no significance.

that SIRPA mediates the increase of islet β-cell viability induced by HSF1 and targeting HSF1/SIRPα pathway may be a novel strategy for the treatment of T1DM.

HSF1 is a highly conserved DNA binding protein, which regulates gene expression at the transcriptional level[25]. HSF1 activates gene transcription by identifying a DNA binding site called heat shock element[26]. The abnormal regulation of HSF1 has been proved to be closely related to human diseases[27-30]. Neurodegenerative diseases have been shown to be associated with reduced activation of HSF1[27,28]. In our study, we found that HSF1 expression was lower in islets from T1DM compared to normal mice. What is the mechanism of the decrease of HSF1 expression in islets from T1DM? Studies have shown that proteasome degradation is an important mechanism regulating HSF1 level[31-33]. Site-specific acetylation modification is involved in the proteasome degradation of HSF1[32]. In the absence of stress, the level of HSF1 is regulated by histone acetyltransferase E1A binding protein P300 (EP300), which subsequently reduces the degradation of HSF1 by acetylating specific lysine residues (Lys208 and Lys298) on HSF1 protein[32]. Silencing of EP300 in HeLa cells increases the proteasome degradation of HSF1 and then decreases the protein level of HSF1[32]. In islet cells, whether decreased HSF1 expression is associated with acetylation modification still needs further study.

HSF1 is the main regulator of heat shock response, which helps to improve the viability of cells in response to harmful conditions such as high temperature, ischemia, inflammation and oxidative stress[34]. Under stress, HSF1 drives the transcription of heat shock proteins (HSPs) and then upregulates their expression[35]. These HSPs further assists in the folding of new polypeptides and the refolding of damaged proteins[36]. In addition, HSF1 also affects a series of cellular processes by regulating the expression of key survival genes, such as genes involved in protein translation, glucose metabolism, cell cycle and cell proliferation[25]. In recent years, studies have reported the role of HSF1 in the regulation of glycemia[14,37]. In our present study, we observed that overexpression of HSF1 decreased the apoptosis of islet β-cells, the death of which is one of the most important mechanisms of T1DM. We also showed that HSF1 decreased the apoptosis of islet β-cells via transcriptionally upregulating SIRPα expression. It has been reported that HSPs are associated with glycemia regulation. However, whether HSPs were involved in the regulation of islet β-cell death by HSF1 need further study.

Conclusions

Collectively, our study reveals that HSF1 expression was lower in islets from T1DM compared to normal mice. We found that overexpression of HSF1 decreased the apoptosis of islet β-cell lines. Moreover, we found that HSF1 decreased the apoptosis of islet β-cells via increasing the expression of SIRPα. In terms of mechanism, HSF1 upregulated SIRPα expression by activating its gene promoter region. The binding site (-1809 to -1795) was required for HSF1-induced increase of SIRPα gene promoter activity. These results indicate that the low expression of HSF1/SIRPα may be one of the mechanisms of islet β-cell death, and the HSF1/SIRPα pathway may be a novel target for the treatment of T1DM.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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