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Original Article

MiR-144 affects proliferation and apoptosis of high glucose-induced AC16 cardiomyocytes by regulating CTRP3/JNK signaling

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Abstract: Background: Diabetic cardiomyopathy (DCM) is a common complication of diabetes and can lead to heart failure, arrhythmia, and sudden death. microRNAs (miRNAs) are reportedly involved in many human disease, including DCM. However, little is known about the biologic functions of miR-144 in DCM progression. Methods: The expression levels of miR-144 and C1q/TNF-related protein-3 (CTRP3) were measured by quantitative real-time polymerase chain reaction (qRT-PCR). Western blot was used to determine the protein levels of CTRP3, phosphorylated c-Jun amino-terminal kinase (p-JNK), JNK, Bax, Bcl-2, and cleaved-caspase-3. Cell proliferation and apoptosis were detected by Cell Counting Kit-8 (CCK-8) assay and flow cytometry, respectively. The potential binding sites between miR-144 and CTRP3 were predicted by microRNA.org databases and further determined using a dual-luciferase assay. AC16 cardiomyocytes were cultured in high glucose (HG, 30 mmol/L) to mimic hyperglycemia. Results: MiR-144 expression level was enhanced, while CTRP3 expression was reduced in HG-induced AC16 cardiomyocytes. Knockdown of miR-144 or overexpression of CTRP3 dramatically promoted cell proliferation and reduced apoptosis of AC16 cardiomyocytes treated with HG. Inhibition of miR-144 evidently decreased the protein levels of Bax and p-JNK, but elevated Bcl-2 expression in HG-induced AC16 cardiomyocytes. Moreover, CTRP3 was a direct target of miR-144, and its abrogation reversed the effects of miR-144 knockdown on proliferation and apoptosis in HG-induced AC16 cardiomyocytes. SP600125 (a JNK inhibitor, 10 μmol/L) attenuated the si-CTRP3-mediated inhibition of proliferation and promotion of apoptosis in AC16 cardiomyocytes transfected with anti-miR-144 and stimulated with HG. Conclusion: MiR-144 regulates proliferation and apoptosis of HG-induced AC16 cardiomyocytes through targeting the CTRP3/JNK signaling pathway, providing a novel avenue for treatment of DCM.

Keywords: Diabetic cardiomyopathy, miR-144, CTRP3, JNK signaling pathway

Introduction

Diabetic cardiomyopathy (DCM) is defined as myocardial dysfunction that occurs in patients with diabetes, without coronary artery disease and hypertension [1], It is a major diabetic complication and a common cause of sudden cardiac death and congestive heart failure. Increasing evidence has demonstrated that hyperglycemia as an independent risk factor, directly causes cardiac damage, resulting in DCM [2-5]. DCM is characterized by early impaired diastolic function, accompanied by development of cardiac hypertrophy, cardiac fibrosis, and cardiomyocyte apoptosis [5]. Cardiomyocyte apoptosis was thought to be an important consequence of inflammatory response and oxidative stress, which could be attributed to hyperglycemia in cardiac tissue. This may be a vital pathologic change in DCM [6]. Although various morphologic characteristics associated with DCM have been identified, the underlying molecular mechanisms of DCM remain largely unknown.

MicroRNAs (miRNAs) are small (19-22nt), single-stranded, noncoding RNAs, which can regulate gene expression through complementary binding to the 3'-untranslated region (3'-UTR) of messenger RNAs (mRNAs), leading to mRNA degradation or inhibition of mRNA translation. In recent years, it has been reported that the majority of miRNAs play critical roles in many biologic processes, including cell proliferation, apoptosis, and autophagy, glucose and lipid metabolism, and signal transduction [7]. Evid-

ence showed that dysregulation of certain miRNAs may contribute to human diseases, including DCM [8, 9]. For example, miR-30c level was observed to be downregulated in DCM rats and patients, and in high glucose (HG)-induced cardiomyocytes [10]. Upregulation of miR-200b protected diabetes-induced cardiac functional and structural changes [11]. MiR-144 has been reported to be increased in human type 2 diabetes blood samples [12]. It also was found that inhibition of miR-144 level abolished oxidative stress and decreased apoptosis in the hearts of streptozotocin-treated diabetic mice [13]. However, the biologic functions of miR-144 in DCM progression still need further elaboration.

miRNAs participate in several crucial biologic processes through direct interaction with their target mRNA. C1q/TNF-related protein-3 (CTRP3), a novel adipokine, is a member of the CTRP superfamily with roles in multiple cellular processes. Recently, it has been reported that CTRP3 has various effects, such as lowering glucose levels, suppressing gluconeogenesis in the liver, promoting angiogenesis, and anti-inflammation [14, 15]. However, little is known about the interaction between CTRP3 and miR-144, and the exact roles of CTRP3 in development of DCM.

The present study was designed to explore the expression levels of CTRP3 and miR-144 in HG-induced AC16 cardiomyocyte. The JNK signaling pathway and the interaction between CTRP3 and miR-144 were also investigated in this study. Study findings provide a potential therapeutic strategy against DCM.

Materials and methods

Cardiomyocyte culture

Human cardiomyocyte cell line AC16 was purchased from EMD Millipore (Billerica, MA, USA) and maintained in Dulbecco's Modified Eagles Medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen) in a humidified incubator at 37°C with 5% CO_2.

Cell transfection

GenePharma (Jiangsu, China) provided the miR-144 inhibitor (anti-miR-144), miR-144 mimics, pcDNA3.1-CTRP3, small interfering RNA (siRNA) against CTRP3 (si-CTRP3), and their matched controls. Cell transfection was performed using Lipofectamine 2000 reagent (Invitrogen) following the manufacturer's requirements. Following transfection, cells were stimulated with 30 mmol/L HG for 24 h, and 5.5 mmol/L normal glucose as control.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

For detection of miR-144 expression, total RNA was extracted from AC16 cardiomyocytes using a mirVana kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The miRNA was converted to complementary DNA (cDNA) using the TaqMan microRNA reverse transcription kit (Applied Biosystems, Foster City, CA). The qRT-PCR was performed with the mirVanaTM miRNA detection kit (Ambion) with miR-144 specific primer (Ambion) using ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The expression level of miR-144 was normalized to U6 small nuclear RNA (U6-snRNA).

For CTRP3 expression detection, total RNA was extracted from AC16 cardiomyocytes using TRIzol reagent (Invitrogen) following the manufacturer's instructions, and was reverse-transcribed into cDNA using TaqMan Reverse Transcription Kit (Applied Biosystems), The qRT-PCR was conducted using SYBR Premix Ex Taq (TaKaRa, Dalian, China) on ABI PRISM 7000 Sequence Detection System. The primers sequences of CTRP3 and GAPDH were as follows: CTRP3, forward 5'-GAGTCTCCACAAACCGGAGG-3' and reverse 5'-TCACCTTTGTCGCCCTTCTC-3'; GAPDH, forward 5'-GACTCCACTCACGGCAAATTCA-3', reverse 5'-TCGCTCCTGGAAGATGGTGAT-3'. The level of CTRP3 was evaluated by the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH (endogenous control).

Western blot assay

Total protein was extracted by lysing cells in radio-immunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, Wilmington, DE, USA) containing the protease inhibitors (Roche, Basel, Switzerland). Lysates were incubated on ice for 20 min and centrifuged at 12,000 × g for 10 min to remove cellular debris. Total protein concentrations were determined

by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Appleton, WI, USA). Then, equal amounts of protein were separated by 10-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat milk in Tris-buffer saline containing 0.1% Tween 20 (TBST) for 2 h at room temperature, and then incubated overnight at 4°C with the primary antibodies against CTRP3 (1:500, ab36870, Abcam, Cambridge, UK), phospho-JNK (1:500, sc-293136, Thr 183/Tyr 185, SantaCruz Biotechnology, Santa Cruz, CA, USA), JNK (1:500, sc-7345, Santa Cruz Biotechnology) Bax (1:500, ab53154, Abcam), Bcl-2 (1:1000, ab196495, Abcam), cleaved-caspase-3 (1:1000, ab2302, Abcam), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:2500, ab9485, Abcam). Next, the membranes were washed with TBST and incubated with horseradish peroxidase (HRP)-linked secondary antibody (Abcam). Finally, protein bands were visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific). The protein levels were normalized to GAPDH and quantitated using ImageJ software.

Cell proliferation assay

Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) was utilized to detect cell proliferation. Briefly, AC16 cardiomyocytes (2×10^4 cells/mL) were seeded in 96-well plates (100 μL/well) and transfected/treated with anti-miR-144, pcDNA3.1-CTRP3, anti-miR-144+si-CTRP3, SP600125, anti-miR-144+si-CTRP3+SP600125 or their matched controls, and then exposed to HG. After transfection/treatment for 24 h, 48 h and 72 h, respectively, CCK-8 solution (10 μL) was added to each well. Then, the plates were incubated for another 2 h at 37°C. The absorbance of each well was detected at 450 nm using a microplate reader (Bio-Teck, Winooski, VT, USA).

Cell apoptosis assay

Cultured AC16 cardiomyocytes were collected and washed with ice cold phosphate-buffered saline (PBS). Subsequently, AC16 cardiomyocytes were resuspended in binding buffer and stained with Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) (Sangon Biotech, Shanghai, China) according to the protocols of manufacturer. The apoptotic rate analyzed by using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Dual-luciferase reporter assay

Wild type (WT) or mutant (MUT) 3'-UTR of CTRP3 were cloned into the pGL3-Basic vector (Promega, Madison, WI, USA) respectively, and then constructed pGL3/CTRP3-WT and pGL3/CTRP3-MUT recombinant vector. AC16 cardiomyocytes cultured in 24-well plates were co-transfected with 100 nmol/L miR-144 mimics or miR-NC and pGL3/CTRP3-WT or pGL3/CTRP3-MUT using Lipofectamine 2000 reagent (Invitrogen). AC16 cardiomyocytes were harvested and lysed after 48-h transfection, using Renilla fluorescence as internal reference, dual-luciferase reporter assay was performed by dual luciferase reporter assay system (Promega) according to the manufacturer's protocols.

Statistical analysis

All data are presented as mean ± standard deviation (SD) from at least three independent experiments. The differences between two groups were assessed by Student's *t*-test. All statistical analyses were performed using GraphPad Prism software version 5.0 (GraphPad Software, San Diego California, USA). $P<0.05$ was considered significant.

Results

The expression of miR-144 was upregulated and CTRP3 expression was downregulated in HG-induced AC16 cardiomyocytes

To examine the potential roles of miR-144 and CTRP3 in DCM, we determined the expression levels of miR-144 and CTRP3 by qRT-PCR and western blot in AC16 cardiomyocytes treated with HG (30 mmol/L), respectively. Results demonstrated that the level of miR-144 was evidently increased in HG-induced AC16 cardiomyocytes (**Figure 1A**). However, the mRNA and protein level of CTRP3 was apparently decreased in HG-induced AC16 cardiomyocytes (**Figure 1B** and **1C**). JNK signaling pathway was involved in the regulation of cell proliferation, differentiation and apoptosis. To investigate whether HG could activate JNK signaling path-

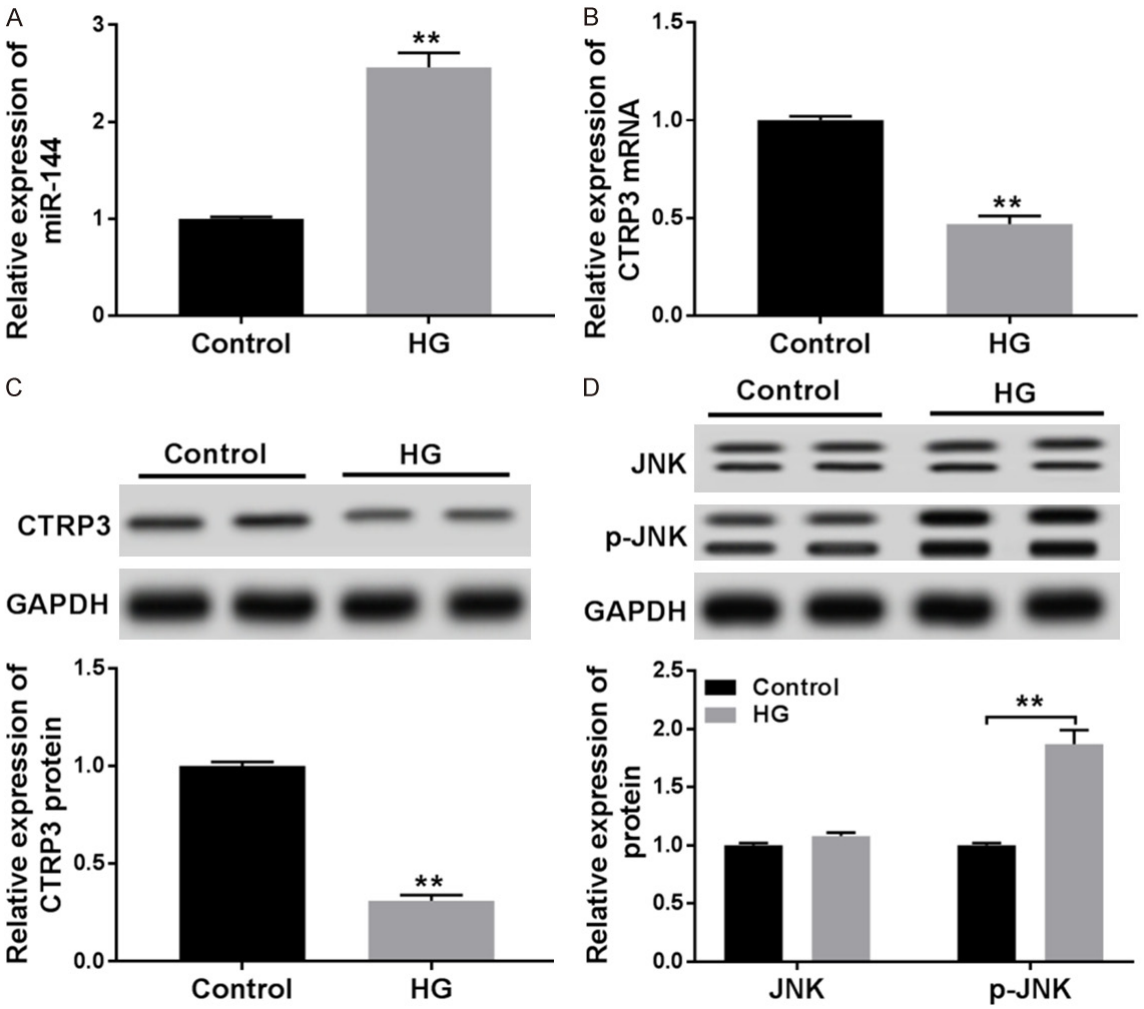

Figure 1. Expression levels of miR-144, CTRP3, JNK and p-JNK in HG-induced AC16 cardiomyocytes. AC16 cardiomyocytes were treated with high glucose (HG, 30 mmol/L) or normal glucose (control, 5.5 mmol/L) for 24 h. A and B. The expressions levels of miR-144 and CTRP3 were determined by qRT-PCR. C and D. The protein levels of CTRP3, JNK and p-JNK were measured by western blot. ***P*<0.01.

way in AC16 cardiomyocytes, western blot was used to detect the phosphorylation level of JNK. As displayed in **Figure 1D**, treatment of HG prominently elevated the level of p-JNK in AC16 cardiomyocytes, but there was no change in JNK protein. From the above, we conclude that miR-144 and CTRP3 might play vital roles in the development of DCM, and HG can activate JNK signaling pathway.

Knockdown of miR-144 attenuates the HG-mediated progression of DCM

To analyze the effects of miR-144 on HG-induced AC16 cardiomyocyte proliferation, apoptosis, and the JNK signaling pathway, anti-miR-144 or anti-NC was transfected into AC16 cardiomyocytes and then treated them with HG. Results showed that inhibition of miR-144 reversed the HG-induced promotion of miR-144 expression in AC16 cardiomyocytes (**Figure 2A**). In addition, cell proliferation was drastically inhibited in HG-induced AC16 cardiomyocytes compared with the control group, which was abated by inhibition of miR-144 (**Figure 2B**). Moreover, exposure to HG conspicuously induced cell apoptosis, which was abolished by knockdown of miR-144 (**Figure 2C** and **2D**). The protein expression of Bax (pro-apoptotic), cleaved-caspase-3 (a key executor in the apoptotic process), and p-JNK were prominently upregulated, while Bcl-2 (anti-apoptotic) expression was downregulated in HG-induced AC16 cardiomyocytes, and a deficiency of miR-144 attenuated these effects (**Figure 2E**). Taken together, these results indicate that inhibition of miR-

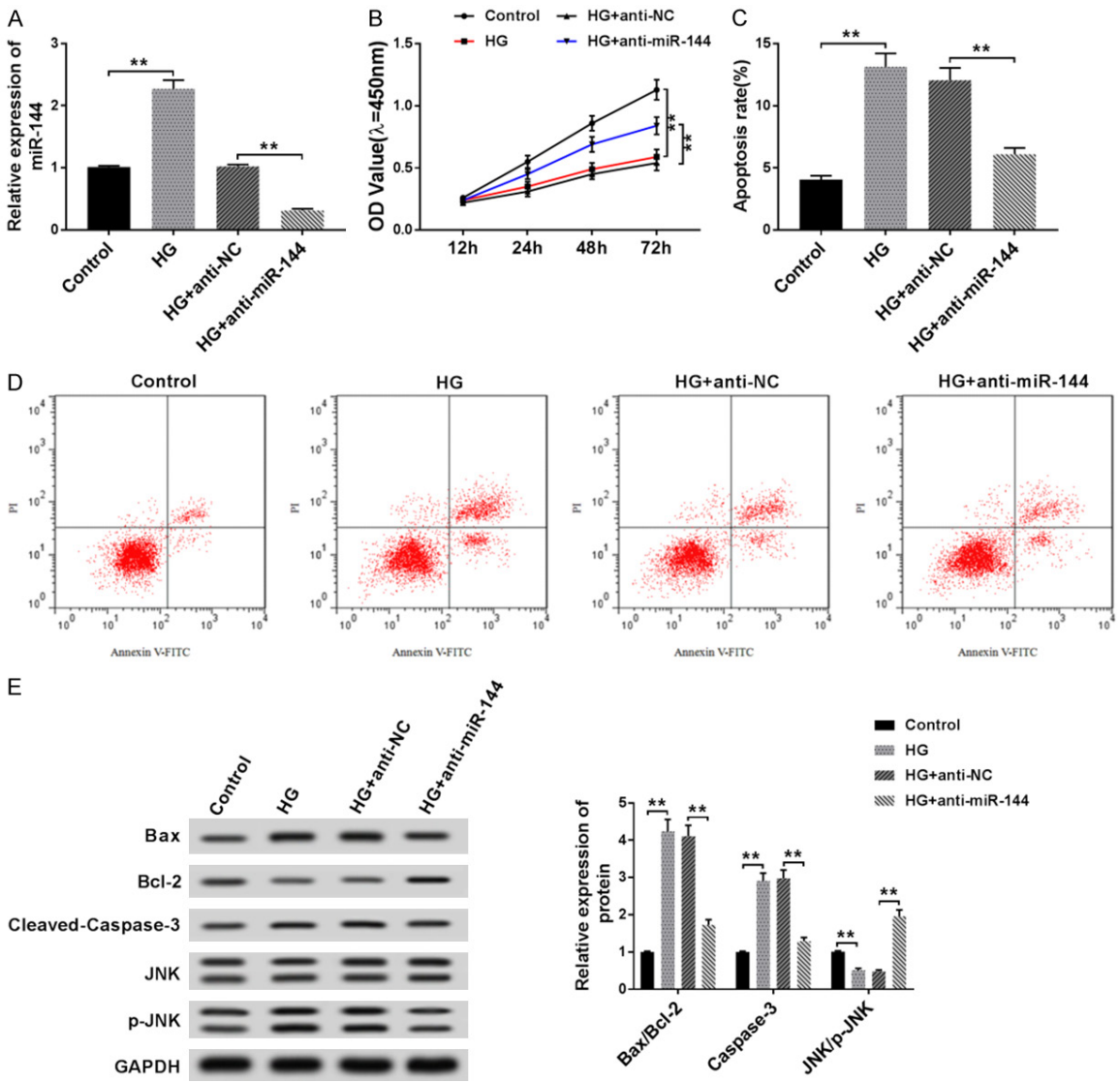

Figure 2. Effects of miR-144 on proliferation and apoptosis in HG-induced AC16 cardiomyocytes. AC16 cardiomyocytes were transfected with anti-miR-144 or anti-NC and then exposed to HG. A. The abundance of miR-144 was analyzed by qRT-PCR. B. CCK-8 assay was utilized to evaluate cell proliferative capacity. C and D. Cell apoptosis was measured by flow cytometry. E. The protein levels of CTRP3, Bax, Bcl-2, cleaved-caspase-3, p-JNK, and JNK were detected by western blot. $^{**}P<0.01$.

144 can reverse the HG-mediated proliferation, apoptosis, and JNK signaling pathway in AC16 cardiomyocytes.

CTRP3 is a direct target of miR-144

To explore the interaction between miR-144 and CTRP3 in AC16 cardiomyocytes, the potential binding sites of miR-144 and CTRP3 were predicted by the microRNA.org resource. As presented in **Figure 3A**, CTRP3 contained binding sites for miR-144. To validate the relationship between miR-144 and CTRP3, the CTRP3 containing wild-type or mutant binding sites of miR-144 (CTRP3-WT or CTRP3-MUT) was cloned and inserted into a luciferase reporter vector. Results showed that addition of miR-144 dramatically suppressed luciferase activity of CTRP3-WT, but no effect was observed in CTRP3-MUT (**Figure 3B**). In addition, enforced expression of miR-144 apparently downregulated the protein level of CTRP3, while its knockdown showed an opposite effect in AC16 cardiomyocytes (**Figure 3C**). CCK-8 analysis indicated that overexpression of CTRP3 apparently inhibited cell proliferation in HG-induced

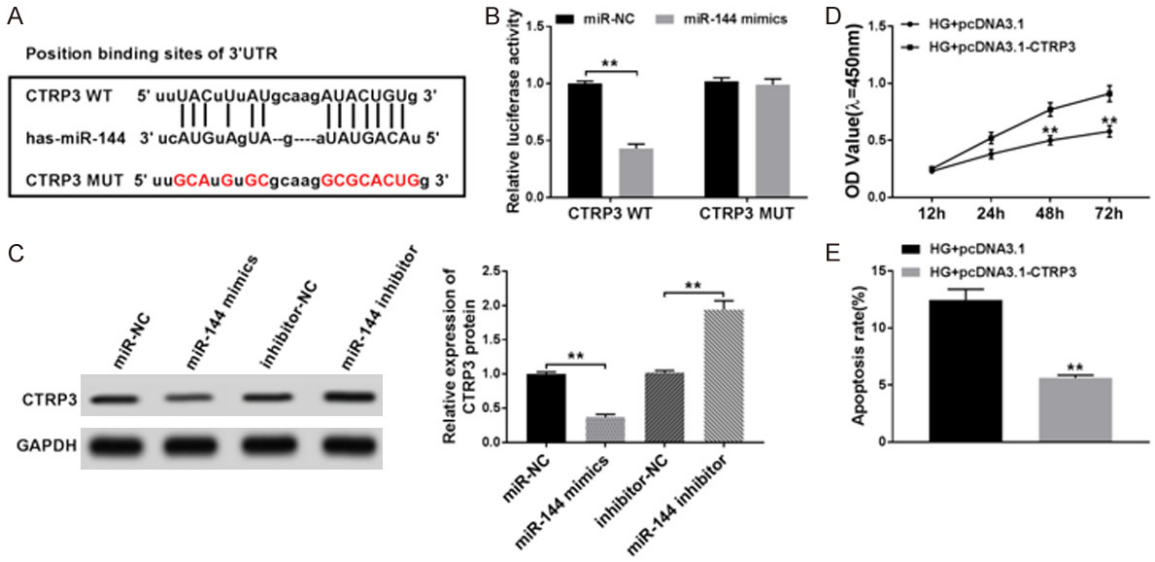

Figure 3. MiR-144 directly targets CTRP3. A. The predicted binding sites for miR-144 in the 3'UTR of CTRP3 and the mutations in the binding sites are shown. B. The luciferase activity of the CTRP3-WT and CTRP3-MUT co-transfected with miR-144 mimics or miR-NC was determined. C. The protein level of CTRP3 in AC16 cardiomyocytes transfected with miR-144 mimics, miR-NC, miR-144 inhibitor, or inhibitor-NC was determined by western blot. D. CCK-8 assay was utilized to evaluate cell proliferation in AC16 cardiomyocytes transfected with pcDNA3.1-CTRP3 or pcDNA3.1 and stimulated with HG. E. Apoptosis was measured by flow cytometry. $^{**}P<0.01$.

AC16 cardiomyocytes (**Figure 3D**). Besides, transfection of pcDNA3.1-CTRP3 markedly decreased cell apoptosis in HG-treated AC16 cardiomyocytes (**Figure 3E**). These findings suggest that CTRP3 is a direct target of miR-144 and its overexpression limits cell proliferation but induces cell apoptosis in HG-treated AC16 cardiomyocytes.

Knockdown of CTRP3 attenuates effects of miR-144 inhibition on cell proliferation and apoptosis in HG-induced AC16 cardiomyocytes

To further investigate the relationship between miR-144 and CTRP3 in HG-treated cardiomyocytes, anti-miR-144, anti-NC, anti-miR-144+si-NC, or anti-miR-638+si-CTRP3 was transfected into AC16 cardiomyocytes and then stimulated with HG. We found that the protein level of CTRP3 in HG-induced AC16 cardiomyocytes transfected with miR-144 inhibitor was much higher than in HG-induced AC16 cardiomyocytes transfected with NC inhibitor, whereas it was abated by inhibition of CTRP3 (**Figure 4A**). Moreover, silencing of CTRP3 could reverse the proliferative effect of miR-144 inhibition on HG-induced AC16 cardiomyocytes (**Figure 4B**). Next, our results showed that abrogation of CTRP3 abrogated the inhibitory effect of miR-144 knockdown on apoptosis in AC16 cardiomyocytes stimulated with HG (**Figure 4C**). Therefore, our data clearly confirm that deficiency of CTRP3 attenuated miR-144 inhibition-mediated promotion of cell proliferation and inhibition of apoptosis in HG-induced AC16 cardiomyocytes.

MiR-144 regulates cell proliferation and apoptosis by affecting CTRP3 expression and the JNK signaling pathway in HG-induced AC16 cardiomyocytes

Finally, we detected the effects of miR-144 and CTRP3 on the JNK signaling pathway in HG-treated AC16 cardiomyocytes. Results in **Figure 5A** proved that the protein level of p-JNK was remarkably downregulated in HG+anti-miR-144 group compared to HG+anti-NC group, while inhibition of CTRP3 showed an opposite effect. SP600125 is a synthetic polyaromatic chemical that suppresses JNK signaling though interfering with phosphorylation of c-Jun. As shown in **Figure 5B**, the growth rate was remarkably elevated in HG-stimulated AC16 cardiomyocytes treated with SP600125 (Calbiochem, La Jolla, CA, USA) compared with the cells treated with 0.1% dimethylsulphoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA). Moreover, the rate

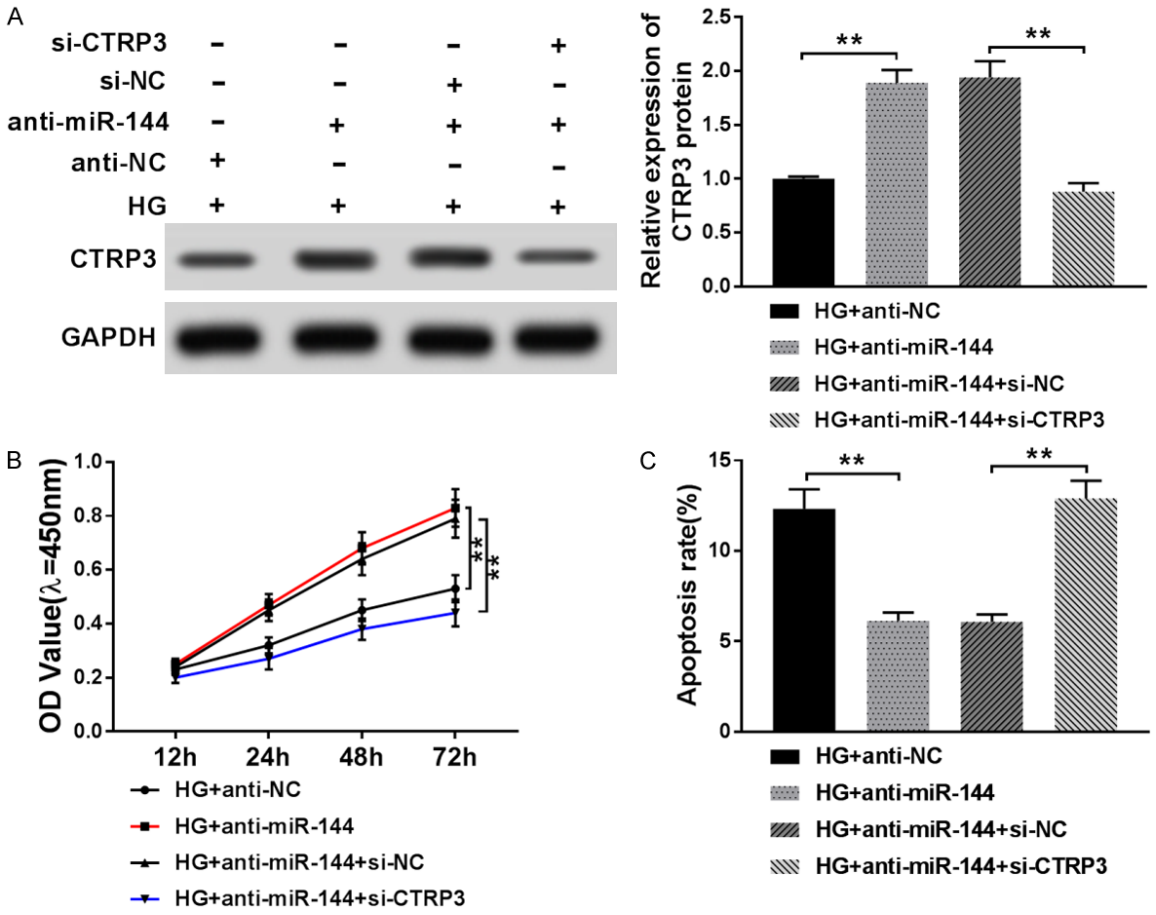

Figure 4. CTRP3 knockdown attenuated the effects of miR-144 inhibition on proliferation and apoptosis in HG-induced AC16 cardiomyocytes. AC16 cardiomyocytes were transfected with anti-NC, anti-miR-144, anti-miR-144+si-CTRP3, or anti-miR-144+si-NC and then treated with HG. A. Western blot was used to detect the protein level of CTRP3. B. Cell proliferation was measured by CCK-8 assay. C. Apoptosis was measured by flow cytometer. $^{**}P<0.01$.

of apoptosis strikingly decreased in HG-induced AC16 cardiomyocytes treated with SP600125 (**Figure 5C**). **Figure 5D** and **5E** illustrate that si-CTRP3 transfection notably reduced cell proliferation and promoted apoptosis in AC16 cardiomyocytes transfected with miR-144 inhibitor and stimulated with HG, whereas treatment ofSP600125, abolished these effects. Thus, these findings demonstrate that knockdown of miR-144 might inhibit progression of DCM by affecting the JNK signaling pathway and CTRP3 expression.

Discussion

DCM is a leading cause of morbidity and mortality for diabetics [16, 17]. Recent studies showed that miRNAs extensively participate in the development of various heart diseases, including DCM [18, 19]. Although the therapeutic regulation of miRNAs has caused widespread interest in diabetic heart disease research, there are still few reports on the underlying mechanism of miRNAs in DCM development. Hyperglycemia was considered to be a central trigger in the pathophysiology of DCM. In this study, we established an *in vitro* DCM model of AC16 cardiomyocytes induced by HG to explore the biologic functions of miR-144 in DCM progression.

MiR-144 has been found to be dysregulated in many human diseases and to participate in multiple cell behaviors, such as proliferation, apoptosis, migration, epithelial mesenchymal transition (EMT), and autophagy [20, 21]. Accumulating evidence suggests that miR-144 acts as a tumor suppressor in some malignancies, including lung cancer [21], hepatocellular carcinoma [22], thyroid cancer [23], bladder

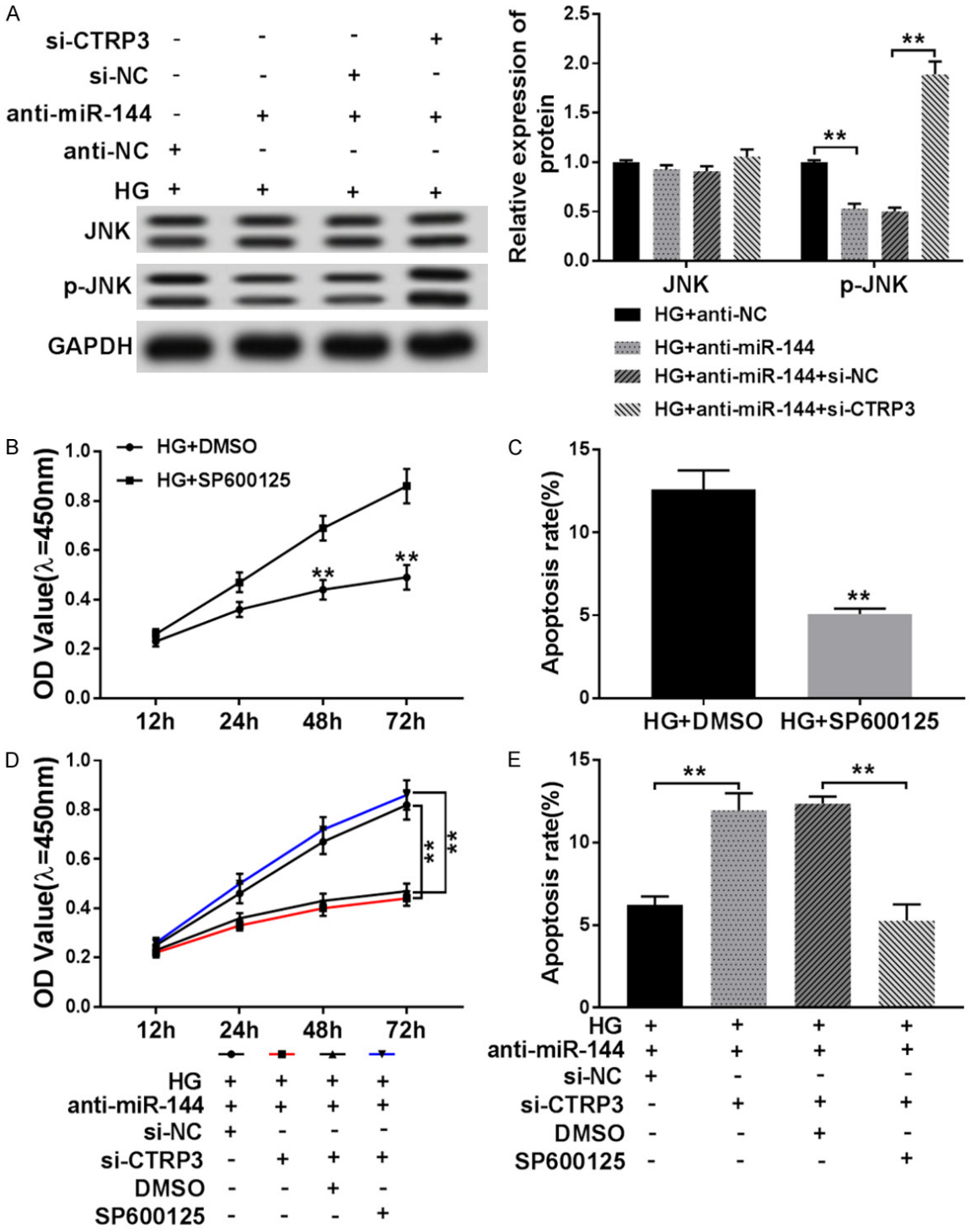

Figure 5. MiR-144 regulates cell proliferation and apoptosis through CTRP3/JNK signaling pathway in HG-treated AC16 cardiomyocytes. A. Western blot was used to detect the protein levels of p-JNK and JNK in AC16 cardiomyocytes transfected with anti-NC, anti-miR-144, si-CTRP3, or si-NC and stimulated with HG. B. CCK-8 assay was performed to evaluate cell proliferation in HG-induced AC16 cardiomyocytes treated with SP60025. C. Apoptosis was evaluated in HG-induced AC16 cardiomyocytes treated with SP60025 by flow cytometer. D. CCK-8 assay was used to evaluate cell proliferation in AC16 cardiomyocytes transfected with anti-miR-144+si-NC, anti-miR-144+si-CTRP3, anti-miR-144+si-CTRP3+DMSO, or anti-miR-144+si-CTRP3+SP60025 and exposed to HG. E. Apoptosis was evaluated by flow cytometer. $^{**}P<0.01$.

cancer [24]. Moreover, miR-144-3p has been suggested to be upregulated in patients with Type 2 diabetes mellitus (T2D) compared with those of healthy subjects [25]. Besides, miR-144 was found to be increased the tissues from diabetic rats and T2D patients, and it impaired insulin signaling by suppressing the level of insulin receptor substrate 1 in TD2 [26]. However, the exact role of miR-144 in DCM and the molecular mechanism by which miR-144 exerts its function remain poorly understood. Here, we also found that miR-144 was upregulated in HG-induced AC16 cardiomyocytes. A growing body of evidence showed that a critical consequence of hyperglycemia is cardiomyocyte apoptosis, which leads to loss of contractile tissue and causes cardiac remodeling [27-29]. The Bcl-2 family consists of pro-apoptotic (e.g., Bax) and anti-apoptotic (e.g., Bcl-2) family members that regulate apoptosis [30]. In our study, we proved that cell proliferation was drastically inhibited in HG-treated AC16 cardiomyocytes compared with the control group, which was abolished by inhibition of miR-144. Additionally, knockdown of miR-144 weakened the HG-mediated promotion of apoptosis of AC16 cardiomyocytes, and miR-144 inhibition attenuated the promotion of Bax and cleaved-caspase-3 protein levels but reduction of Bcl-2 protein expression in AC16 cardiomyocytes treated with HG. These results show that inhibition of miR-144 promoted proliferation and inhibited apoptosis in HG-induced AC16 cardiomyocytes.

Most miRNAs mainly perform their biologic functions through regulation of target mRNA translation [31]. Bioinformatic analysis was performed to investigate whether CTRP3 was a target of miR-144. The microRNA.org databases identified that CTRP3 mRNA contained potential binding sites ofmiR-144. In this study, we first showed that miR-144 directly targeted CTRP3. Previous studies demonstrated that CTRP3 could regulate hepatic glucose output, attenuate hepatic steatosis and inhibit inflammatory responses [32-34]. CTRP3 has been demonstrated to be decreased in diabetic rat hearts and to protect against HG-induced oxidative stress, inflammation, and apoptosis *in vitro* (14). In line with previous findings, we revealed that the mRNA and protein levels of CTRP3 were apparently downregulated in HG-induced AC16 cardiomyocytes. The study also showed that overexpression of CTRP3 markedly enhanced proliferation and reduced apoptosis of AC16 cardiomyocytes treated with HG. Downregulation of CTRP3 reversed the effects of miR-144 knockdown on proliferation and apoptosis. Therefore, these findings suggested that miR-144 exerted its biologic functions through regulation of CTRP3 expression.

The JNKs are members of the mitogen-activated protein kinase (MAPK) family [35]. A great deal of evidence has demonstrated that JNK signaling pathway is involved in regulation of multiple cellular events, such as growth control, transformation, and programmed cell death (apoptosis) [36]. The former finding has revealed that the HG was shown to activate the JNK signaling pathway in AC16 cardiomyocytes, and the JNK pathway was found to participate in the regulation of cardiac apoptosis induced by HG in AC16 cardiomyocytes [37]. Consistent with previous studies, we found that HG could activate the JNK signaling pathway. Moreover, abrogation of miR-144 blocked the JNK signaling pathway, which was overturned by inhibition of CTRP3. Furthermore, treatment of SP600125 attenuated the si-CTRP3-mediated inhibition of proliferation and promotion of apoptosis in AC16 cardiomyocytes transfected with anti-miR-144 and stimulated with HG, suggesting that miR-144 regulates proliferation and apoptosis of AC16 cardiomyocytes treated with HG by affecting the CTRP3/JNK signaling pathway.

Conclusion

The expression level of miR-144 was upregulated and CTRP3 expression was downregulated in HG-induced AC16 cardiomyocytes. Knockdown of miR-144 weakened the HG-mediated reduction of proliferation, promotion of apoptosis, and activation of the JNK signaling pathway in AC16 cardiomyocytes, which was reversed by inhibition of CTRP3. Moreover, we first identified that miR-144 directly targeted CTRP3 in AC16 cardiomyocytes. Overexpression of CTRP3 promoted proliferation and suppressed apoptosis in HG-stimulated AC16 cardiomyocytes. Besides, exposure of SP600125 attenuated the si-CTRP3-mediated inhibition of proliferation and promotion of apoptosis in AC16 cardiomyocytes transfected with anti-miR-144 and stimulated with HG. Collectively, miR-144 regulated proliferation and apoptosis of AC16 cardiomyocytes treated with HG by regulating

the CTRP3/JNK signaling pathway. Understanding the mechanism underlying miR-144 and CTRP3 involvement in DCM progression would enable us to use them in the development of new strategies for DCM treatment.

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Disclosure of conflict of interest

None.

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