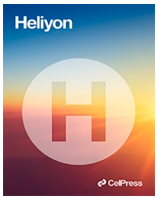
Heliyon 10 (2024) e24604

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

LncRNA HCG18 affects diabetic cardiomyopathy and its association with miR-9-5p/IGF2R axis

Yuhui Luo [a,1], Yi Jiang [b,1], Tingting Zhong [a], Zhenggong Li [a], Jia He [c], Xiaoli Li [a], Kun Cui [a,*]

[a] Department of Cardiology, Chongqing General Hospital, University of Chinese Academy of Sciences, Chongqing, 40013, China
[b] Department of Geriatrics, Chongqing Emergency Medical Center, Central Hospital of Chongqing University, Chongqing, 40013, China
[c] Department of Echocardiogram, Chongqing General Hospital, University of Chinese Academy of Sciences, Chongqing, 40013, China

ARTICLE INFO

Keywords:
Diabetic cardiomyopathy
IGF2R
HCG18
miR-9-5p

ABSTRACT

This paper aimed to investigate the role of lncRNA HCG18 (HCG18) in the progression of diabetic cardiomyopathy (DCM) and potential mechanisms. Streptozocin (STZ) was used to induce DCM model in rats, which was confirmed by blood glucose concentration, body weight, and HE staining. Myocardial apoptosis was detected by TUNEL. H9c2 cardiomyocytes were used to construct cell models of DCM through treatment of high glucose. The results showed that HCG18 was overexpressed in STZ induced DCM rat model and high glucose induced H9c2 cardiomyocytes. Si-HCG18 significantly increased cell viability, reduced cell apoptosis, attenuated activities of myocardial enzymes and enhanced activities of antioxidant enzymes in STZ induced DM model and high glucose induced H9c2 cardiomyocytes, while the results of upregulation of HCG18, in high glucose induced H9c2 cardiomyocytes, were opposite with that of si-HCG18. MiR-9-5p was a target of HCG18, and which was down-regulated in cardiomyocytes of DCM. The overexpression of miR-9-5p could neutralize the high glucose induced cardiomyocyte injury, and the silence of miR-9-5p could reverse the effect of si-HCG18 on high glucose induced cardiomyocytes. MiR-9-5p could directly target to IGF2R, and IGF2R was overexpressed in cardiomyocytes of DCM. Up-regulation of IGF2R can reverse the protective effect of si-HCG18 on cardiomyocytes. Taken together, HCG18 is significantly increased in cardiomyocytes of DCM. Down-regulation of HCG18 can improve cardiomyocyte injury through miR-9-5p/IGF2R axis in DCM.

1. Introduction

Diabetic cardiomyopathy (DCM) refers to the specific myocardial lesions in diabetes mellitus (DM), which belongs to the organic lesions of microvessels [1]. DCM could cause metabolic disorders, microvascular lesions, extensive myocardial necrosis, arrhythmia, and heart failure [2,3]. The pathogenesis of DCM is very complex and has not been fully elucidated. Studies have shown that hyperglycemia leads to insulin resistance, increase of oxidative stress, mitochondrial dysfunction and increase of cardiomyocyte

* Corresponding author. Department of Cardiology, Chongqing General Hospital, University of Chinese Academy of Sciences, No. 312, 1st Zhongshan Road, Yuzhong District, Chongqing, 40013, China.
E-mail address: KunCuiL@tom.com (K. Cui).
[1] They make the same contribution.

https://doi.org/10.1016/j.heliyon.2024.e24604
Received 7 April 2023; Received in revised form 9 January 2024; Accepted 10 January 2024
Available online 26 January 2024

apoptosis in DCM [1,4]. Therefore, it is of great significance to explore molecular mechanisms underlying DCM for its diagnosis and treatment.

LncRNA can change gene expression by complementary pairing with DNA or RNA bases, covering splicing points or covering miRNA binding sites and promoters. In addition, some studies have shown that lncRNA may act as a "sponge" of endogenous miRNAs, and which regulate effect of miRNA on mRNA [5]. More and more studies have shown that lncRNA participated in regulating the development of DCM [6]. Li et al. found that overexpression of H19 could significantly increase the levels of glutathione peroxidase (GSH PX) and superoxide dismutase (SOD), while significantly decrease the MDA level, myocardial apoptosis index, and expression of mitochondrial apoptosis related proteins [7]. In the DCM model of AC16 cells, the cell viability was decreased, the apoptosis rate was increased, MEG3 level was up-regulated, and miR-145 level was down-regulated. In addition, MEG3 could inhibit the expression of miR-145 act as a ceRNA, thus attenuate the inhibition of PDCD4 expression modulated by miR-145. Knockdown of MEG3 could protect human cardiomyocytes from apoptosis induced by high glucose via regulating miR-145/PDCD4 axis [8]. Human leukocyte antigen complex 18 (HCG18) is a lncRNA, which is located on chromosome 6p22 1 of the 2430-bp [9]. Abnormal expression of HCG18 causes a variety of human diseases, including cancer, vascular disease and diabetes mellitus [10–12]. The lncRNA HCG18 facilitated the progression of diabetic peripheral neuropathy by promoting M1 macrophage polarization through regulation of the miR-146a/TRAF6 axis [12]. It had been demonstrated that HCG18 promoted development of bladder cancer through combining with microRNA-34c-5p [13]. HCG18 was closely related to lumbar disc degeneration [14]. However, the role of HCG18 in DCM was yet unclear.

In this study, we assumed that silencing of HCG18 could attenuate injury of H9c2 cardiomyocytes to improve progression of DCM. Through target gene prediction, it was found that HCG18 could target miR-9-5p, and IGF2R was a target of miR-9-5p. We extensively investigated the expression of HCG18 in matched DCM tissues and cardiomyocytes induced by high glucose, explored its biological activities, and potential molecular mechanisms. Our research aims to offer novel insights into the role of HCG18 and reveals its potential as a prognostic indicator and therapeutic target for DCM.

2. Material and methods

2.1. Animals

All animal experimental operations were performed on the basis of the requirements of the Guide for the Care and Use of Laboratory Animals of Chongqing General Hospital, and approved by the Animal Ethics Committee of Chongqing General Hospital SYXK (YU) 2018-0004. 24 healthy SD rats (8 weeks old) were obtained from Shanghai Lab. Animal Research Center. They were fed with 12 h light/12 h dark cycle, conventional temperature and humidity for 1 week. The rats were randomly divided into four group (Control, DM, DM + sh-NC, and DM + sh-HCG18), 6 rats per group. Rats from DM, DM + sh-NC, and DM + sh-HCG18 groups were intraperitoneal injected STZ solution (600 mg/kg) twice a week to constructed type 2 diabetic mellitus rats. Rats from control group were injected equivalent amount of physiological saline. One week later, blood samples were collected from tail vein, and blood glucose was measured with a glucometer (Accu-Chek, Roche Diagnostics). If the blood glucose was more than 16.7 mmol/L, and the urine volume and drinking water volume were significantly increased, it was considered that the type 2 diabetic mellitus rats were successfully constructed. According to previous studies, diabetic rats showed onset of cardiac dysfunction after 8 weeks of STZ injection [15,16]. After 8 weeks of STZ injection, type 2 diabetic mellitus rats from DM + sh-NC group and DM + sh-HCG18 group were respectively transfected with sh-NC and sh-HCG18, which was obtained from GenePharma (GenePharma, China); 50 μL sh-HCG18 or sh-NC was injected into the tail vein of type 2 diabetic mellitus rats. After 8 weeks, the rats were weighed, and then all rats were euthanized by pentobarbital sodium (120 mg/kg, intraperitoneal injection), and the death of rats was determined by observing the cardiac arrest and pupil dilation. The heart was removed and the right ventricle was separated. Some tissues were used to isolated RNAs and proteins through Trizol Reagent (Invitrogen) and RIPA lysate (Beyotime) respectively. Some tissues were fixed with 4 % paraformaldehyde for the pathological examination.

2.2. HE staining

4 μm paraffin sections were prepared after 4 % paraformaldehyde fixation for 24 h. The sections were treated with xylene to remove the wax, dehydrated using a gradient of ethanol, rinsed with distilled water, and then stained with a hematoxylin solution for 5 min at room temperature. They were differentiated using 1 % hydrochloric acid alcohol for 5 s, and finally stained with a 1 % eosin solution for 1 min at room temperature before being sealed with neutral resin. The pathological changes of myocardial tissue were observed and analyzed under a light microscope (Nikon, Tokyo).

2.3. TUNEL assay

The slices were dewaxed in xylene for 5–10 min, treated with absolute ethanol for 5 min, 90 % ethanol for 2 min, 70 % ethanol for 2 min, and distilled water for 2 min. The enzyme K without DNase was added by dropping, and then the slices were incubated at 37 °C for 20 min, and washed with PBS for 3 times. The mixture of TUNEL reaction was added, and the slices were incubated at 37 °C for 1 h at the dark room, and washed with PBS for 3 times. The slices were incubated with DAB solution at room temperature for 5 min, and washed with PBS for 3 times. Hematoxylin staining and 1 % hydrochloric acid ethanol differentiation was performed, and the slices were treated for 10 min with distilled water, then the slices were sealed using neutral resin. Under the light microscope (Nikon, Tokyo), the sections were observed in the light microscope by an investigator who was initially blinded to treatment groups, and five randomly

selected fields of each slide were analyzed.

2.4. Cardiomyocyte culture

Rat H9c2 cardiomyocytes were purchased from American Type Culture Collection (ATCC, USA), and cultured using Dulbecco's modified eagle's medium (DMEM, Gibico, USA) containing 10 % fetal bovine serum (FBS, Gibico, USA) and 1 % streptomycin (Gibico, USA). The cells were seeded in 6-well plate or 12-well plate and cultured for 24 h at a concentration of 1×10^5/ml in a carbon dioxide incubator (37 °C and 5 % CO_2). Then H9c2 cardiomyocytes were cultured for 48 h using DMEM containing 5.5 mmol/L glucose (control) or 30 mmol/L glucose (HG).

2.5. Cell transfection

Si-HCG18 (5′-TTGGCTTCAGTCCTGTTCATCAG-3′), si-contorl (si-con, 5′-GGCCAAGCCUUGUGUAAAU-3′), pcDNA-HCG18, pcDNA-con, miR-9-5p mimics (miR-9-5p, 5′-UCUUUGGUUAUCUAGCUGUAUGA-3′), mimics control (miR-con, 5′-UUCUCCGAACGUGUCACGUTT-3′), miR-9-5p inhibitor (anti-miR-9-5p, 5′-UCAUACAGCUAGAUAACCAAAGA-3′), inhibitor contol (anti-miR-con, 5′-CAGUACUUUUGUGUAGUACAA-3′), pcDNA-IGF2R (IGF2R) and were synthesized by GenePharma Co., Ltd. (Shanghai, China). Cardiomyocytes were cultured in DMEM medium without antibiotics at 37 °C for 24 h, then transfected with plasmids (2 μg each hole) or miR-9-5p inhibitors, mimics, or nontargeting negative control at a final concentration of 50 nM using Lipofectamine 2000 (Invitrogen) at 37 °C for 24 h, and then cultured in DMEM medium with 5.5 mmol/l glucose or 30 mmol/L glucose at 37 °C for 48 h.

2.6. Reverse transcription-Polymerase chain reaction (RT-PCR)

Trizol Reagent (Invitrogen) was used to extract total RNA from myocardial tissues and cells according to the instructions, and mirVana RNA isolation kit (Ambion) was used for extraction of miRNA. The cDNA was obtained using prime script reverse transcription reagent kit (Thermo Fisher Scientific). RT-PCR was carried out with SYBR Green qPCR master mixtures (Thermo Fisher Scientific), and the mRNA levels were detected by light cycler 480 SYBR green fluorescent quantitative PCR. TaqMan MicroRNA Assays were used to quantify miRNA expression (Applied Biosystems). U6 was used as a miRNA internal control, and GAPDH was an mRNA internal control. The primer sequence is shown as follow, HCG18: forward 5′- GCTAGGTCCTCTACTTTCTG-3′ and reverse 5′-CAGAAAGTAGAGGACCTAGC-3′, miR-9-5p: forward 5′-GTGCAGGGTCCGAGGT-3′ and reverse 5′-GCGCTCTTTGGTTATCTAGC-3′, IGF2R: forward 5′-CCGGCGTGCTCTGGA-3′ and reverse 5′-CCAGAGGGTCACAGTGGAAGA-3′, U6: forward 5′-ATTGGAACGATACAGAGAAGATT-3′ and reverse 5′-GGAACGCTTCACGAATTTG-3′, GAPDH: forward 5′- CATGAGAAGTATGACAACAGCCT-3′ and reverse 5′-AGTCCTTCCACGATACCAAAGT-3′. Reaction conditions: pre-denaturation at 95 °C for 5min, and then 35 cycles (denaturation at 94 °C for 30s, annealing at 60 °C for 30s, extending at 72 °C for 30s) were performed, final extension was at 72 °C for 5min. The relative expression of mRNAs in each group were calculated according to $2^{-\Delta\Delta Ct}$ method.

2.7. Cell viability assay

The cells were inoculated into 96 well plates at 1 x 10^4 per well [17,18]. After 48 h, 10 μL 5 g/L MTT solution was added. After 4 h, the supernatant was discarded, and cells were treated with 150 μL DMSO in each well. The results were measured at 570 nm by a microplate reader (BioTek, VT, USA).

2.8. ELISA assay

The cells were lysed with RIPA (Beyotime) and centrifuged at 12000 rpm/min for 10 min at 4 °C. The levels of inflammatory factor (IL-1β, IL-6 and TNF-α), myocardial enzymes (LDH, CK-MB, AST) and antioxidant enzymes (SOD, CAT) were detected according to the instructions of ELISA kit (Nanjing Jiangcheng Bioengineering Institute). The OD value was detected at 450 nm using a microplate reader (BioTek, VT, USA).

2.9. Flow cytometry

After washed by PBS for 3 times, the cells were collected and prepared for single cell suspension (1×10^6/ml) in serum-free DMEM medium. Cell suspension (5 ml) was mixed with 1.25 μl Annexin V-FITC and incubated for 15 min at room temperature. The cells were centrifuged at 1000×*g* for 5 min, resuspended with 0.5 ml of precooled binding buffer, mixed with 10 μl Propidium Iodide (PI), and incubated overnight at 4 °C. Cell apoptosis was detected by CytoFLEX S (Beckman Coulter, Inc.).

2.10. Western blot

The total protein of myocardial tissue and cardiomyocyte was extracted by RIPA lysate (Beyotime), and BCA protein concentration detection kit (Beyotime) was used for the measurement of protein concentration. The same amount of total protein was separated in SDS-PAGE (12 %), and then transferred to PVDF membrane by electroporation. After washed for 3 times, the membranes were sealed for 1 h at room temperature with 5 % skimmed milk powder. After washed for 3 times, the membranes were incubated overnight at

4 °C with appropriate primary antibody. After washed for 3 times, the membranes were incubated for 2 h at room temperature with corresponding secondary antibody. After washed for 3 times, the bands were detected. Used antibodies were as follow: anti-Bcl-2 (ab196495, 1:1000), anti-Bax (ab53154, 1:1000), anti-IGF2R (ab124767, 1:1000), anti-β-actin (ab8226, 1:1000) and anti-GAPDH (ab8245, 1:1000). All antibodies were acquired from Abcam (Cambridge, USA). GAPDH or β-actin was used as normalizations.

2.11. Detection of double luciferase

The target sequence and mutation sequence (the sequence based on the 3′-UTR of HCG18 or IGF2R combined with miR-9-5p) was constructed by Genepharma Co. Ltd. (Shanghai, China), and inserted into pmirGLO plasma (Promega, Madison, WI, USA) to obtain the luciferase reporter vectors, HCG18-WT, HCG18-Mut, IGF2R-WT and IGF2R-Mut, respectively. The vectors and miR-9-5p were transfected into H9c2 cardiomyocytes using Lipofectamine 2000 (Invitrogen). After transfected for 48 h, the fluorescence intensity of double luciferase was detected by the Dual Luciferase Reporter Assay System (Promega).

2.12. Bioinformatic analysis

Online prediction of downstream target genes of miR-9-5p was performed through Targetscan 7.2 (https://www.targetscan.org/cgi-bin/targetscan/vert_72/view_gene.cgi?rs=ENST00000356956.1&taxid=9606&members=miR-9-5p&showcnc=0&shownc=0&shownc_nc=&showncf1=&showncf2=&subset=1).

2.13. Statistical analysis

In this study, statistical analysis was performed using SPSS statistics 17.0. All data were expressed as mean ± standard deviation (SD). The data in this article followed a normal distribution, a parametric one-way ANOVA was conducted followed by Tukey post hoc test, for comparisons between more than two groups. The comparison between the two groups was performed by Student's *t*-test. $P < 0.05$ was represented to be significant difference.

3. Results

3.1. HCG18 was overexpressed in DCM

After STZ injected for a week, the blood glucose concentration of DM model group was significantly more than 16.7 mmol/L compared with control (Fig. 1A). After 8 weeks, all rats were weighed, the weight of DM group was significantly lower than that of control group (Fig. 1B). Our gene silencing treatment occurred after 8 weeks of STZ injection. After another 8 weeks, the results of HE staining showed that cardiomyocytes were hypertrophic, and myofibers were disordered in DM group (Fig. 1C), which were typical

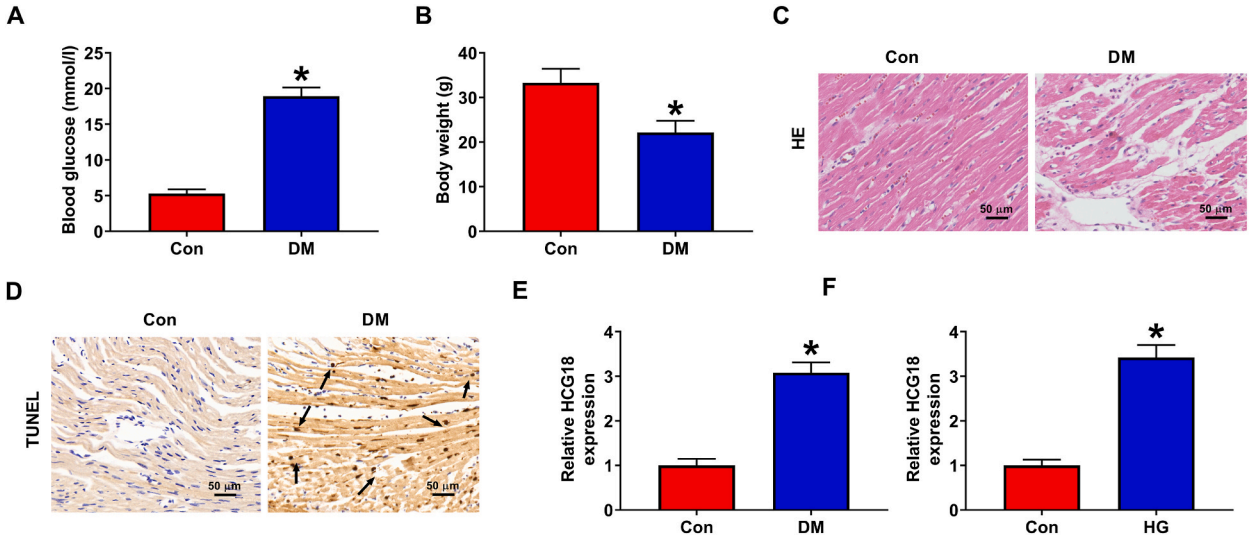


Fig. 1. Expression of HCG18 was overexpression in myocardium of DM rat induced by STZ and H9c2 cardiomyocytes induced by HG. (A) Blood glucose was detect using a glucometer (Accu-Chek, Roche Diagnostics) once a week, the results of eighth weeks are presented. (B) The weight of the rats was measured once a week, the results of eighth weeks are presented. (C) The pathological changes of myocardium in diabetic rats were measured by HE staining. (D) Cardiomyocyte apoptosis in myocardium of diabetic rats was detected by TUNEL. (E) Expression of HCG18 was detected in myocardium of DM rat induced by STZ through RT-PCR. (F) Expression of HCG18 was detected in H9c2 cardiomyocytes induced by HG through RT-PCR. *P < 0.05.

pathological changes of DM. Cardiomyocyte apoptosis was detected by TUNEL. The results showed that the cell apoptotic in DM group was more than that in control group (Fig. 1D). We then detected the expression of HCG18 in myocardium by RT-PCR. The data showed that HCG18 was overexpressed in DM group (Fig. 1E). In order to determine whether abnormal expression of HCG18 regulates cardiomyocyte function, we treated cardiomyocytes with HG medium (DMEM medium containing 30 mmol/L glucose) and detected the expression of HCG18 by RT-PCR (Fig. 1F). The results indicated that HCG18 was up-regulated in cardiomyocytes treated with HG.

3.2. *Down-regulation of HCG18 improved H9c2 cardiomyocyte injury induced by HG*

In order to clarify the role of HCG18 in the injury of cardiomyocytes in DM, HG induced H9c2 cardiomyocytes were transfected with si-HCG18 or si-con. si-HCG18 exerted the significant inhibition effect on HCG18 expression in H9c2 cardiomyocytes. (Fig. 2A). The results of MTT assay indicated that the down-regulation of HCG18 could inhibit the effect of HG on cardiomyocyte activity

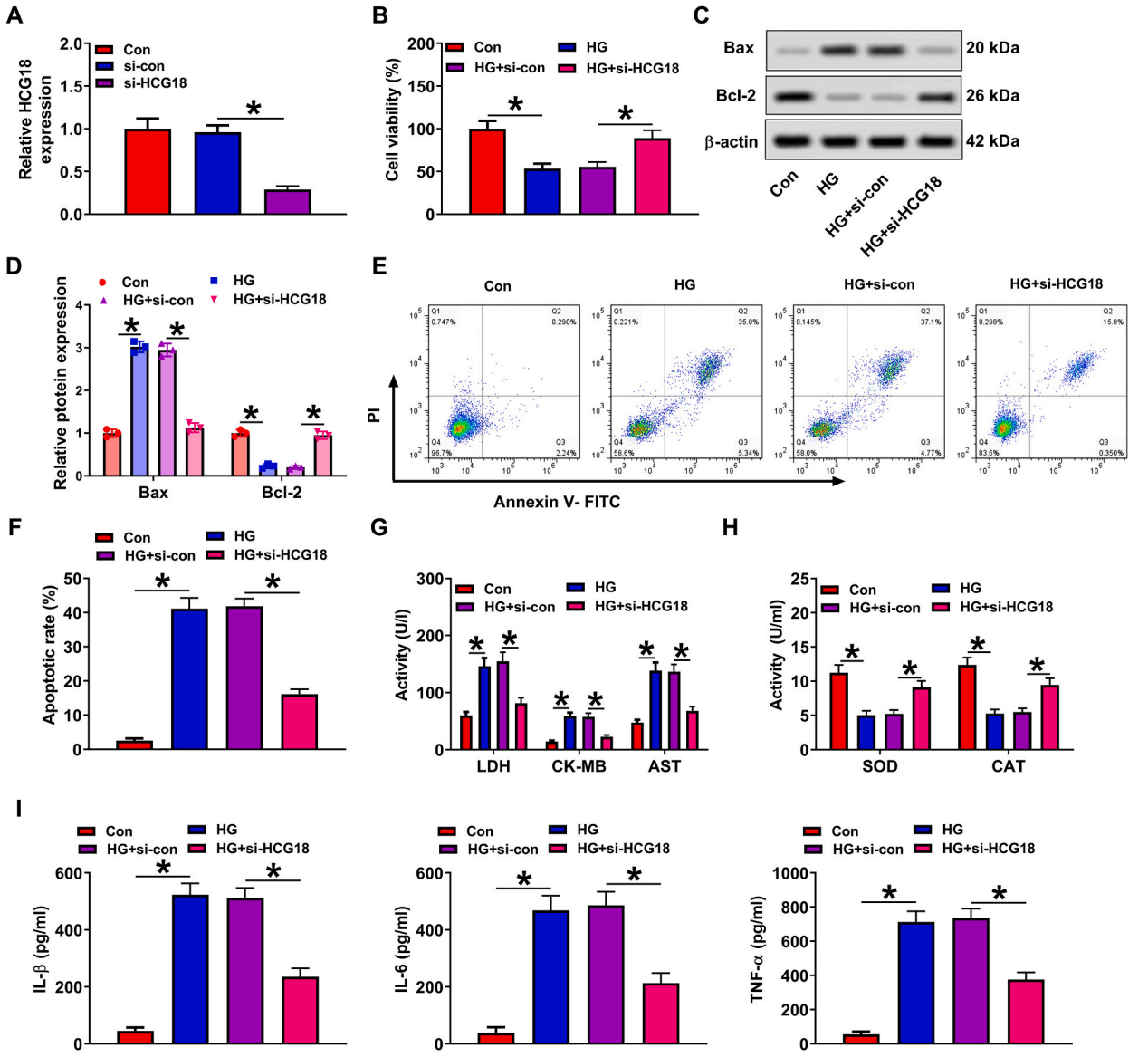


Fig. 2. Down-regulation of HCG18 ameliorated H9c2 cardiomyocytes injury induced by HG. (A) After si-HCG18 transfected, the expression of HCG18 was detected by RT-PCR in H9c2 cardiomyocytes. (B) After si-HCG18 transfected, cell viability was measured by MTT assay in H9c2 cardiomyocytes induced by HG. (C and D) After si-HCG18 transfected, the expression of Bax and Bcl-2 was detected by western blot in H9c2 cardiomyocytes induced by HG. (E and F) After si-HCG18 transfected, cell apoptosis was measured by flow cytometry in H9c2 cardiomyocytes induced by HG. (G and H) After si-HCG18 transfected, myocardial enzyme (LDH, CK-MB, AST) and antioxidant enzyme (SOD, CAT) levels were detected by ELISA in H9c2 cardiomyocytes induced by HG. (I) Inflammatory factor (IL-1β, IL-6 and TNF-α) were measured by ELISA in H9c2 cardiomyocytes induced by HG. *P $<$ 0.05.

(Fig. 2B). it was found that the down-regulation of HCG18 could reverse the HG induced decrease of Bcl-2 expression and increase of Bax expression in H9c2 cardiomyocytes (Fig. 2C and D). Furthermore, the results of flow cytometry showed that the down-regulation of HCG18 attenuated the increase of cardiomyocyte apoptosis induced by HG (Fig. 2E and F). Besides, we detected the activities of myocardial enzymes (LDH, CK-MB, AST) and antioxidant enzymes (SOD, CAT) by ELISA. The results demonstrated that HG significantly increased the activities of myocardial enzymes (LDH, CK-MB, AST) and decreased the activities of antioxidant enzymes (SOD, CAT) in H9c2 cardiomyocytes, whereas down-regulation of HCG18 could reverse the effect (Fig. 2G and H). In addition, knockdown of HCG18 recovered HG-induced increase of inflammatory factor (IL-1β, IL-6 and TNF-α) (Fig. 2I). Those data indicated that silencing of HCG18 attenuated H9c2 cardiomyocyte injury induced by HG.

3.3. *HCG18 could band to miR-9-5p directly*

Through the analysis of Starbase database, HCG18 and miR-9-5p had potential binding sites. The binding sites were shown in Fig. 3A. MiR-9-5p or anti-miR-9-5p, as well as pcDNA-HCG18 or si-HCG18, was transfected into H9c2 cardiomyocytes. The results showed that miR-9-5p/pcDNA-HCG18 upregulated expression of miR-9-5p/HCG18 and anti-miR-9-5p/si-HCG18 downregulated expression of miR-9-5p/HCG18 (Fig. 3B and C). In order to demonstrate our prediction, 3′-UTR of HCG18 was cloned into pmirGLO plasma, and double luciferase reporter gene detection was performed. The results illustrated that up-regulation of miR-9-5p could obviously inhibit luciferase activity in WT-HCG18 group, but the effect was dissolved in MUT-HCG18 (Fig. 3D). Moreover, overexpression of HCG18 could inhibit the expression of miR-9-5p, while down-regulation of HCG18 could increase the expression of miR-9-5p in H9c2 cardiomyocytes (Fig. 3E). Furthermore, miR-9-5p was down-regulated in STZ induced myocardial tissue and HG induced H9c2 cardiomyocytes compared with control group (Fig. 3F and G). These results showed that HCG18 negatively regulated the

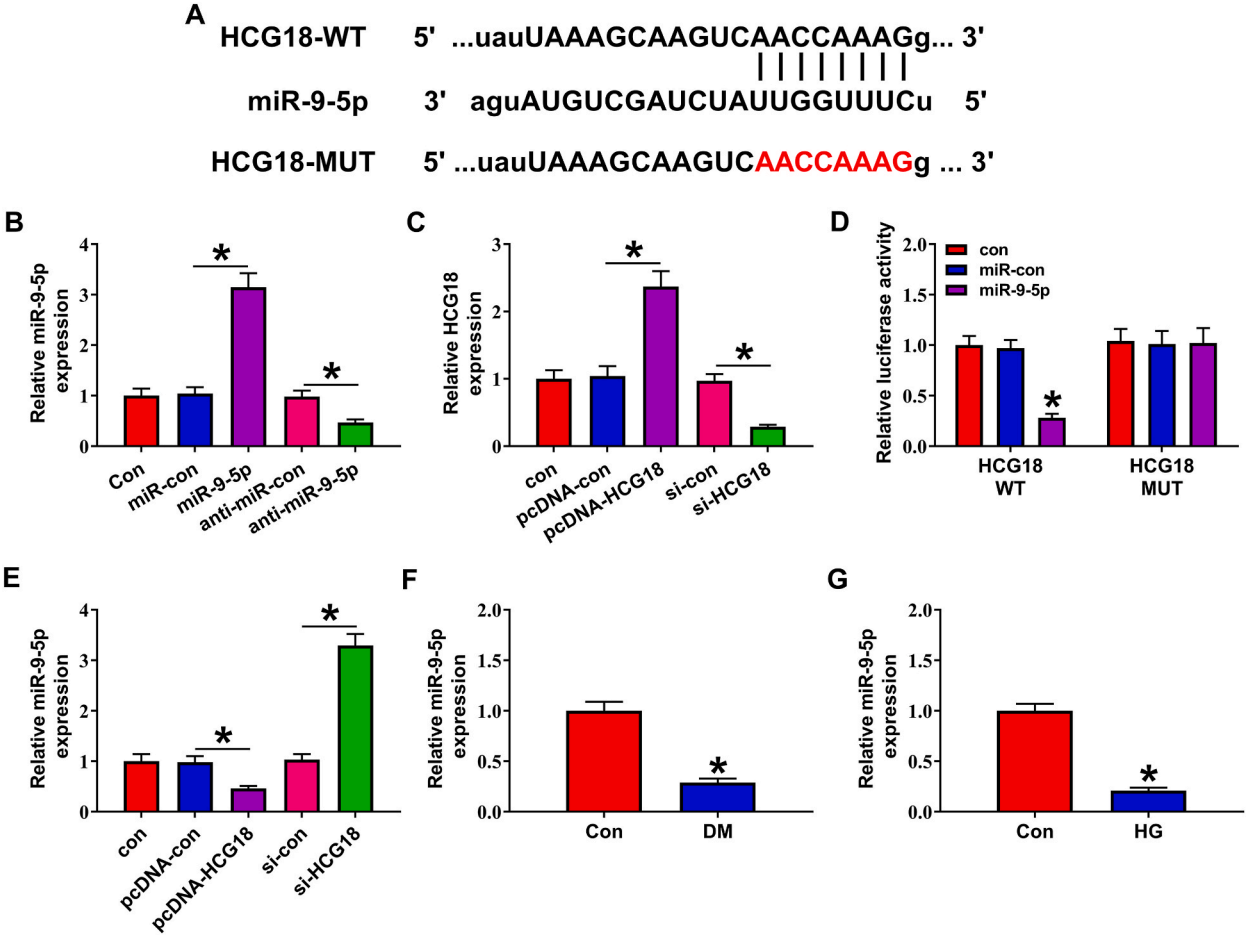


Fig. 3. HCG18 had targeted binding sites with miR-9-5p. (A) The target binding sites of HCG18 and miR-9-5p were presented. (B) The transfection effects of miR-9-5p and anti-miR-9-5p were detected by RT-PCR. (C) The transfection effects of pcDNA-HCG18 and si-HCG18 were detected by RT-PCR. (D) Double luciferase assay was used to verify the targeted binding of HCG18 with miR-9-5p. (E) The effect of overexpression or knockdown of HCG18 on miR-9-5p expression was detected by RT-PCR. (F) The expression of miR-9-5p in myocardium of STZ induced diabetic rats was detected by RT-PCR. (G) The expression of miR-9-5p in H9c2 cardiomyocytes induced by HG was detected by RT-PCR. *$P < 0.05$.

expression of miR-9-5p in H9c2 cardiomyocytes.

3.4. MiR-9-5p could target to IGF2R

Through the analysis of Targetscan software, the results showed that miR-9-5p and IGF2R have potential binding sites (Fig. 4A). It was predicted that miR-9-5p could bind to the promoter of IGF2R mRNA through the binding site, thus inhibiting the transcription and expression of IGF2R. The results of double luciferase reporter gene assay indicated that miR-9-5p could inhibit luciferase activity in IGF2R-WT group, while not effect in IGF2R-mut group (Fig. 4B). IGF2R mRNA and protein expression in STZ induced myocardial tissue and HG induced H9c2 cardiomyocytes were significantly higher than those in control group (Fig. 4C–F). Furthermore, the results indicated that down-regulation of HCG18 could inhibit IGF2R expression, and down-regulation of miR-9-5p could reverse this effect in H9c2 cardiomyocytes (Fig. 4G). These results indicated that miR-9-5p bound to IGF2R, and reduced the expression IGF2R in H9c2 cardiomyocytes.

3.5. Si-HCG18 attenuates HG induced H9c2 cardiomyocyte injury via miR-9-5p/IGF2R axis

The role of miR-9-5p/IGF2R axis was investigated in HG induced H9c2 cardiomyocytes. Si-HCG18, si-HCG18 + anti-miR-9-5p, or si-HCG18 + IGF2R was transfected in H9c2 cardiomyocytes induced by HG. Anti-miR-9-5p and IGF2R could reverse the down-regulation of IGF2R induced by si-HCG18 in HG induced H9c2 cardiomyocytes (Fig. 5A). Moreover, anti-miR-9-5p and IGF2R inhibited cell viability in H9c2 cardiomyocytes co-treated by HG and si-HCG18 (Fig. 5B). In H9c2 cardiomyocytes co-treated with HG and si-HCG18, decreased expression of Bax and increased expression of Bcl-2 were attenuated by anti-miR-9-5p or IGF2 (Fig. 5C and D). Inhibition of apoptosis induced by si-HCG18 was reversed by anti-miR-9-5p and IGF2R (Fig. 5E and F). In addition, the effects of si-HCG18 on myocardial enzymes (LDH, CK-MB, AST) and antioxidant enzymes (SOD, CAT) were reversed by anti-miR-9-5p or IGF2R in H9c2 cardiomyocytes induced by HG (Fig. 5G and H). Besides, anti-miR-9-5p and IGF2R could block si-HCG18-induced the decrease of inflammatory factor (IL-1β, IL-6 and TNF-α) (Fig. 5I). Those results suggested that HCG18 prevented HG induced H9c2 cardiomyocyte injury through modulating miR-9-5p/IGF2R axis.

3.6. HCG18 regulates miR-9-5p/IGF2R axis in STZ induced diabetic rats

DM model was constructed by intraperitoneal injection of STZ. After 8 weeks, DM rats were injected sh-HCG18 via tail vein (twice a

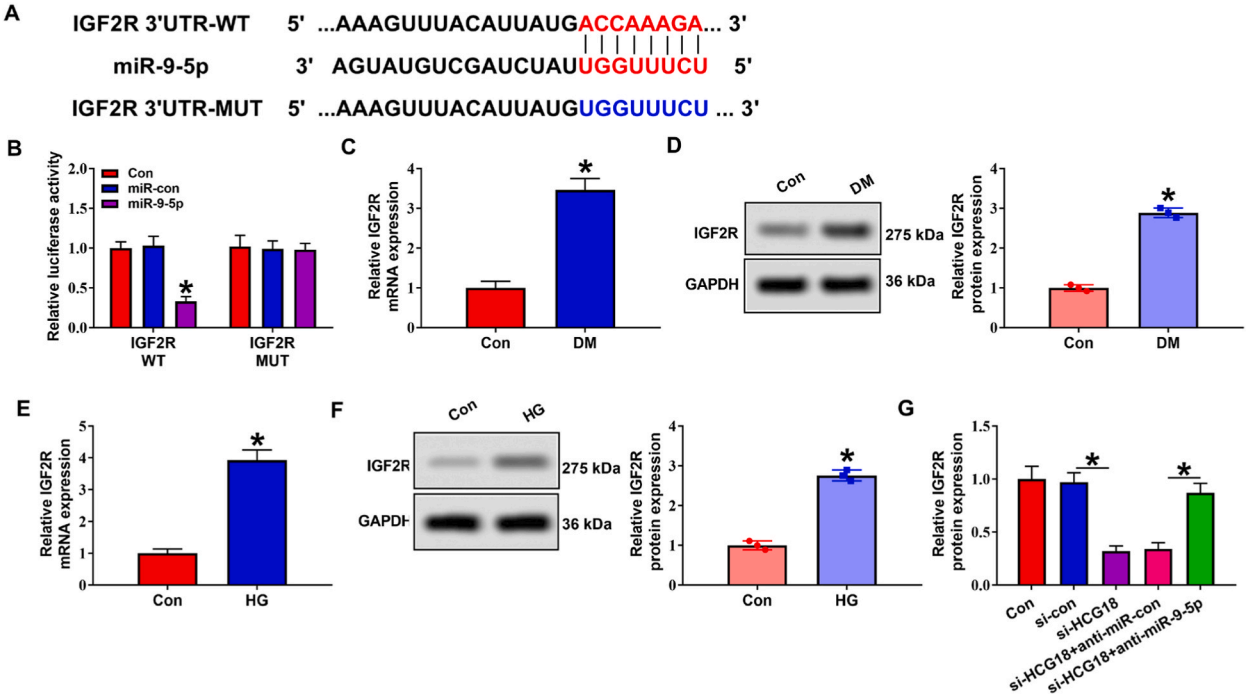


Fig. 4. MiR-9-5p directly targeted to IGF2R. (A) The target binding sites of miR-9-5p and IGF2R were presented. (B) Double luciferase assay was used to demonstrated the targeted binding of miR-9-5p with IGF2R. (C and D) mRNA and protein expression of IGF2R in myocardium of diabetic rats induced by STZ through RT-PCR and western blot, respectively. (E and F) mRNA and protein expression of IGF2R in H9c2 cardiomyocytes induced by HG through RT-PCR and western blot, respectively. (G) The effect of knockdown of HCG18 and miR-9-5p on IGF2R expression was detected by RT-PCR. *P < 0.05.

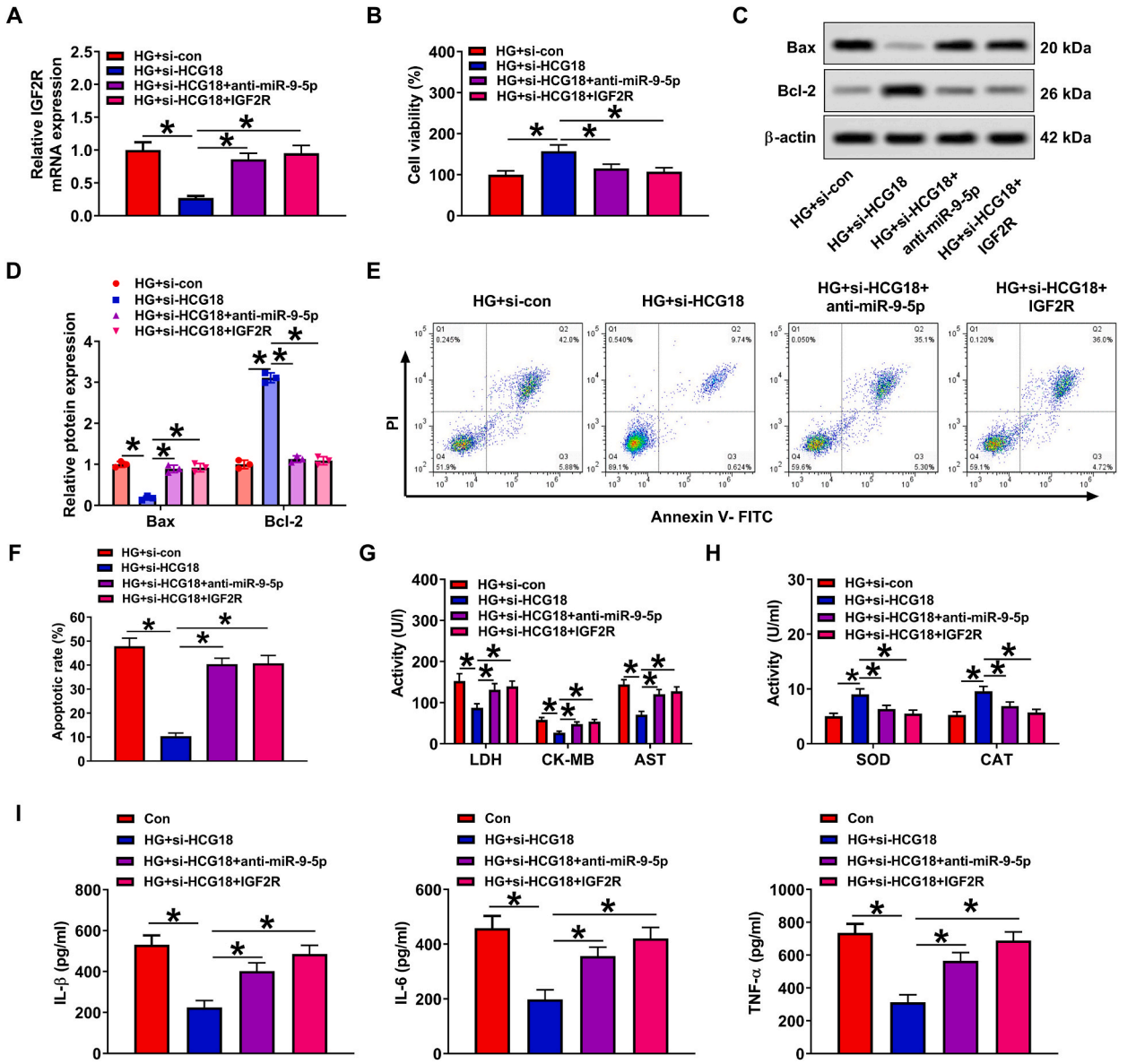


Fig. 5. HCG18 regulated the cell biological behavior of H9c2 cardiomyocytes induced by HG via miR-9-5p/IGF2R axis. (A) After transfection of si-HCG18 or/and anti-miR-9-5p (IGF2R), IGF2R expression was measured by RT-PCR. (B) Transfection of si-HCG18 or/and anti-miR-9-5p (IGF2R), cell viability was measured by MTT assay in H9c2 cardiomyocytes induced by HG. (C and D) After transfection of si-HCG18 or/and anti-miR-9-5p (IGF2R), the expression of Bax and Bcl-2 was detected by western blot in H9c2 cardiomyocytes induced by HG. (E and F) After transfection of si-HCG18 or/and anti-miR-9-5p (IGF2R), cell apoptosis was measured by flow cytometry in H9c2 cardiomyocytes induced by HG. (G and H) Transfection of si-HCG18 or/and anti-miR-9-5p (IGF2R), myocardial enzyme (LDH, CK-MB, AST) and antioxidant enzyme (SOD, CAT) levels were detected by ELISA in H9c2 cardiomyocytes induced by HG. (I) Inflammatory factor (IL-1β, IL-6 and TNF-α) were measured by ELISA in H9c2 cardiomyocytes induced by HG. *P < 0.05.

week). After 8 weeks, HE staining were carried out to estimate the histopathological changes. Si-HCG18 restrained the cardiomyocyte hypertrophy and inflammation induced by STZ (Fig. 6A). Blood glucose, cTnl and BNP in diabetic mice were significantly amplified, but ameliorated in the DM + sh-HCG18 groups (Fig. 6B, C and 6D). Compared with control group, HCG18 and IGF2R were significantly overexpressed in DM group, while miR-9-5p was reduced. However, sh-HCG18 could attenuate STZ induced increase of HCG18 and IGF2R expression and decrease of miR-9-5p (Fig. 6E, F, 6G, and 6H). Compared with the control group, the apoptosis was enhanced in DM group, and sh-HCG18 reversed the effect of STZ on apoptosis (Fig. 6I). We also found that the increase of myocardial enzyme (LDH, CK-MB, AST) and the decrease of antioxidant enzymes (SOD, CAT) in DM group could be restored by sh-HCG18 (Fig. 6J and K). Furthermore, the results showed that sh-HCG18 could inhibit the increase of IL-1β, IL-6 and TNF-α expression induced by STZ (Fig. 6L). These results indicated that downregulation of HCG18 improved myocardial damage of DM rats through miR-9-5p/IGF2R axis in *vivo*.

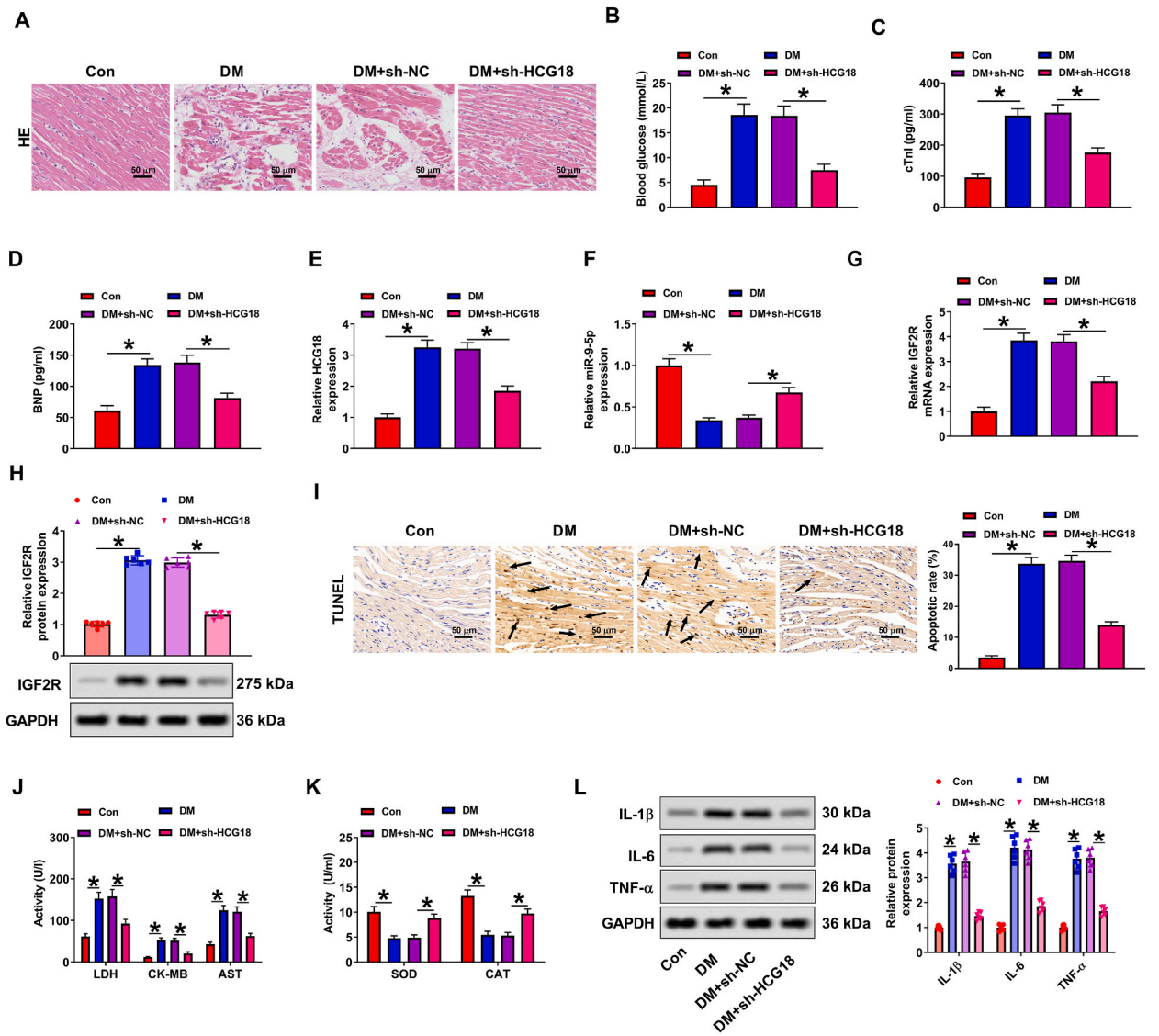


Fig. 6. Down-regulation of HCG18 improved the myocardium injury of diabetic rats induced by STZ. (A) HE staining were performed for each group. (B, C and D) Blood glucose, cTnl and BNP levels were evaluated by ELISA (E, F G and H) The expressions of HCG18, miR-9-5p and IGF2R were detected by RT-PCR and western blot in myocardium of diabetic rats induced by STZ. (I) The apoptosis was measured by TUNEL in myocardium of diabetic rats induced by STZ. (J and K) myocardial enzyme (LDH, CK-MB, AST) and antioxidant enzyme (SOD, CAT) levels were detected by ELISA in myocardium of diabetic rats induced by STZ. (L) The expressions of IL-1β, IL-6 and TNF-α were assessed by western blot. *P < 0.05.

4. Discussion

DCM is an independent heart disease, the pathogenesis of which may include metabolism disorders, myocardial interstitial fibrosis, cardiac parasympathetic regulation impairment and cardiac microvascular disease [1]. There is still no effective prevention and treatment method for DCM. The main treatment principle is to control blood glucose, blood pressure, blood lipid and improve cardiac function [19]. It is very important to study and clarify the molecular mechanism of DCM.

In recent years, the abnormal expression of lncRNA has been confirmed to regulate the pathological development of DCM at the molecular level [20,21]. Zhang et al. found that the expression of MHRT was significantly increased in H_2O_2 induced oxidative stress injury model in cardiomyocytes. When MHRT was down-regulated by siRNA, the cell apoptosis was reduced in oxidative stress model, which indicated that down-regulation of MHRT could protect cardiomyocytes from oxidative stress [22]. NONRAT007560.2 was significantly up-regulated in HG induced cardiomyocyte. When NONRATT007560.2 was down-regulated, cardiomyocyte apoptosis was inhibited and ROS level was decreased [23]. HCG18 is an immune-related gene, which is involved in regulating a variety of biological functions in a variety of diseases [24–26]. HCG18 had protective impacts on blood glucose and fat deposition by regulating

miR-197-3p in non-alcoholic fatty liver disease [27]. HCG18 promoted M1 macrophage polarization and further enlarged the development of diabetic peripheral neuropathy by regulating miR-146a/TRAF6 axis [12]. In this study, compared with control, blood glucose, apoptosis of cardiomyocytes, and myocardial pathology injury were enhanced, and body weight was decreased in DM rats. Further, we found that HCG18 was overexpressed in the myocardium of DM rats and H9c2 cardiomyocytes treated with HG. Moreover, previous studies had shown that silencing HCG18 could increase cell viability and reduce apoptosis of papillary thyroid cancer cell via miR-106a-5p/PPP2R2A axis [28]. knockdown of HCG18 prevented the progression of myasthenia gravis through suppressing Jurkat cell apoptosis and promoted cell proliferation by sponging miR-145-5p/CD28 [29]. In this study, downregulation of HCG18 attenuated HG induced the decrease of cell activity and the increase of apoptosis in H9c2 cardiomyocytes. SOD and CAT were often used as biomarkers of reactive oxygen species [30]. At present, the increase of AST, CK and LDH is the early diagnosis basis of myocardial ischemia or infarction [31]. Our data showed that silencing of HCG18 enhanced antioxidant enzyme (SOD, CAT) levels and reduced myocardial enzyme (LDH, CK-MB, AST) levels in H9c2 cardiomyocytes. Inflammatory response plays an important role in the development of DCM [32]. Nonspecific inflammatory factors such as IL-6, TNF-α and IL-1β are closely related to cardiovascular dysfunction in diabetic patients [33,34]. Interestingly, our results indicated that and inflammatory factor (IL-1β, IL-6 and TNF-α) were upregulated in H9c2 cardiomyocytes induced by HG, which was recovered by diminished HCG18. The results demonstrated that HCG18 could affect the progression of DCM.

MiR-9-5p is located on chromosome 15 [35]. Current studies have shown that miR-9-5p could regulate insulin secretion, promote insulin resistance, and participate in regulating myocardial biological function [36,37]. Kai et al. found that miR-9-5p might participate in the occurrence and development of DCM by affecting myocardial fibrosis [38]. Therefore, we investigated whether the effect of HCG18 on cardiomyocytes was mediated by miR-9-5p. In this study, HCG18 could directly target miR-9-5p in H9c2 cardiomyocytes. pcDNA-HCG18 decreased miR-9-5p expression, but si–HCG18 increased miR-9-5p expression. In addition, we found that the expression of miR-9-5p was significantly reduced in STZ induced DM rats and HG induced H9c2 cardiomyocytes. Up-regulation of miR-9-5p could increase cell viability, inhibit apoptosis, decrease myocardial enzyme activities and increase antioxidant enzyme activities in H9c2 cardiomyocytes induced by HG. Although we analyzed that HCG18 could target and regulate miR-9-5p in HG induced cardiomyocytes, but whether other miRNAs are regulated by HCG18 needs further investigation. This is a limitation of our research.

IGF2R protein is a single strand penetrating receptor, which is expressed in all tissues and cells. IGF2R, as an antagonist of IGF2, is a regulatory polypeptide. When combined with IGF2, IGF2R causes the degradation of IGF2 in lysosomes and inhibits the activation of tyrosine kinase mediated by IGF, thus playing a cell growth inhibition role [39]. Studies had shown that IGF2R signaling pathway is abnormally activated in H9c2 cells as well as in STZ induced diabetic rat hearts, and leads to cardiomyocyte apoptosis [40]. MiR-193-5p can inhibit angiogenesis through targeting IGF2 gene in type 2 diabetic goto Kakizaki (GK) rats' myocardial microvascular endothelial cells [41]. It was predicted that miR-9-5p could target IGF2R to affect cardiomyocytes. The results indicated that miR-9-5p could bind to 3′-UTR region of IGF2R. Our finding showed that si-HCG18 could down-regulate the expression of IGF2R, and miR-9-5p could attenuate this effect.

HCG18, as a ceRNA could sponging miRNAs to regulate multiple signaling Pathway [42–44]. Previous studies had shown that HCG18 can target miR-9 as ceRNA *in vitro* model of cerebral ischemia-reperfusion injury and gastric cancer cells [45,46]. Our studies indicated that HCG18 regulated high glucose induced cardiomyocyte injury by targeting miR-9-5p. In addition, IGF2R is one of the predictive targets of miR-9-5p. Many studies have shown that IGF2R is involved in the regulation of cardiomyocyte injury [40,47,48]. Therefore, we speculate that the miR-9-5p/IGF2R axis might be related to the effect of HCG18 on cardiomyocyte injury. Down-regulation of miR-9-5p could counteract the protective effect of si-HCG18 on H9c2 cardiomyocytes induced by HG. Moreover, up-regulation of IGF2R could neutralize the protective effect of si-HCG18 in H9c2 cardiomyocytes induced by HG. The results revealed that si-HCG18 protected H9c2 cardiomyocytes induced by HG through modulating miR-9-5p/IGF2R axis. However, whether other targets of miR-9-5p are also involved in the effect of HCG18 on cardiomyocyte injury still needs further study.

GAS5 attenuated the histological abnormalities and reversed the decreased LC3B II and increased p62 expression levels of DM model rats via the miR-221-3p/p27 axis [49]. Similarly, *in vivo*, our studies showed that the expression of HCG18 and IGF2R was decreased and the expression of miR-9-5p was increased in DCM rats treated with sh-HCG18, compared with that in DM rats treated with sh-NC. Sh-HCG18 significantly reduced the expression of IL-1β, IL-6 and TNF-α in DM rats. Myocardial enzyme activities were down-regulated and antioxidant enzyme activities were up-regulated by silencing HCG18 in DM rats. The results further confirmed the effect of HCG18 on cardiomyopathy in DM, and its association with miR-9-5p/IGF2R axis.

5. Conclusions

Taken together, this study revealed the HCG18 was significantly up-regulated in H9c2 cardiomyocytes induced by HG and myocardial tissue of STZ induced DM rats. Besides, silencing of HCG18 promoted the expression of miR-9-5p, and enhanced the negative regulation of miR-9-5p on IGF2R, which increased cell viability, inhibited cell apoptosis, down-regulated myocardial enzyme activities and up-regulated antioxidant enzyme activities in H9c2 cardiomyocytes induced by HG and myocardial tissue of STZ induced DM rats. Whether HCG18 had other mechanisms on the occurrence and development of DCM needs further study. This study might provide a new potential target for the treatment of DCM. The study on the clinical application of HCG18 as a therapeutic target for DCM is upcoming.

Funding

No funding in this research.

Availability of data and materials

This study do not contain any data that need to be deposited into a publicly available repository. The data supporting the results or analyses presented in the paper could obtain from the corresponding author on reasonable request.

CRediT authorship contribution statement

Yuhui Luo: Data curation, Supervision, Writing – original draft. **Yi Jiang:** Data curation, Supervision, Writing – original draft. **Tingting Zhong:** Methodology, Resources, Software. **Zhenggong Li:** Methodology, Resources, Writing – review & editing. **Jia He:** Investigation, Resources, Writing – review & editing. **Xiaoli Li:** Formal analysis, Resources, Software, Writing – review & editing. **Kun Cui:** Conceptualization, Project administration, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24604.

References

[1] G. Jia, A. Whaley-Connell, J.R. Sowers, Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease, Diabetologia 61 (1) (2018) 21–28, https://doi.org/10.1007/s00125-017-4390-4. PMID: PMC5720913.
[2] I. Falcão-Pires, A.F. Leite-Moreira, Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment, Heart Fail. Rev. 17 (3) (2012) 325–344, https://doi.org/10.1007/s10741-011-9257-z. PMID.
[3] G. Jia, V.G. DeMarco, J.R. Sowers, Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy, Nat. Rev. Endocrinol. 12 (3) (2016) 144–153, https://doi.org/10.1038/nrendo.2015.216. PMID: PMC4753054.
[4] S. Wu, Q. Lu, Y. Ding, Y. Wu, Y. Qiu, P. Wang, X. Mao, K. Huang, Z. Xie, M.H. Zou, Hyperglycemia-driven inhibition of amp-activated protein kinase α2 induces diabetic cardiomyopathy by promoting mitochondria-associated endoplasmic reticulum membranes in vivo, Circulation 139 (16) (2019) 1913–1936, https://doi.org/10.1161/circulationaha.118.033552. PMID: PMC6465113.
[5] S. Jathar, V. Kumar, J. Srivastava, V. Tripathi, Technological developments in lncrna biology, Adv. Exp. Med. Biol. 1008 (2017) 283–323, https://doi.org/10.1007/978-981-10-5203-3_10. PMID.
[6] W. Zhang, W. Xu, Y. Feng, X. Zhou, Non-coding rna involvement in the pathogenesis of diabetic cardiomyopathy, J. Cell Mol. Med. 23 (9) (2019) 5859–5867, https://doi.org/10.1111/jcmm.14510. PMID: PMC6714214.
[7] X. Li, H. Wang, B. Yao, W. Xu, J. Chen, X. Zhou, Lncrna h19/mir-675 axis regulates cardiomyocyte apoptosis by targeting vdac1 in diabetic cardiomyopathy, Sci. Rep. 6 (2016) 36340, https://doi.org/10.1038/srep36340. PMID: PMC5087087.
[8] Y. Chen, Z. Zhang, D. Zhu, W. Zhao, F. Li, Long non-coding rna meg3 serves as a cerna for microrna-145 to induce apoptosis of ac16 cardiomyocytes under high glucose condition, Biosci. Rep. 39 (6) (2019), https://doi.org/10.1042/bsr20190444. PMID: PMC6554216.
[9] Y. Zou, Z. Sun, S. Sun, Lncrna hcg18 contributes to the progression of hepatocellular carcinoma via mir-214-3p/cenpm axis, J. Biochem. 168 (5) (2020) 535–546, https://doi.org/10.1093/jb/mvaa073. PMID.
[10] W. Li, T. Pan, W. Jiang, H. Zhao, Hcg18/mir-34a-5p/hmmr axis accelerates the progression of lung adenocarcinoma, Biomed. Pharmacother. 129 (110217) (2020), https://doi.org/10.1016/j.biopha.2020.110217. PMID.
[11] Y. Lu, J. Guo, S. Zhu, H. Zhang, Q. Zhu, Y. Li, Lncrna hcg18 is critical for vascular smooth muscle cell proliferation and phenotypic switching, Hum. Cell 33 (3) (2020) 537–544, https://doi.org/10.1007/s13577-020-00366-2. PMID.
[12] W. Ren, G. Xi, X. Li, L. Zhao, K. Yang, X. Fan, L. Gao, H. Xu, J. Guo, Long non-coding rna hcg18 promotes m1 macrophage polarization through regulating the mir-146a/traf6 axis, facilitating the progression of diabetic peripheral neuropathy, Mol. Cell. Biochem. 476 (1) (2021) 471–482, https://doi.org/10.1007/s11010-020-03923-3. PMID.
[13] Z. Xu, B. Huang, Q. Zhang, X. He, H. Wei, D. Zhang, Notch1 regulates the proliferation and migration of bladder cancer cells by cooperating with long non-coding rna hcg18 and microrna-34c-5p, J. Cell. Biochem. 120 (4) (2019) 6596–6604, https://doi.org/10.1002/jcb.27954. PMID.
[14] Y. Xi, T. Jiang, W. Wang, J. Yu, Y. Wang, X. Wu, Y. He, Long non-coding hcg18 promotes intervertebral disc degeneration by sponging mir-146a-5p and regulating traf6 expression, Sci. Rep. 7 (1) (2017) 13234, https://doi.org/10.1038/s41598-017-13364-6. PMID: PMC5643303.
[15] V.A. Lacombe, S. Viatchenko-Karpinski, D. Terentyev, A. Sridhar, S. Emani, J.D. Bonagura, D.S. Feldman, S. Györke, C.A. Carnes, Mechanisms of impaired calcium handling underlying subclinical diastolic dysfunction in diabetes, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (5) (2007) R1787–R1797, https://doi.org/10.1152/ajpregu.00059.2007. PMID: PMC2413069.
[16] Y. Ti, G.L. Xie, Z.H. Wang, X.L. Bi, W.Y. Ding, J. Wang, G.H. Jiang, P.L. Bu, Y. Zhang, M. Zhong, W. Zhang, Trb3 gene silencing alleviates diabetic cardiomyopathy in a type 2 diabetic rat model, Diabetes 60 (11) (2011) 2963–2974, https://doi.org/10.2337/db11-0549. PMID: PMC3198078.

[17] A. Rahimi, I. Amiri, A.M. Roushandeh, Z.G. Choshali, Z. Alizadeh, T. Artimani, S. Afshar, S.S. Asl, Sublethal concentration of h(2)o(2) enhances the protective effect of mesenchymal stem cells in rat model of spinal cord injury, Biotechnol. Lett. 40 (3) (2018) 609–615, https://doi.org/10.1007/s10529-017-2499-7. PMID.
[18] M. Hasan Abdali, S. Afshar, A. Sedighi Pashaki, D. Dastan, M.H. Gholami, R. Mahmoudi, M. Saidijam, Investigating the effect of radiosensitizer for ursolic acid and kamolonol acetate on hct-116 cell line, Bioorg. Med. Chem. 28 (1) (2020) 115152, https://doi.org/10.1016/j.bmc.2019.115152. PMID.
[19] S. Paolillo, F. Marsico, M. Prastaro, F. Renga, L. Esposito, F. De Martino, P. Di Napoli, I. Esposito, A. Ambrosio, M. Ianniruberto, R. Mennella, R. Paolillo, P. Gargiulo, Diabetic cardiomyopathy: definition, diagnosis, and therapeutic implications, Heart Fail. Clin. 15 (3) (2019) 341–347, https://doi.org/10.1016/j.hfc.2019.02.003. PMID.
[20] Y. Chen, S. Tan, M. Liu, J. Li, Lncrna tincr is downregulated in diabetic cardiomyopathy and relates to cardiomyocyte apoptosis, Scand. Cardiovasc. J. 52 (6) (2018) 335–339, https://doi.org/10.1080/14017431.2018.1546896. PMID.
[21] Q. Li, P. Li, J. Su, S. Liu, X. Yang, Y. Yang, S. Niu, Lncrna nkila was upregulated in diabetic cardiomyopathy with early prediction values, Exp. Ther. Med. 18 (2) (2019) 1221–1225, https://doi.org/10.3892/etm.2019.7671. PMID: PMC6601402.
[22] J. Zhang, C. Gao, M. Meng, H. Tang, Long noncoding rna mhrt protects cardiomyocytes against h2o2-induced apoptosis, Biomol Ther (Seoul) 24 (1) (2016) 19–24, https://doi.org/10.4062/biomolther.2015.066. PMID: PMC4703348.
[23] M. Yu, X. Shan, Y. Liu, J. Zhu, Q. Cao, F. Yang, Y. Liu, G. Wang, X. Zhao, Rna-seq analysis and functional characterization revealed lncrna nonratt007560.2 regulated cardiomyocytes oxidative stress and apoptosis induced by high glucose, J. Cell. Biochem. 120 (10) (2019) 18278–18287, https://doi.org/10.1002/jcb.29134. PMID.
[24] Silencing of long non-coding rna-hcg18 inhibits the tumorigenesis of gastric cancer through blocking pi3k/akt pathway [retraction], OncoTargets Ther. 13 (2020) 12195, https://doi.org/10.2147/ott.s293126. PMID: PMC7705277.
[25] S. Wang, Y. Lin, Y. Zhong, M. Zhao, W. Yao, X. Ren, Q. Wang, X. Guo, Q.Q. Zhang, J. Dai, The long noncoding rna hcg18 participates in pm2.5-mediated vascular endothelial barrier dysfunction, Aging (Albany NY) 12 (23) (2020) 23960–23973, https://doi.org/10.18632/aging.104073. PMID: PMC7762519.
[26] X. Pan, J. Guo, C. Liu, Z. Pan, Z. Yang, X. Yao, J. Yuan, Lncrna hcg18 promotes osteosarcoma growth by enhanced aerobic glycolysis via the mir-365a-3p/pgk1 axis, Cell. Mol. Biol. Lett. 27 (1) (2022) 5, https://doi.org/10.1186/s11658-021-00304-6. PMID.
[27] Y. Xia, Y. Zhang, H. Wang, Upregulated lncrna hcg18 in patients with non-alcoholic fatty liver disease and its regulatory effect on insulin resistance, Diabetes Metab Syndr Obes 14 (2021) 4747–4756, https://doi.org/10.2147/dmso.s333431. PMID: PMC8651094.
[28] Y. Zhu, J. Zhao, L. Tan, S. Lin, M. Long, X. Peng, Lncrna-hcg18 regulates the viability, apoptosis, migration, invasion and epithelial-mesenchymal transition of papillary thyroid cancer cells via regulating the mir-106a-5p/ppp2r2a axis, Pathol. Res. Pract. 221 (2021) 153395, https://doi.org/10.1016/j.prp.2021.153395. PMID.
[29] S. Li, X. Wang, T. Wang, H. Zhang, X. Lu, L. Liu, L. Li, C. Bo, X. Kong, S. Xu, S. Ning, J. Wang, L. Wang, Identification of the regulatory role of lncrna hcg18 in myasthenia gravis by integrated bioinformatics and experimental analyses, J. Transl. Med. 19 (1) (2021) 468, https://doi.org/10.1186/s12967-021-03138-0. PMID: PMC8600732.
[30] S. Ivanović-Matić, M. Mihailović, S. Dinić, V. Martinović, D. Bogojević, I. Grigorov, G. Poznanović, The absence of cardiomyopathy is accompanied by increased activities of cat, mnsod and gst in long-term diabetes in rats, J. Physiol. Sci. 60 (4) (2010) 259–266, https://doi.org/10.1007/s12576-010-0093-z. PMID.
[31] Y. Bai, Z. Li, W. Liu, D. Gao, M. Liu, P. Zhang, Biochanin a attenuates myocardial ischemia/reperfusion injury through the tlr4/nf-κb/nlrp3 signaling pathway, Acta Cir. Bras. 34 (11) (2019) e201901104, https://doi.org/10.1590/s0102-865020190110000004. PMID: PMC6917477.
[32] I. Portig, A. Wilke, M. Freyland, M.J. Wolf, A. Richter, V. Ruppert, S. Pankuweit, B. Maisch, Familial inflammatory dilated cardiomyopathy, Eur. J. Heart Fail. 8 (8) (2006) 816–825, https://doi.org/10.1016/j.ejheart.2006.02.010. PMID.
[33] N. Kaur, Y. Guan, R. Raja, A. Ruiz-Velasco, W. Liu, Mechanisms and therapeutic prospects of diabetic cardiomyopathy through the inflammatory response, Front. Physiol. 12 (2021) 694864, https://doi.org/10.3389/fphys.2021.694864. PMID: PMC8257042.
[34] V. Ruppert, B. Maisch, Molecular signatures and the study of gene expression profiles in inflammatory heart diseases, Herz 37 (6) (2012) 619–626, https://doi.org/10.1007/s00059-012-3662-5. PMID.
[35] L. Yang, Y. Mu, H. Cui, Y. Liang, X. Su, Mir-9-3p augments apoptosis induced by h2o2 through down regulation of herpud1 in glioma, PLoS One 12 (4) (2017) e0174839, https://doi.org/10.1371/journal.pone.0174839. PMID: PMC5400238.
[36] K.H. Ho, P.H. Chen, E. Hsi, C.M. Shih, W.C. Chang, C.H. Cheng, C.W. Lin, K.C. Chen, Identification of igf-1-enhanced cytokine expressions targeted by mir-181d in glioblastomas via an integrative mirna/mrna regulatory network analysis, Sci. Rep. 7 (1) (2017) 732, https://doi.org/10.1038/s41598-017-00826-0. PMID: PMC5429683.
[37] G. Sebastiani, M. Valentini, G.E. Grieco, G. Ventriglia, L. Nigi, F. Mancarella, S. Pellegrini, G. Martino, V. Sordi, L. Piemonti, F. Dotta, Microrna expression profiles of human ipscs differentiation into insulin-producing cells, Acta Diabetol. 54 (3) (2017) 265–281, https://doi.org/10.1007/s00592-016-0955-9. PMID.
[38] K. Huang, S. Wen, J. Huang, F. Wang, L. Pang, Y. Wang, X. Sun, Integrated analysis of hub genes and mirnas in dilated cardiomyopathy, BioMed Res. Int. 2020 (8925420) (2020), https://doi.org/10.1155/2020/8925420. PMID: PMC7512046.
[39] P. Bella, A. Farini, S. Banfi, D. Parolini, N. Tonna, M. Meregalli, M. Belicchi, S. Erratico, P. D'Ursi, F. Bianco, M. Legato, C. Ruocco, C. Sitzia, S. Sangiorgi, C. Villa, G. D'Antona, L. Milanesi, E. Nisoli, P. Mauri, Y. Torrente, Blockade of igf2r improves muscle regeneration and ameliorates duchenne muscular dystrophy, EMBO Mol. Med. 12 (1) (2020) e11019, https://doi.org/10.15252/emmm.201911019. PMID: PMC6949491.
[40] C.C. Feng, S. Pandey, C.Y. Lin, C.Y. Shen, R.L. Chang, T.T. Chang, R.J. Chen, V.P. Viswanadha, Y.M. Lin, C.Y. Huang, Cardiac apoptosis induced under high glucose condition involves activation of igf2r signaling in h9c2 cardiomyoblasts and streptozotocin-induced diabetic rat hearts, Biomed. Pharmacother. 97 (2018) 880–885, https://doi.org/10.1016/j.biopha.2017.11.020. PMID.
[41] F. Yi, Y. Shang, B. Li, S. Dai, W. Wu, L. Cheng, X. Wang, Microrna-193-5p modulates angiogenesis through igf2 in type 2 diabetic cardiomyopathy, Biochem. Biophys. Res. Commun. 491 (4) (2017) 876–882, https://doi.org/10.1016/j.bbrc.2017.07.108. PMID.
[42] S. Li, T. Wu, D. Zhang, X. Sun, X. Zhang, The long non-coding rna hcg18 promotes the growth and invasion of colorectal cancer cells through sponging mir-1271 and upregulating mtdh/wnt/β-catenin, Clin. Exp. Pharmacol. Physiol. 47 (4) (2020) 703–712, https://doi.org/10.1111/1440-1681.13230. PMID.
[43] Y. Yang, P. Gong, D. Yao, D. Xue, X. He, Lncrna hcg18 promotes clear cell renal cell carcinoma progression by targeting mir-152-3p to upregulate rab14, Cancer Manag. Res. 13 (2021) 2287–2294, https://doi.org/10.2147/cmar.s298649. PMID: PMC7959199.
[44] F. Zhang, B.H. Luo, Q.H. Wu, Q.L. Li, K.D. Yang, Lncrna hcg18 upregulates traf4/traf5 to facilitate proliferation, migration and emt of epithelial ovarian cancer by targeting mir-29a/b, Mol Med 28 (1) (2022) 2, https://doi.org/10.1186/s10020-021-00415-y. PMID: PMC8725507.
[45] Y. Ren, X.P. Gao, H. Liang, H. Zhang, C.Y. Hu, Lncrna kcnq1ot1 contributes to oxygen-glucose-deprivation/reoxygenation-induced injury via sponging mir-9 in cultured neurons to regulate mmp8, Exp. Mol. Pathol. 112 (2020) 104356, https://doi.org/10.1016/j.yexmp.2019.104356. PMID.
[46] L. Feng, H. Li, F. Li, S. Bei, X. Zhang, Lncrna kcnq1ot1 regulates microrna-9-lmx1a expression and inhibits gastric cancer cell progression, Aging (Albany NY) 12 (1) (2020) 707–717, https://doi.org/10.18632/aging.102651. PMID: PMC6977675.
[47] W.K. Chen, W.W. Kuo, D.J. Hsieh, H.N. Chang, P.Y. Pai, K.H. Lin, L.F. Pan, T.J. Ho, V.P. Viswanadha, C.Y. Huang, Creb negatively regulates igf2r gene expression and downstream pathways to inhibit hypoxia-induced h9c2 cardiomyoblast cell death, Int. J. Mol. Sci. 16 (11) (2015) 27921–27930, https://doi.org/10.3390/ijms161126067. PMID: PMC4661925.
[48] C.H. Chu, C.Y. Huang, M.C. Lu, J.A. Lin, F.J. Tsai, C.H. Tsai, C.Y. Chu, W.H. Kuo, L.M. Chen, L.Y. Chen, Enhancement of ag1024-induced h9c2 cardiomyoblast cell apoptosis via the interaction of igf2r with galpha proteins and its downstream pka and plc-beta modulators by igf-ii, Chin. J. Physiol. 52 (1) (2009) 31–37, https://doi.org/10.4077/cjp.2009.amh004. PMID.
[49] D. Chen, M. Zhang, Gas5 regulates diabetic cardiomyopathy via mir-221-3p/p27 axis-associated autophagy, Mol. Med. Rep. 23 (2) (2021), https://doi.org/10.3892/mmr.2020.11774. PMID: PMC7751493.