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miRNA-711-SP1-collagen-I pathway is involved in the anti-fibrotic effect of pioglitazone in myocardial infarction

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Although microRNAs (miRNAs) have been intensively studied in cardiac fibrosis, their roles in drug-mediated anti-fibrotic therapy are still unknown. Previously, Pioglitazone attenuated cardiac fibrosis and increased miR-711 experimentally. We aimed to explore the role and mechanism of miR-711 in pioglitazone-treated myocardial infarction in rats. Our results showed that pioglitazone significantly reduced collagen-I levels and increased miR-711 expression in myocardial infarction heart. Pioglitazone increased the expression of miR-711 in cardiac fibroblasts, and overexpression of miR-711 suppressed collagen-I levels in angiotensin II (Ang II)-treated or untreated cells. Transfection with antagomir-711 correspondingly abolished the pioglitazone-induced reduction in collagen-I levels. Bioinformatics analysis identified SP1, which directly promotes collagen-I synthesis, as the putative target of miR-711. This was confirmed by luciferase assay and western blot analysis. Additionally, increased SP1 expression was attenuated by pioglitazone in myocardial infarction heart. Furthermore, transfection of antagomir-711 attenuated pioglitazone-reduced SP1 expression in cardiac fibroblasts with or without Ang II stimulation. We conclude that pioglitazone up-regulated miR-711 to reduce collagen-I levels in rats with myocardial infarction. The miR-711-SP1-collagen-I pathway may be involved in the anti-fibrotic effects of pioglitazone. Our findings may provide new strategies for miRNA-based anti-fibrotic drug research.

pioglitazone, miR-711, cardiac fibrosis

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Interstitial fibrosis is a major aspect of myocardial remodeling following myocardial infarction (MI), which contributes to impaired cardiac contractility and loss of pump function [1]. The extracellular matrix (ECM), which mainly includes collagen deposition, contributes to adverse cardiac interstitial fibrosis after MI. Therefore, inhibiting the process of collagen synthesis may be clinically relevant for the prevention and treatment of heart failure after MI. Since specific anti-fibrotic agents are not currently available, considerable efforts have focused on exploring new therapeutic signaling pathways that can attenuate adverse cardiac fibrosis.

Pioglitazone is an insulin-sensitizing drug that acts as a high affinity ligand for PPARγ, and has a cardiac protective role in various cardiovascular diseases [2,3]. It attenuated cardiac fibrosis, improved left ventricular failure and reduced myocardial infarct size after experimental MI [4–6].

Anita et al. [7] observed that inhibiting PPARγ completely abolished the up-regulation of miR-711 expression in mouse adipose cells. In addition, the expression levels of miR-711 were markedly changed in myocardial infarction

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[8]. Thus, we suggest that miR-711 may play a role in pioglitazone- mediated anti-fibrotic processes in myocardial infarction.

In the present study, we aimed to investigate whether miR-711 was altered by pioglitazone treatment in MI and its potential signaling pathway.

1 Methods

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication, 8th edition, 2011), and the Guidelines for Animal Experiments, Peking University Health Science Center.

1.1 Animal model preparation

All surgical procedures were performed with male Sprague-Dawley (SD) rats (eight weeks old, 180–200 g) anaesthetized with sodium pentobarbital (50 mg kg^{-1} intraperitoneally). Experimental MI was induced by ligation of the left anterior descending coronary artery as previously described [9]. Control rats were treated in the same way without ligation (sham operation). Rats were randomly subdivided into four groups for treatment: (i) vehicle+sham (n=6): water and sham operation; (ii) pioglitazone+sham (n=6): pioglitazone and sham operation; (iii) vehicle+MI (n=8): water and MI; and (iv) pioglitazone+MI (n=8): pioglitazone and MI. All rats were administered pioglitazone (Takeda Pharmaceuticals, Osaka, Japan; 10 mg kg^{-1} d^{-1}) or water each day for four weeks via gastro-gavage and underwent surgery at the end of week 2. Hearts were removed at two weeks after surgery. The walls of ventricles were fixed with 4% (w/v) paraformaldehyde and the remaining samples were stored in liquid nitrogen.

1.2 Histology

Heart ventricles were fixed with 4% (w/v) paraformaldehyde, dehydrated, and embedded in paraffin. Ventricle sections were stained with Sirius red to observe interstitial fibrosis. To determine the degree of cardiac fibrosis, the cardiac collagen volume fraction was calculated as the ratio of Sirius red-stained fibrosis area to total myocardium area with the use of Image-Pro Plus 6.0.

1.3 Quantitative real-time PCR

Total RNA was extracted by Trizol Reagent method (Invitrogen, Carlsbad, CA, USA). Relative quantification by real-time PCR involved SYBR-green detection of PCR products in real time with the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Invitrogen). Rat 18s ribosomal RNA was amplified as a reference standard. Reactions were prepared in triplicate and heated to 95°C for 5 min, followed by 40 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s.

Small RNA was isolated using the miRcute miRNA isolation kit (Tiangen Biotech, Beijing, China). The miRcute microRNA first-strand cDNA synthesis and qPCR detection kits (Tiangen) were used for miRNA analysis. Quantitative PCR was performed using the ABI PRISM 7700 Sequence Detection System. Rat 5s ribosomal RNA was used as an internal control. The primer sequences are shown in Table S1.

1.4 Cell culture, transfection and luciferase assay

Cardiac fibroblasts were isolated as previously described [10], grown to 80% confluence and serum starved for 24 h in serum-free medium before treatment with angiotensin II (0.1 μmol) or being transfected with agomir (100 nmol; RibioBio, Guangzhou, China) or antagomir (50 nmol; RibioBio). HEK293 cells were transfected with plasmid DNA (PGL_3-SP1-3′UTR/PGL_3-SP1-3′UTR deletion 200 ng, RTK 20 ng) and miRNA mimics (5 nmol; RibioBio) using Lipofectamine 2000 according to the manufacturer's methods (Invitrogen). Luciferase activity was measured by the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

1.5 Western blot analysis

Total protein was extracted by RIPA buffer (CxBio, Shanghai, China) supplemented with phenylmethanesulfonyl (PMSF, CxBio). Protein concentrations were measured by the BCA Protein Assay (Applygen, Beijing, China). The samples were separated on 10% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane blocked in 5% skim milk for 1 h and then incubated with anti-SP1 and eIF5 antibodies (both Santa Cruz Biotechnology, CA, USA) at 4°C overnight. After washing, membranes were incubated with horseradish peroxidase-labeled secondary antibodies for 1 h at room temperature. Bands were visualized using a chemiluminescence detection system. Autoradiographs were quantified by densitometry (Science Imaging System, Bio-Rad, USA). EIF5 levels were used as internal controls for protein normalization.

1.6 Enzyme-linked immunosorbent assay (ELISA)

Collagen-I was analyzed in proteins from heart tissue and cardiac fibroblasts by a rat collagen-I ELISA kit (Cat. No. CK-E30413; Qxbio, Beijing, China). Heart tissues were prepared with lysis buffer (10 mmol L^{-1} Tris pH 7.4, 0.1 mol L^{-1} NaCl, 1 mmol L^{-1} EDTA, 1 mmol L^{-1} EGTA, 1 mmol L^{-1} NaF, 20 mmol L^{-1} $Na_4P_2O_7$, 2 mmol L^{-1} Na_3VO_4, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100,

10% glycerol) with the addition of PMSF (1 mmol L^{-1}) and aprotinin (1 μg mL^{-1}). Cells were lysed by pipetting in complete cell extraction buffer (Cat. No. FNN0011, Invitrogen) with the addition of PMSF (1 mmol L^{-1}) and protease cocktail (50 μL mL^{-1}) (Cat. No. P-2714; Sigma, St. Louis, MO, USA). Collagen-I protein content was normalized to that of total protein.

1.7 Bioinformatics analyses

We used TargetScan (http://www.targetscan.org) and PITA (http://genie.weizma-nn.ac.il/pubs/mir07/mir07data.html) for miRNA target gene prediction.

1.8 Statistical analysis

Results are presented as mean±standard error of the mean (SEM). One-way ANOVA or two-way ANOVA with a Bonferroni post-hoc test was used as applicable. $P<0.05$ was considered statistically significant.

2 Results

2.1 Pioglitazone attenuated cardiac fibrosis after MI *in vivo*

Rat heart sections were stained with Sirius red to examine collagen levels (Figure 1A). Collagen deposition appeared to increase following MI but was attenuated with pioglitazone treatment. Interstitial fibrosis assessed by collagen volume fraction was markedly reduced by 75% following pioglitazone treatment compared with control treatment, with no significant difference in sham-operated hearts (Figure 1B). In MI hearts, the relative protein content of myocardial collagen-I was significantly increased compared with sham-treated hearts but decreased by 38% compared with the pioglitazone-treated group (Figure 1C).

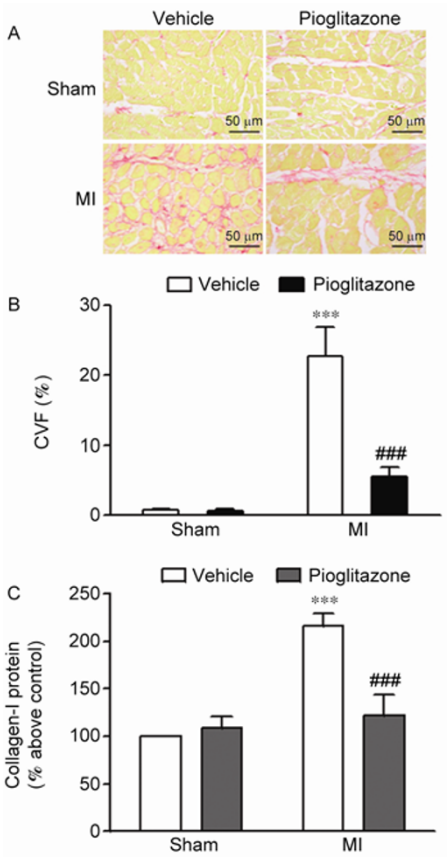

Figure 1 Effect of pioglitazone on cardiac fibrosis induced by myocardial infarction (MI) in rats. A, Representative micrographs of Sirius red staining of heart sections from MI and sham-operated groups (400×). B, Interstitial collagen volume fraction (CVF) in Sirius red-stained cross-sections of heart ventricles (n=6). C, ELISA of collagen-I expression (n=6–8). Data are mean±SEM. ***, $P<0.001$ vs. vehicle+sham; ###, $P<0.001$ vs. vehicle+MI.

2.2 Effect of pioglitazone on miR-711 expression

The miR-711 expression profile following MI detected by real-time PCR is shown in Figure S1.

MiR-711 levels were significantly down-regulated by 80% in MI rats with vehicle treatment ($P<0.001$), and the down-regulation was significantly inhibited by pioglitazone treatment ($P<0.05$) (Figure 2A).

To confirm the direct effects of pioglitazone on miR-711, a dose-response experiment was performed in neonatal rat cardiac fibroblasts. The results showed that miR-711 was up-regulated dose-dependently with pioglitazone (1–10 μmol) for 24 h (Figure 2B).

We further evaluated the effect of pioglitazone on miR-711 levels in Ang II-stimulated cardiac fibroblasts. MiR-711 expression was not affected by Ang II treatment (Figure S2), but miR-711 was up-regulated after pre-treatment with pioglitazone (10 μmol) for 1 h and Ang II (0.1 μmol) for up to 24 h (Figure 2C).

2.3 MiR-711 participated in pioglitazone-induced inhibition of collagen-I expression

To detect the effects of miR-711 on collagen-I levels, cardiac fibroblasts were transfected with agomir-711, antagomir-711 or nothing for the negative control (NC). The overexpression of agomir-711 and antagomir-711 was identified by quantification of miR-711 levels (Figure S3). Collagen-I protein levels were significantly down-regulated by agomir-711 (Figure 3A) and up-regulated by antagomir-711 treatment (Figure 3B) compared with NC. To determine whether pioglitazone inhibited collagen-I protein expression via miR-711, cardiac fibroblasts were incubated with pioglitazone after transfection of antagomir-711. The down-regulation of collagen-I after pioglitazone treatment was attenuated by antagomir-711 transfection (Figure 3C).

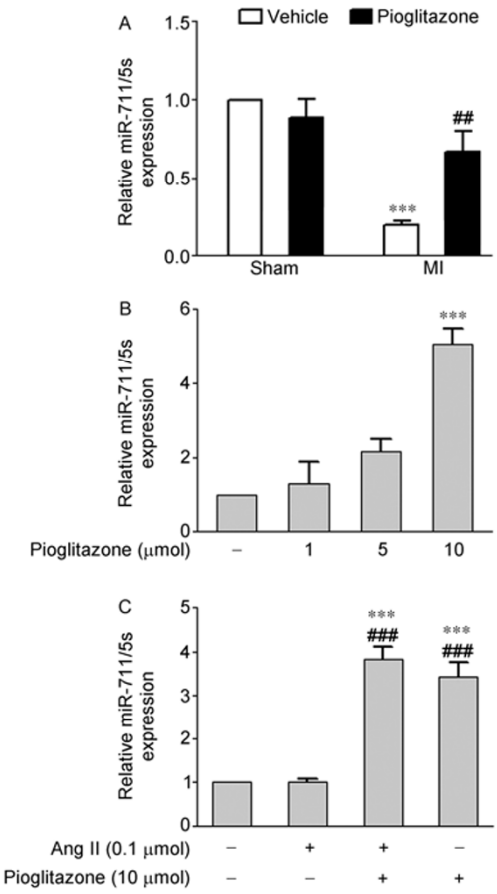

Figure 2 Effect of pioglitazone on miR-711 expression in rat myocardial tissue and neonatal rat cardiac fibroblasts. A, Real-time PCR analysis of miR-711 (n=6–8). Expression of miR-711 was standardized to 5s ribosomal RNA level. Data are mean±SEM. ***, P<0.001 vs. vehicle+sham; ##, P<0.01 vs. vehicle+MI. B, Cells were treated with pioglitazone (1, 5 and 10 μmol) for 24 h and then miR-711 expression was detected (n=3) (control was treated with DMSO). ***, P<0.001 vs. control. C, Cells were pre-incubated with pioglitazone (10 μmol) for 1 h, treated with angiotensin II (Ang II) (0.1 μmol) for up to 24 h, then miR-711 expression was detected (n=3). ***, P<0.001 vs. control; ###, P<0.001 vs. Ang II.

In addition, collagen-I expression was increased approximately 1.6- to 1.9-fold in Ang II-stimulated cardiac fibroblasts compared with controls (P<0.001). Agomir-711 inhibited increased collagen-I expression by about 31% (P<0.001) (Figure 3D). We transfected cardiac fibroblasts with antagomir-711 to antagonize pioglitazone-induced miR-711 levels by Ang II stimulation. Collagen-I expression was increased compared with Ang II and pioglitazone treatment (P<0.001) (Figure 3E).

2.4 Downstream signaling of miR-711

To explore the underlying mechanism of miR-711 mediated down-regulation of collagen-I content, we used bioinformatics analysis to predict the potential targets of miR-711 (Table S2). We revealed one predicted binding site of miR-711 within the 3′ untranslated region of SP1, which promotes collagen-I synthesis in tissue fibrosis. The complementary nucleotides between the miR-711 seed sequences and the SP1 binding site were highly conserved among species, including humans, rats and mice (Figure 4A).

To confirm that SP1 is the direct target of miR-711, we transfected HEK293 cells with a luciferase reporter plasmid pGL_3-SP1-3′UTR or control scramble target sites (pGL_3-SP1-deletion) for sequence specificity. Luciferase activity of the pGL_3-SP1-3′UTR reporter was significantly suppressed in cells transfected with miRNA mimic-711 compared with NC normalized to a control vector containing Renilla luciferase, pRL-TK. Transfection with the pGL_3-SP1-deletion reporter abolished this effect (Figure 4B).

To examine further whether miR-711 regulated SP1 expression, cardiac fibroblasts were transfected with agomir and antagomir. The protein levels of SP1 were significantly decreased with miR-711 overexpression and significantly increased with miR-711 inhibition (Figure 4C). However, mRNA levels were not significantly changed by miR-711 treatment (Figure 4D).

2.5 Pioglitazone inhibited SP1 expression via miR-711 *in vivo* and *in vitro*

SP1 protein levels were significantly up-regulated in rats with MI compared with controls (P<0.001), and markedly reduced with pioglitazone treatment (31%, P<0.05) (Figure 5A).

Treatment with pioglitazone in cardiac fibroblasts for 24 h dose-dependently inhibited SP1 protein levels (Figure 5B). To determine whether pioglitazone inhibited SP1 protein expression via miR-711, cardiac fibroblasts were transfected with antagomir-711, then incubated with pioglitazone for 24 h. SP1 protein expression was greater with pioglitazone and antagomir-711 than with pioglitazone alone (P<0.05) (Figure 5C).

Overexpressing miR-711 with agomir inhibited increased SP1 protein levels compared with Ang II stimulation alone (P<0.01) (Figure 5D). In addition, the up-regulation of SP1 protein expression with Ang II stimulation was attenuated by pioglitazone treatment (P<0.01) (Figure 5E). To determine whether miR-711 had an effect in the process, we used antagomir to antagonize miR-711, and the down-regulation of SP1 protein levels with Ang II and pioglitazone was attenuated (P<0.001) (Figure 5F).

3 Discussion

Cardiac fibrosis is one of the leading causes of cardiac dysfunction in heart diseases. Pioglitazone has been reported to attenuate cardiac fibrosis and improve left ventricular failure [4–6]. The current study also demonstrated that pioglitazone inhibited collagen-I expression (Figure 1C) and

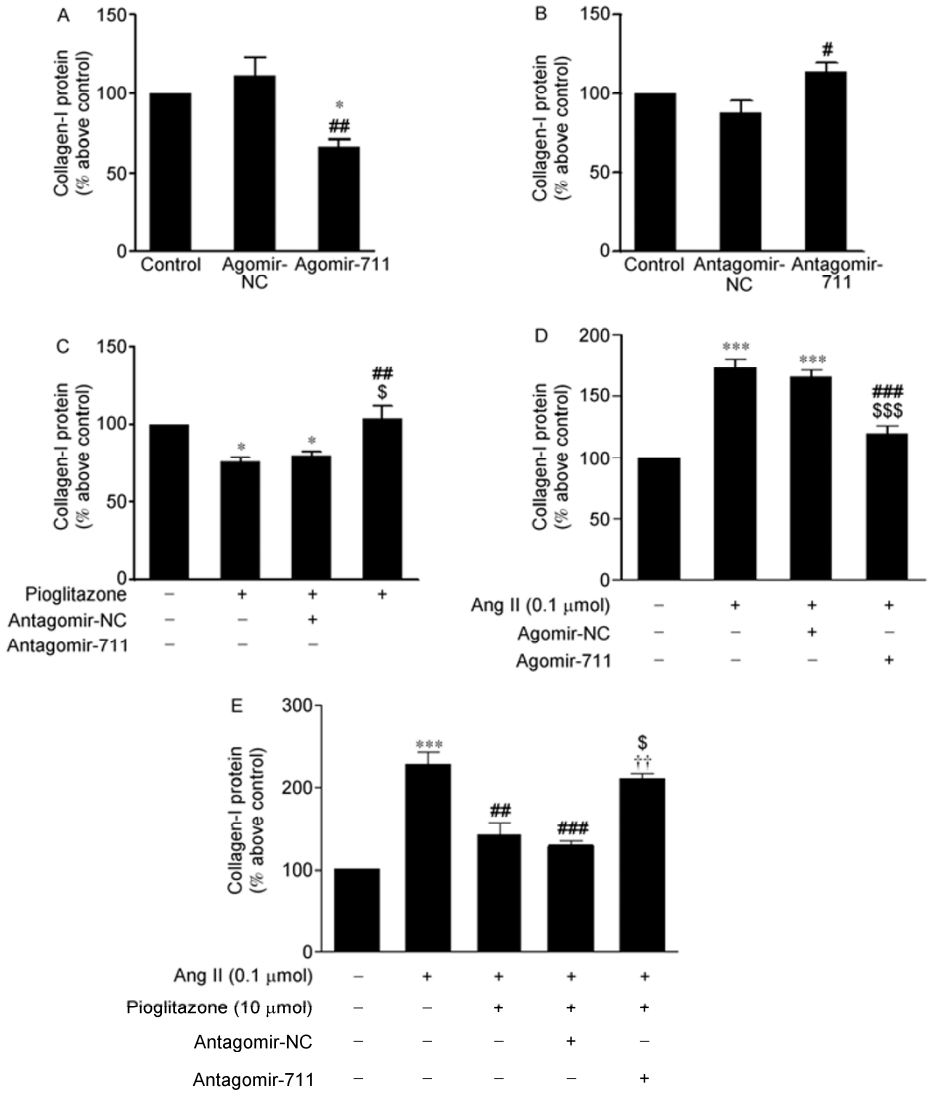

Figure 3 Pioglitazone inhibits collagen-I expression via miR-711 in cardiac fibroblasts. ELISA of collagen-I protein expression. Results are shown as a percentage of the control. A and B, Cardiac fibroblasts were transfected with agomir-711 and antagomir-711, collagen-I expression was detected (n=4). *, P<0.05 vs. control; #, P<0.05 vs. NC; ##, P<0.01 vs. NC. C, After transfection with antagomir-711, cardiac fibroblasts were incubated with pioglitazone (10 μmol) for up to 48 h, then collagen-I expression was detected (n=4). *, P<0.05 vs. control; ##, P<0.01 vs. pioglitazone; $, P<0.05 vs. pioglitazone+antagomir-NC. D, After transfection with agomir-711, cells were stimulated with angiotensin II (Ang II) (0.1 μmol) for up to 48 h, then collagen -I expression was detected (n=4). ***, P<0.001 vs. control; ###, P<0.001 vs. Ang II; $$$, P<0.001 vs. Ang II+agomir-negative control (NC). E, After transfection with antagomir-711 or NC, cells were pre-incubated with pioglitazone (10 μmol) for 1 h and then treated with Ang II (0.1 μmol) for up to 48 h, then collagen -I expression was detected (n=4). ***, P<0.001 vs. control; ##, P<0.01 vs. Ang II; ###, P<0.001 vs. Ang II; $, P<0.05 vs. Ang II+pioglitazone; ††, P<0.01 vs. Ang II+pioglitazone+antagomir-NC.

ameliorated cardiac fibrosis in myocardial infarction hearts (Figure 1A and B). Additionally, our results showed that pioglitazone up-regulated endogenous miR-711 levels (Figure 2B and C), and attenuated down-regulation of miR-711 in myocardial infarction hearts (Figure 2A). In addition, our results showed that down-regulation of collagen-I expression by pioglitazone in Ang II-treated cardiac fibroblasts was attenuated by transfection of antagomir-711 (Figure 3E). These results demonstrate that pioglitazone reduced collagen-I expression via miR-711 in cardiac fibroblasts.

Although the precise molecular mechanism of miR-711 regulation by pioglitazone is unclear, miR-711 could be regulated by PPARγ in mouse adipose cells [7]. We speculate that the anti-fibrotic effect of pioglitazone mediated by the miR-711 pathway occurs in a PPARγ-dependent manner. A vast amount of data from recent years suggested that the anti-fibrotic effects of pioglitazone mediated by PPARγ occur by various mechanisms, including control of inflammation [11], cross-regulation of signaling events implicated in fibrogenesis [12–14], reducing oxidative stress-induced NAPDH oxidase-mediated ROS activity [13], and impacting on the key molecules involved in both inflammation or

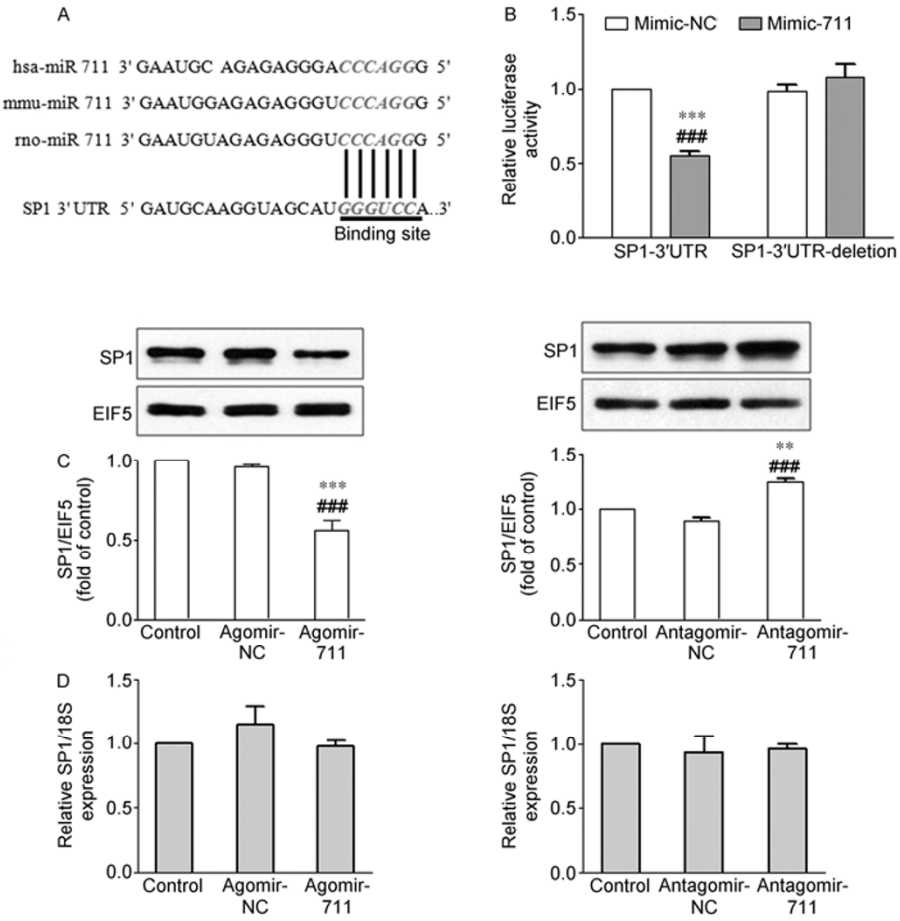

Figure 4 MiR-711 targeted by SP1. A, MiR-711 sequences and the binding site between miR-711 and SP1. B, HEK293 cells were co-transfected with PGL_3-SP1-3′UTR and miRNA mimic-711/NC, PGL_3-SP1-3′UTR-deletion and miRNA mimic-711/NC, for 48 h, then luciferase activity was detected (SP1-3′UTR, PGL_3-luciferase plasmid containing the seed sequences of 3′UTR of SP1, SP1-3′UTR-deletion, PGL_3-luciferase plasmid containing the deleted sequences/GGGUCC of 3′UTR of SP1) (n=4). ***, P<0.001 vs. SP1-3′UTR+Mimic-NC; ###, P<0.001 vs. SP1-3′UTR-deletion+Mimic-711. C and D, Western blot and real-time PCR analysis SP1 protein and mRNA expression, respectively, in cardiac fibroblasts transfected with agomir-711 and antagomir-711 (n=3). Expression of SP1 protein was standardized to eIF5 levels, and SP1 mRNA levels were standardized to that of 18s ribosomal RNA. ***, P<0.001 vs. control; ###, P<0.001 vs. NC; ##, P<0.01 vs. NC.

fibrosis, such as, NF-κB [15]. In light of these mechanisms, the effects of the pioglitazone-miR-711 pathway on inflammatory cells, fibrogenesis, molecular signaling and ROS require further exploration. It is worth noting that we observed binding sites between NF-κB and the promoter of the host gene for miR-711 by bioinformatic analysis. Thus, we speculate the anti-fibrotic effects of pioglitazone mediated by up-regulating miR-711 may be induced by the PPARγ-NF-κB pathway. This requires further elucidation.

Previous studies indicated that PPARγ activated by pioglitazone reduced SP1 protein expression [16], but the mechanisms involved were unclear. Our results (Figure 5B) suggest a possible mechanism where PPARγ may down-regulate SP1 protein levels via miR-711. Ang II-induced SP1 expression has pro-fibrotic effects in cardiac fibroblasts [17], but does not affect the expression of miR-711 (Figure S2). Thus, Ang II stimulation was used to observe the effect of pioglitazone-induced up-regulation of miR-711.

Recently, miR-711 levels were shown significantly changed after organs injury [8,18,19]. We also observed that miR-711 levels were down-regulated in rats with MI (Figure S1). Thus, miR-711 may play a key role in the process of cardiac fibrosis. Indeed, our results verify that miR-711 regulated collagen-I expression in cardiac fibroblasts (Figure 3A, B and D), but whether the anti-fibrotic effects of pioglitazone was mediated by miR-711 *in vivo* requires further study by inhibiting miR-711 with antagomir-711 in rats with MI.

Emerging evidence has shown that miRNAs may regulate cardiac fibrosis by targeting many different mRNAs [20]. Analysis of the predicted targets of miR-711 (Table S2) demonstrated only SP1 as a key factor involved in the process of fibrosis [21–23]. We identified SP1 protein as a target of miR-711 using luciferase assay and Western blot analysis (Figure 4B and C). SP1 protein is negatively regulated by miR-711 in cardiac fibroblasts, and its mRNA expression is not altered by miR-711 (Figure 4D). Thus, miR-711 may negatively regulate SP1 expression at the

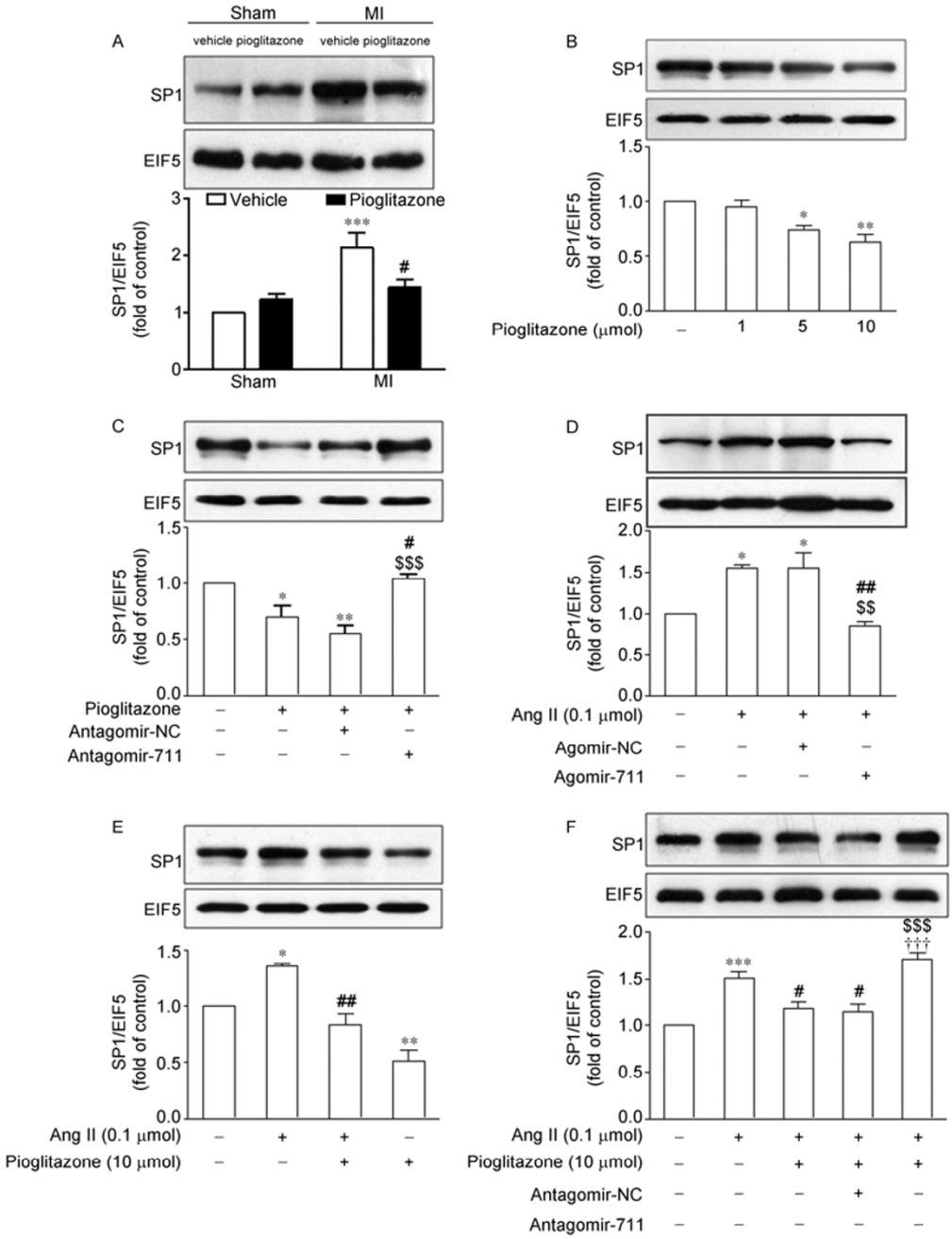

Figure 5 Pioglitazone inhibited SP1 expression via miR-711. Western blot analysis of SP-1 protein expression was standardized to eIF5 levels. A, Western blot analysis of SP1 protein expression in rat myocardium tissue (n=6). ***, P<0.001 vs. vehicle+sham; #, P<0.05 vs. vehicle+MI. B, Cardiac fibroblasts were treated with pioglitazone (1, 5 and 10 μmol) for 24 h, then SP1 expression was detected (n=4) (control was treated with DMSO). *, P<0.05 vs. control; **, P<0.01 vs. control. C, After transfection with antagomir-711, cardiac fibroblasts were incubated with pioglitazone (10 μmol) for up to 48 h, then SP1 expression was detected (n=4). *, P<0.05 vs. control; **, P<0.01 vs. control; #, P<0.05 vs. pioglitazone; $$$, P<0.001 vs. pioglitazone+antagomir-NC. D, After transfection with agomir-711, cells were stimulated with Ang II (0.1 μmol) for up to 48 h, then SP1 expression was detected (n=4). ***, P<0.001 vs. control; ##, P<0.01 vs. Ang II; $$, P<0.01 vs. Ang II+agomir-NC. E, Cardiac fibroblasts were pre-treated with pioglitazone (10 μmol) for 1 h, treated with Ang II (0.1 μmol) for up to 48 h, then SP1 expression was detected (n=4). *, P<0.05 vs. control; **, P<0.01 vs. control; ##, P<0.01 vs. Ang II. F, After transfection of antagomir-711/NC, cells were pre-incubated with pioglitazone (10 μmol) for 1 h, treated with Ang II (0.1 μmol) for up to 48 h, then SP1 expression was detected (n=4). ***, P<0.001 vs. control; #, P<0.05 vs. Ang II; $$$, P<0.001 vs. Ang II+pioglitazone; †††, P<0.001 vs. Ang II+pioglitazone+antagomir-NC.

translational level in cardiac fibroblasts. Furthermore, our results showed pioglitazone inhibited SP1 protein expression in cardiac fibroblasts (Figure 5B and E), and the down-regulation of SP1 protein was attenuated by transfection of antagomir-711 (Figure 5D and F). This indicates that pioglitazone inhibits collagen-I expression via miR-711-SP1.

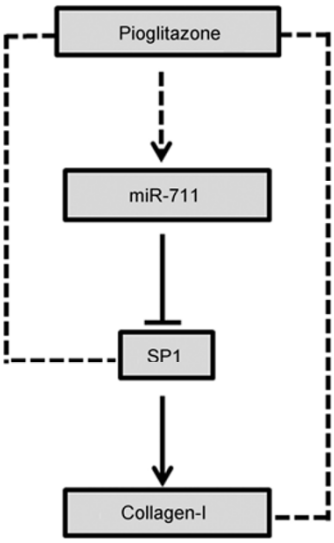

Figure 6 Model for pioglitazone suppression of collagen-I expression via miR-711.

In conclusion, the miR-711-SP1 pathway may be involved in pioglitazone-mediated anti-fibrotic effects (Figure 6), which may provide a new strategy for miRNA-based anti-fibrotic drug research.

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Supporting Information

Figure S1 The miR-711 expression profiles in cardiac fibrosis following myocardial infarction (MI). A, Real-time PCR analysis of miR-711 in myocardium tissue (n=6) of MI rat. B, Real-time PCR analysis of miR-711 expression at different time (2, 7, 14 d) in rats following sham operation (n=6). Expression of miR-711 was standardized to 5s ribosomal RNA level. *, P<0.05 vs. sham; ***, P<0.001 vs. sham.

Figure S2 miR-711 expression in cardiac fibroblasts stimulated by Ang II at different times. Cardiac fibroblasts were treated with Ang II (0.1 μmol) for up to 24 h and then miR-711 expression at different time points were detected. Real-time PCR analysis of expression of miR-711 standardized to 5s mRNA level. MiR-711 expression was not affected by Ang II.

Figure S3 Using agomir to overexpress and antagomir to antagonize the expression of miR-711. Real-time PCR analysis of expression of miR-711 standardized to 5s mRNA level.

Table S1 Real-time PCR primer sequences for amplification

Table S2 The predicted conserved targets of miR-711

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