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Basic Research

Involvement of lncR-30245 in Myocardial Infarction–Induced Cardiac Fibrosis Through Peroxisome Proliferator-Activated Receptor-γ–Mediated Connective Tissue Growth Factor Signalling Pathway

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ABSTRACT

Background: Long noncoding RNAs (lncRNAs) are emerging as important mediators of cardiac pathophysiology. The aim of the present study is to investigate the effects of lncR-30245, an lncRNA, on cardiac fibrogenesis and the underlying mechanism.

Methods: Myocardial infarction (MI) and transforming growth factor (TGF)-β1 were used to induce fibrotic phenotypes. Cardiac fibrosis was detected by Masson's trichrome staining. Cardiac function was evaluated by echocardiography. Western blot, quantitative reverse transcription-polymerase chain reaction, and pharmacological approaches were used to investigate the role of lncR-30245 in cardiac fibrogenesis.

Results: Expression of lncR-30245 was significantly increased in MI hearts and TGF-β1–treated cardiac fibroblasts (CFs). LncR-30245 was mainly located in the cytoplasm. Overexpression of lncR-30245 promoted collagen production and CF proliferation. Knockdown of lncR-30245 significantly inhibited TGF-β1–induced collagen production and CF proliferation. LncR-30245 overexpression inhibited the antifibrotic role of peroxisome proliferator-activated receptor (PPAR)-γ and

RÉSUMÉ

Contexte : Les longs ARN non codants (lncRNA) se révèlent actuellement comme des médiateurs importants de la physiopathologie cardiaque. Le but de la présente étude est d'examiner les effets d'un lncRNA (lncR-30245) sur la fibrogenèse cardiaque et les mécanismes sous-jacents.

Méthodologie : Un infarctus du myocarde (IM) et le facteur de croissance transformant β1 (TGF-β1) ont été utilisés pour produire des phénotypes fibrotiques. Une fibrose cardiaque a été détectée par coloration trichrome de Masson. La fonction cardiaque a été évaluée par échocardiographie. Un transfert de western, une RT-PCR quantitative et des approches pharmacologiques ont été employés pour examiner le rôle du lncR-30245 dans la fibrogenèse cardiaque.

Résultats : L'expression du lncR-30245 était nettement augmentée dans les cœurs ayant subi un IM et les fibroblastes cardiaques (FC) traités par le TGF-β1. Le lncR-30245 était situé surtout dans le cytoplasme. La surexpression du lncR-30245 a favorisé la production de collagène et la prolifération des FC. L'inactivation du lncR-30245 a entraîné une inhibition importante de la production de collagène

Myocardial infarction (MI) is the leading cause of death worldwide. Fibrosis is a common pathological change that occurs after MI. Typical cardiac fibrosis is characterized by excessive proliferation of cardiac fibroblasts (CFs) and deposition of extracellular matrix (ECM) proteins in the myocardium.[1] Excessive ECM deposition leads to myocardial stiffening, cardiac relaxation, and diastolic dysfunction. The ECM is mainly composed of fibrillar collagen types I and III in heart tissue.[2] TGF-β1 is a major causal factor of cardiac fibrosis, which is over-produced and released during MI.[3] TGF-β1 binds to TGF-β1 receptors type I and II and activates the TGF-β1/Smad pathway leading to CF differentiation into myofibroblasts and ECM protein synthesis.[4] Enhanced TGF-β1 expression is often accompanied by increased collagen synthesis, deposition, and myocardial fibrosis.[5]

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increased connective tissue growth factor (CTGF) expression, whereas lncR-30245 knockdown exerted the opposite effects. Rosiglitazone, a PPAR-γ agonist, significantly inhibited lncR-30245–induced CTGF upregulation and collagen production in CFs. In contrast, T0070907, a PPAR-γ antagonist, attenuated the inhibitory effects of lncR-30245 small interfering RNA (siRNA) on TGF-β1–induced CTGF expression and collagen production. LncR-30245 knockdown significantly enhanced ejection fraction and fractional shortening and attenuated cardiac fibrosis in MI mice.

Conclusion: Our study indicates that the lncR-30245/PPAR-γ/CTGF pathway mediates MI-induced cardiac fibrosis and might be a therapeutic target for various cardiac diseases associated with fibrosis.

induite par le TGF-β1 et la prolifération des FC. La surexpression du lncR-30245 a inhibé le rôle antifibrotique du récepteur activé par les proliférateurs de peroxysomes gamma (PPAR-γ) et a augmenté l'expression du facteur de croissance du tissu conjonctif (CTGF), tandis que l'inactivation du lncR-30245 a entraîné les effets inverses. La rosiglitazone, un agoniste du PPAR-γ, a entraîné une inhibition importante de la régulation à la hausse du CTGF induite par le lncR-30245 et la production de collagène dans les FC. En revanche, le T0070907, un antagoniste du PPAR-γ, a atténué les effets inhibiteurs des petits ARN interférents (siRNA) du lncR-30245 sur l'expression du CTGF induite par le TGF-β1 et la production de collagène. Soulignons que l'inactivation du lncR-30245 a entraîné une augmentation notable de la fraction d'éjection et de la fraction de raccourcissement ainsi qu'une diminution de la fibrose cardiaque chez la souris ayant subi un IM.

Conclusion : Notre étude indique que la voie lncR-30245/PPAR-γ/CTGF assure la médiation de la fibrose cardiaque provoquée par un IM et pourrait être une cible thérapeutique pour diverses maladies cardiaques associées à une fibrose.

Noncoding RNAs, including long noncoding RNA (lncRNA), microRNA, and circular RNAs, play important roles in cardiovascular disease.[6-14] LncRNAs belong to a novel class of noncoding RNA of 200 nucleotides to 100 kilobases in length without protein-coding capacity, and they participate in various physiological processes and pathological events.[15] It has been shown that deregulated expression of lncRNA is associated with inflammatory response and apoptosis which are involved in myocardial ischemia–reperfusion injury,[16] and may serve as a therapeutic target for the treatment of ischemia–reperfusion injury.[17] Jiang and Ning[18] revealed that angiotensin II dynamically downregulated the expression of lncRNA-NR024118 in adult rat CFs. A recent study demonstrated that Wisp2 super-enhancer–associated RNA (Wisper) regulates the gene expression programs that are important for cell identity, ECM deposition, proliferation, and survival in CFs, which is a CF-enriched super-enhancer–associated lncRNA and potentially a highly sensitive marker for pathological fibrosis.[19] Our published results showed that an lncRNA MI-associated transcript is remarkably upregulated and acts as a profibrotic lncRNA in a mouse model of MI characterized by cardiac interstitial fibrosis.[20] In another study, we found that lncRNA NONMMUT030245 (lncR-30245) was significantly upregulated in MI mouse heart and was correlated with fibrosis-associated genes.[8] Moreover, lncR-30245 is highly conserved between mouse and human (https://blast.ncbi.nlm.nih.gov/Blast.cgi). However, the functional role of lncR-30245 in the development of cardiac fibrosis remains unknown.

Several pieces of evidence point to peroxisome proliferator-activated receptor (PPAR)-γ as a fibrotic suppressor in cardiac fibrosis.[21,22] PPAR-γ has been shown to prevent or attenuate cardiac fibrosis and functions as an upstream molecule of connective tissue growth factor (CTGF).[23] In this study, we showed that lncR-30245 contributed to the progression of cardiac fibrosis via inhibiting PPAR-γ and simultaneously enhancing CTGF expression. This finding suggested that inhibition of lncR-30245 might represent a novel antifibrotic treatment in cardiac diseases.

Material and Methods

Mouse model of myocardial infarction

Animal care and experimental protocols were in accordance with the Institutional Animal Care and were approved by the Ethics Committee of Harbin Medical University. Male C57BL/6 mice (20-25 g) were housed in the environment at a temperature of 20°C with a 12/12-hour light/dark cycle. The procedures for MI establishment were the same as described previously.[24] Mice were anaesthetized with the intraperitoneal injection of ketamine (60 mg/kg) and xylazine (6 mg/kg). The mice were intubated and ventilated with an artificial respiration machine (UGO Basile Biological Research Apparatus Company, Comerio, Italy). An incision was made through the fourth intercostal space, and the heart was exposed. The left anterior descending coronary artery was permanently ligated with a 7/0 silk thread to establish the MI model. For sham-operated mice, a suture was passed through around the left anterior descending coronary artery without ligation. After surgery, mice were monitored daily for signs of infection and health status. The mice were anaesthetized for echocardiography analysis and killed by cervical dislocation at 4 weeks after MI.

In vivo infection of adenovirus carrying small interfering RNA for lncR-30245

The adenovirus carrying lncR-30245 small interfering RNA (siRNA) (sense: 5'-CCAGAAUCCCACACGUCAATT-3', anti-sense: 5'-UUGACGUGUGGGAUUCUGGTT-3') and the adenovirus carrying scrambled siRNA (sense: 5'-UUCUCCGAACGUGUCACGUTT-3', antisense: 5'-ACGUGACACGUUCGGAGAATT-3') as negative control were constructed by Genechem Co., Ltd. (Shanghai, China). The procedures for intracardiac injection of adenovirus (1.0×10E9 PFU/mouse) were the same as described previously.[25]

Echocardiographic measurements

The procedures of echocardiographic measurements were the same as described previously.[20] Transthoracic

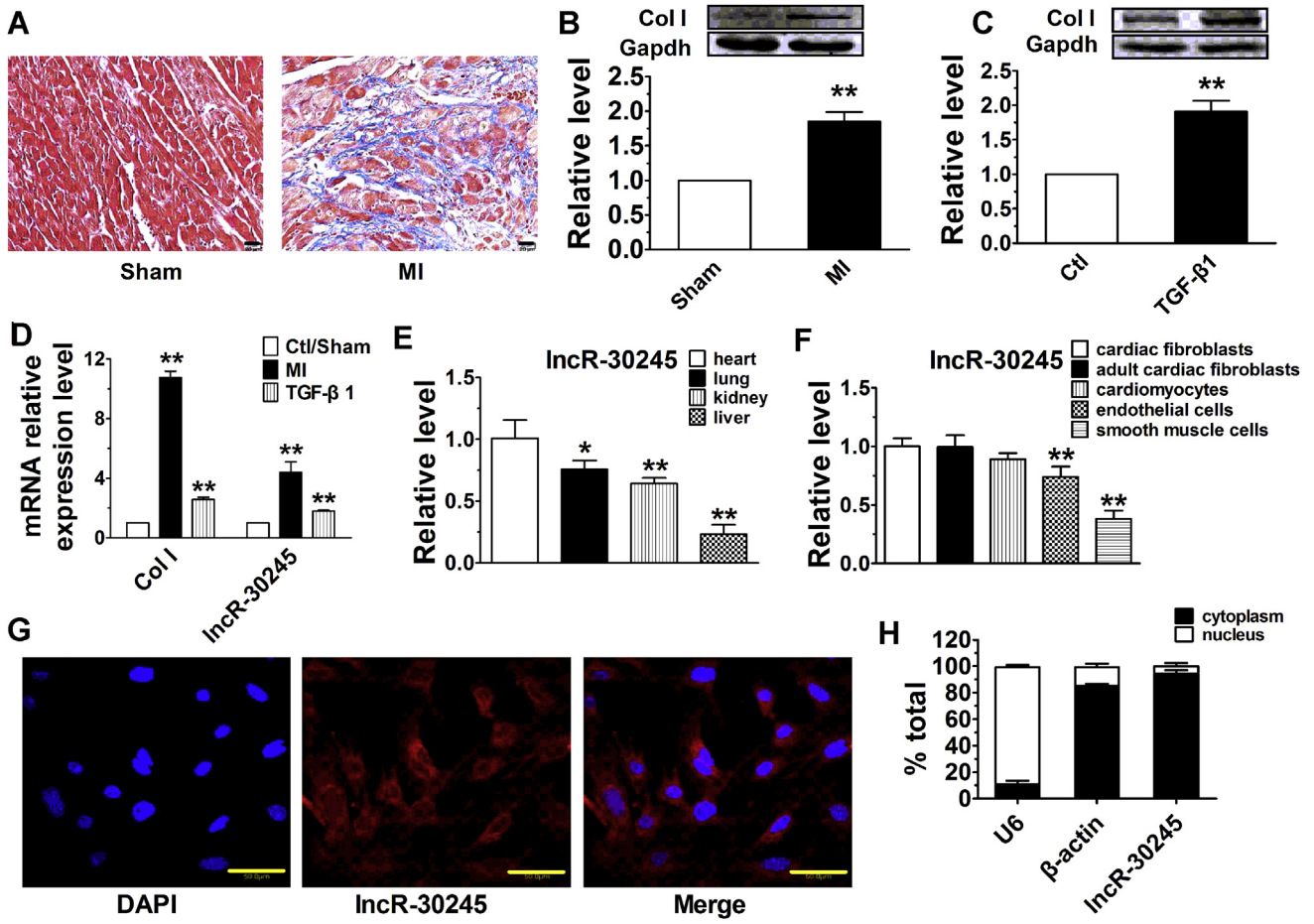

Figure 1. Upregulation of lncR-30245 in myocardial infarction (MI) hearts and transforming growth factor (TGF)-β1–treated cardiac fibroblasts (CFs). (**A**) Masson's trichrome staining of ischemic border zone of mouse heart 4 weeks after MI. **Scale bars**, 20 μm. (**B**) Elevation of collagen I protein level in MI group as determined by Western blot. $^{**}P < 0.01$ vs sham; n = 5 mice in each group. (**C**) Elevation of collagen I protein level in cultured CFs treated with TGF-β1 determined by Western blot. $^{**}P < 0.01$ vs control (Ctl); n = 5 batches of cells in each group. (**D**) Collagen I and lncR-30245 expression in MI hearts and CFs treated with TGF-β1, determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). $^{**}P < 0.01$ vs Ctl/sham; n = 5 batches of cells/mice in each group. (**E**) The expression of lncR-30245 in different tissues determined by qRT-PCR. $^{*}P < 0.05$ vs heart, $^{**}P < 0.01$ vs heart; n = 8 mice in each group. (**F**) The expression of lncR-30245 in different cell types. $^{**}P < 0.01$ vs CFs, n = 5 batches of cells. (**G**) Representative fluorescent in situ hybridization images showing the expression abundance and cellular localization of lncR-30245 in CFs. **Scale bars**, 50 μm. (**H**) The levels of lncR-30245, nuclear control transcript (U6), and cytoplasmic control transcript (β-actin) were assessed by qRT-PCR in nuclear and cytoplasmic fractions of CFs. n = 5 batches of cells in each group.

echocardiography was performed to monitor changes of the left ventricular function using the ultrasound machine Vevo 2100 high-resolution imaging system (VisualSonics, Inc, Toronto, ON) equipped with a 30-MHz phased-array transducer with the M-mode recordings.

Masson's trichrome staining

The hearts of mice from each group were quickly dissected, immersed in 4% paraformaldehyde for 24 hours, and stained with Masson's trichrome as previously described.[26] The extent of collagen deposition was calculated with image analysis software (Image-Pro Plus v4.0; Meida Cybernetics, Bethesda, MD).

Data analysis

Data are expressed as mean ± standard deviation and were analyzed by GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA). Two-group comparisons were performed by Student *t* test. Multi-group comparisons were carried out using 1-way analysis of variance followed by Dunnett's multiple comparison test. Results were considered significant if $P < 0.05$.

Results

LncR-30245 expression is upregulated in cardiac fibrosis

Masson's trichrome staining showed considerable collagen deposition in MI hearts relative to non-MI hearts (sham group) (Fig. 1A). In parallel, the increased protein level of collagen I as the fibrotic substrates was confirmed in the border zone of MI hearts and CFs treated with transforming growth factor (TGF)-β1 (Fig. 1, B and C). Collagen I expression at mRNA level was also increased in MI hearts and TGF-β1–treated CFs (Fig. 1D). Meanwhile, consistent with the microarray data reported in our previous study,[8] the

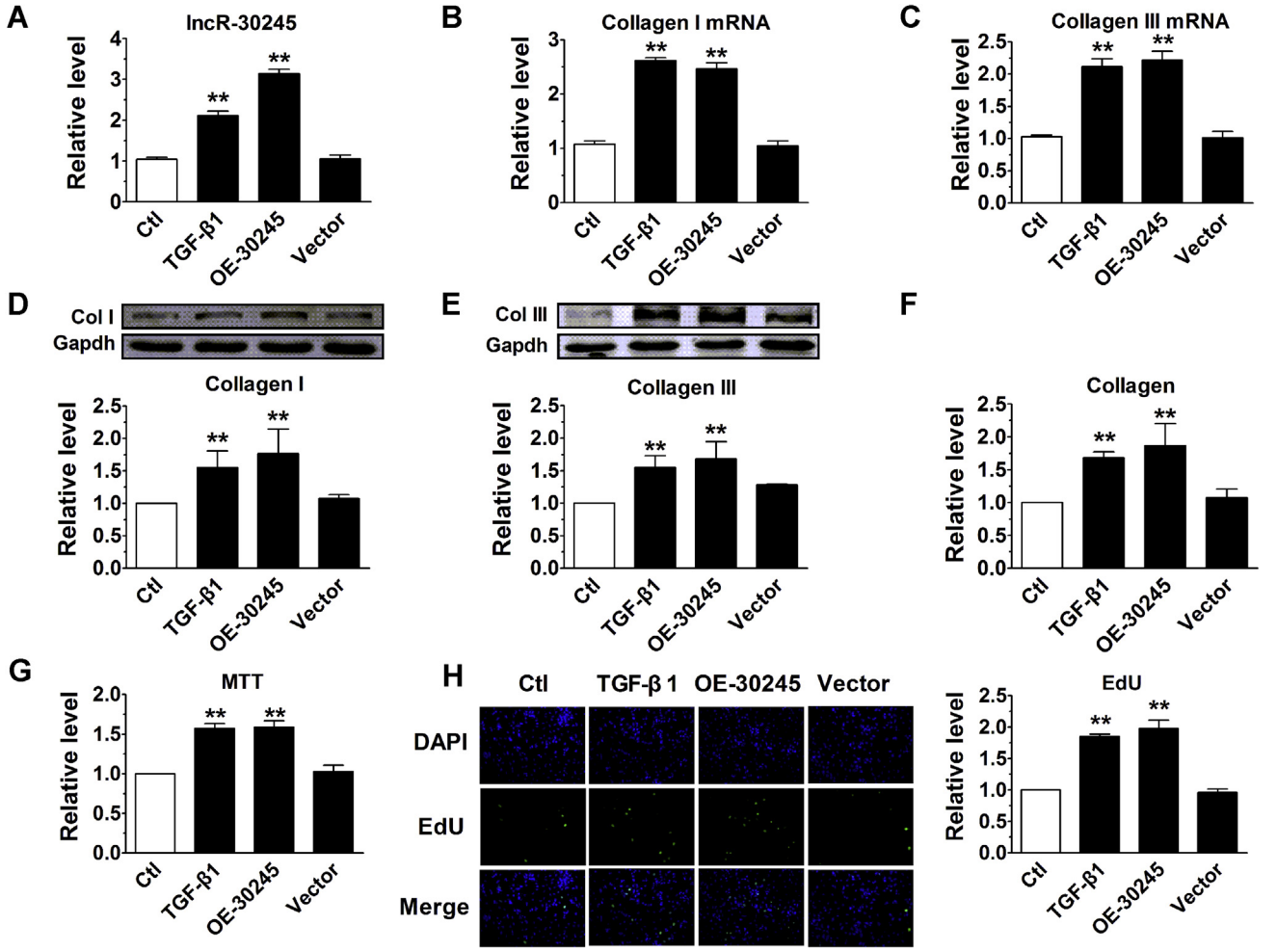

Figure 2. LncR-30245 overexpression promotes collagen production and CF proliferation. (**A-E**) LncR-30245 overexpression (OE-30245) upregulated the expression of collagen I and III at both mRNA and protein levels in CFs. (**F**) LncR-30245 overexpression increased total collagen contents in CFs as determined by collagen kit. (**G**) LncR-30245 overexpression increased viability of CFs as determined by MTT assay. (**H**) LncR-30245 overexpression promoted proliferation of CFs as determined by EdU staining. Cell proliferation was quantified as a percentage of EdU-positive cells over the total number of cells with DAPI-positive staining (magnification × 200). $^{**}P < 0.01$ vs Ctl; n = 5 batches of cells in each group. TGF, transforming growth factor.

quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) results verified that the expression level of lncR-30245 was significantly increased in the border zone of MI hearts and TGF-β1—treated CFs (Fig. 1D). LncR-30245 was ubiquitously expressed in the heart, lung, kidney, and liver, and was most abundant in the heart (Fig. 1E). At the cellular level, the expression of lncR-30245 was higher in neonatal and adult mouse CFs than in mouse cardiomyocytes, endothelial cells, and smooth muscle cells (Fig. 1F).

Fluorescent in situ hybridization assay showed that lncR-30245 was predominantly localized in the cytoplasm of CFs (Fig. 1G). To further confirm localization of lncR-30245 in CFs, we fractionated CFs into nuclear and cytoplasmic fractions. The results revealed that lncR-30245 was mainly distributed in the cytoplasm rather than the nucleus (Fig. 1H).

LncR-30245 overexpression increases collagen production and CF proliferation

Cardiac fibrosis is characterized by overproduction of collagen and enhanced cell proliferation. Our results demonstrated that lncR-30245 expression was significantly upregulated by TGF-β1 or lncR-30245 overexpression plasmid (OE-30245) (Fig. 2A). LncR-30245 overexpression significantly upregulated the expression of collagen I and III at both mRNA and protein levels (Fig. 2, B-E). Collagen content in CFs was also elevated by lncR-30245 (Fig. 2F). In addition, the results from both MTT and EdU assays showed that lncR-30245 overexpression significantly induced CF proliferation (Fig. 2, G and H). These findings indicated that lncR-30245 is a profibrotic molecule in CFs.

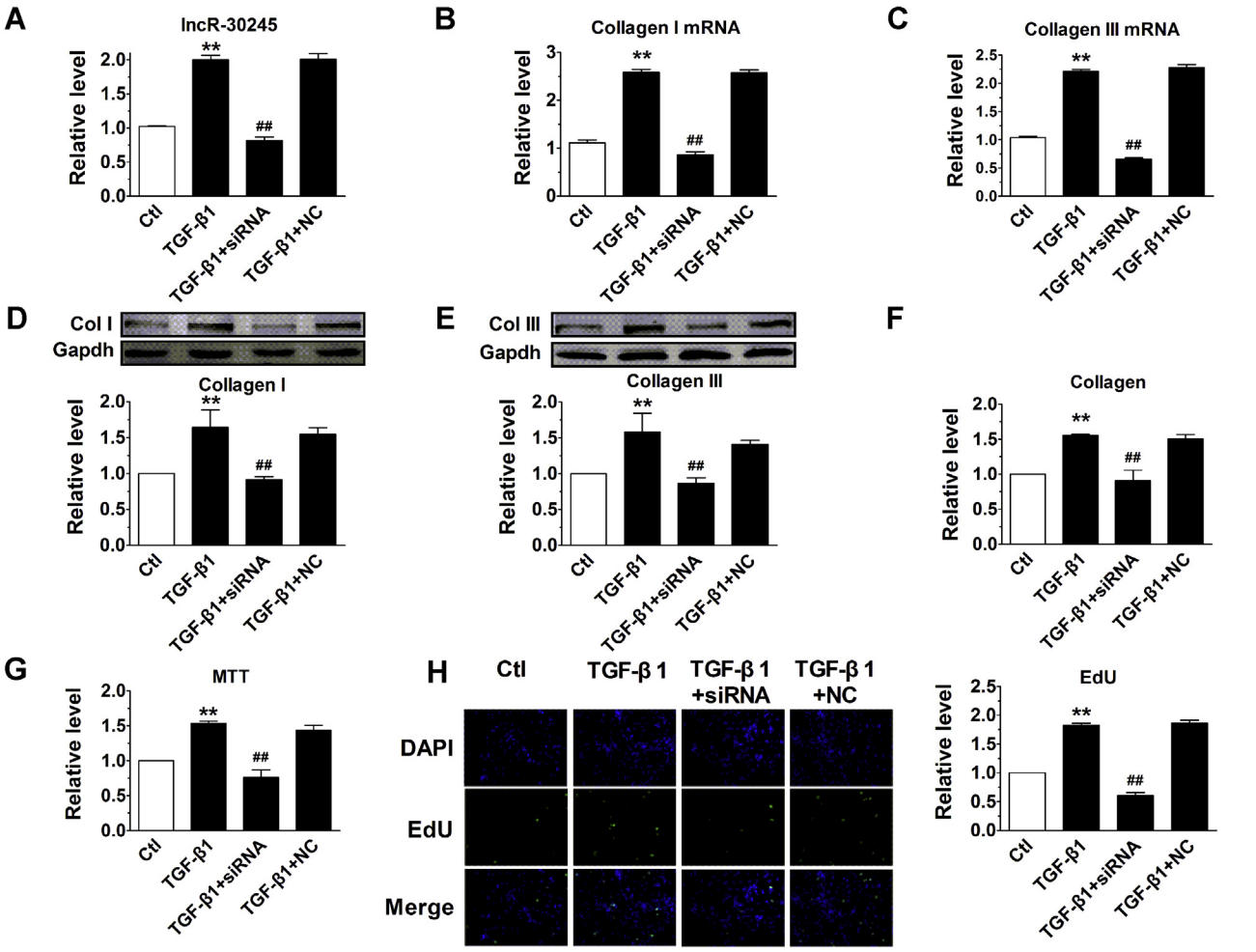

Figure 3. LncR-30245 knockdown attenuates TGF-β1–induced collagen production and CF proliferation. (**A**) Real-time PCR results showing the efficacy of lncR-30245 silencing by its specific siRNA. (**B, C**) LncR-30245 knockdown reduced TGF-β1–induced collagen I and III mRNA expression. (**D, E**) LncR-30245 knockdown decreased TGF-β1–induced collagen I and III protein expression. (**F**) LncR-30245 knockdown diminished TGF-β1–induced collagen synthesis determined by collagen kit. (**G**) LncR-30245 knockdown suppressed TGF-β1–induced CF proliferation as revealed by MTT assay. (**H**) LncR-30245 knockdown inhibited TGF-β1–induced CF proliferation as revealed by EdU staining. Cell proliferation was quantified as a percentage of EdU-positive cells to the total number of cells with DAPI-positive staining (magnification × 200). $^{**}P < 0.01$ vs Ctl; $^{\#\#}P < 0.01$ vs TGF-β1; n = 5 batches of cells in each group. NC, negative control; TGF, transforming growth factor.

LncR-30245 knockdown inhibits TGF-β1–induced collagen production and CF proliferation

Next, we examined the effect of lncR-30245 silencing by its specific siRNA in CFs. As shown in Figure 3A, TGF-β1 substantially increased the expression of lncR-30245, and such an abnormal upregulation was dramatically repressed by lncR-30245 siRNA in CFs, but not by the negative control siRNA. LncR-30245 silencing significantly attenuated TGF-β1–induced expression of collagen I and III at both mRNA (Fig. 3, B and C) and protein (Fig. 3, D and E) levels. In addition, knockdown of lncR-30245 also abolished the elevation of collagen content induced by TGF-β1 (Fig. 3F). Notably, lncR-30245 silencing resulted in a significant reduction in TGF-β1–induced CF proliferation (Fig. 3, G and H).

Regulation of lncR-30245 on PPAR-γ and CTGF expression

CTGF is known to be an important factor in CF activation and excessive ECM deposition.[23] We found that lncR-30245 overexpression significantly increased CTGF expression at mRNA and protein levels (Fig. 4, A and B). Conversely, lncR-30245 siRNA dramatically inhibited TGF-β1–induced CTGF expression at both mRNA and protein levels (Fig. 4, C and D).

PPAR-γ functions as an upstream negative regulator of CTGF.[23] We observed that lncR-30245 overexpression significantly reduced PPAR-γ expression at mRNA and protein levels (Fig. 4, E and F). Conversely, lncR-30245 siRNA restored TGF-β1–induced suppression of PPAR-γ expression (Fig. 4, G and H). These data indicated that lncR-30245

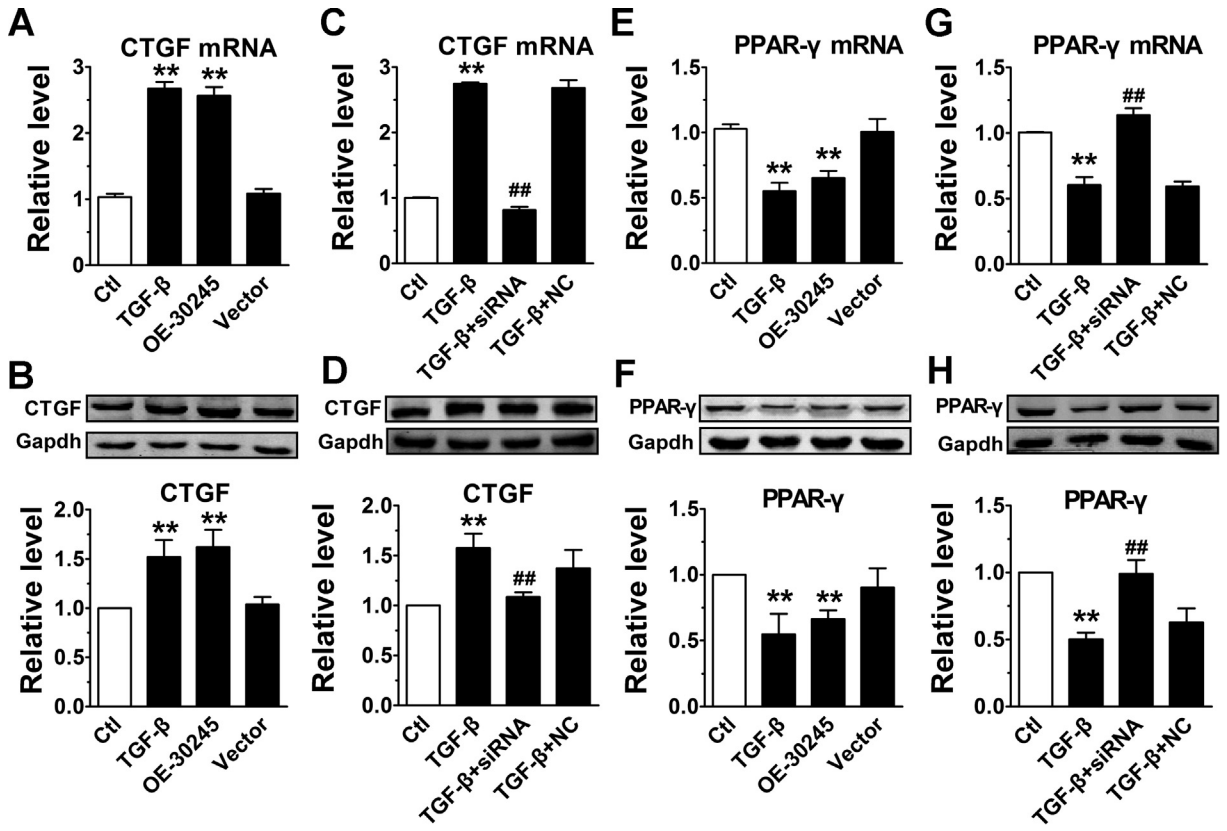

Figure 4. LncR-30245 overexpression promotes CTGF and inhibits PPAR-γ expression. (**A, B**) LncR-30245 overexpression increased CTGF mRNA by qRT-PCR and protein expression by Western blot in CFs. (**C, D**) LncR-30245 knockdown attenuated TGF-β1–induced CTGF mRNA and protein expression in CFs. (**E, F**) LncR-30245 overexpression reduced PPAR-γ mRNA and protein expression in CFs. (**G, H**) LncR-30245 knockdown abolished TGF-β1–induced decrease of PPAR-γ mRNA and protein expression in CFs. $^{**}P < 0.01$ vs Ctl; $^{\#\#}P < 0.01$ vs TGF-β1; n = 5 batches of cells in each group. CTGF, connective tissue growth factor; PPAR, peroxisome proliferator-activated receptor; TGF, transforming growth factor.

promotes cardiac fibrosis via at least inhibiting PPAR-γ that in turn stimulates CTGF.

LncR-30245 stimulates CTGF expression and collagen synthesis via inhibiting PPAR-γ

It has been demonstrated that activation of PPAR-γ suppresses the expression of CTGF and exerts the protective effect on cardiac injury.[23] We found that PPAR-γ agonist rosiglitazone (5 μM) abolished the lncR-30245–induced increases in mRNA and protein expression of CTGF (Fig. 5, A and B), collagen I (Fig. 5, C and D), and collagen III (Fig. 5, E and F) in CFs. Conversely, T0070907 (10 nM), an antagonist of PPAR-γ, almost completely abrogated the inhibitory effects of lncR-30245 siRNA on the TGF-β1–induced CTGF expression (Fig. 5, G and H), collagen I (Fig. 5, I and J), and collagen III (Fig. 5, K and L) in CFs.

LncR-30245 knockdown improves cardiac function and alleviates cardiac fibrosis after MI in mice

To further verify the beneficial action of lncR-30245 knockdown on cardiac fibrosis, we investigated the effects of lncR-30245 knockdown using adenovirus carrying lncR-30245 siRNA (Adv-siRNA) on MI mice. Administration of Adv-siRNA significantly suppressed the upregulation of lncR-30245 in MI hearts (Fig. 6A). LncR-30245 siRNA significantly increased ejection fraction and fractional shortening in MI mice. By comparison, the adenovirus carrying scrambled siRNA produced no effects on cardiac function (Fig. 6, B-D and Supplemental Table S1). Moreover, Masson's trichrome staining showed less fibrotic area was observed in Adv-siRNA–treated MI hearts (Fig. 6, E and F). Compared with the sham group, the mRNA and protein expression of collagen I and III were increased in MI mice, which were significantly reduced by lncR-30245 siRNA (Fig. 6, G-J). In addition, lncR-30245 siRNA decreased CTGF and restored PPAR-γ expression in MI mice (Fig. 6, K-N). These data indicated that suppression of lncR-30245 alleviates MI-induced cardiac fibrosis and preserves cardiac function via mediating PPAR-γ/CTGF pathway.

Discussion

The present study generated 3 important findings. First, lncR-30245 overexpression promoted CF proliferation and collagen production, whereas lncR-30245 knockdown inhibited TGF-β1–induced CF proliferation and collagen production. Second, PPAR-γ agonist rosiglitazone inhibited lncR-30245–induced fibrotic phenotype, whereas PPAR-γ antagonist T0070907 abrogated the antifibrotic effect of lncR-30245 siRNA. Third, lncR-30245 silencing alleviates cardiac fibrosis and improves cardiac function after MI. These

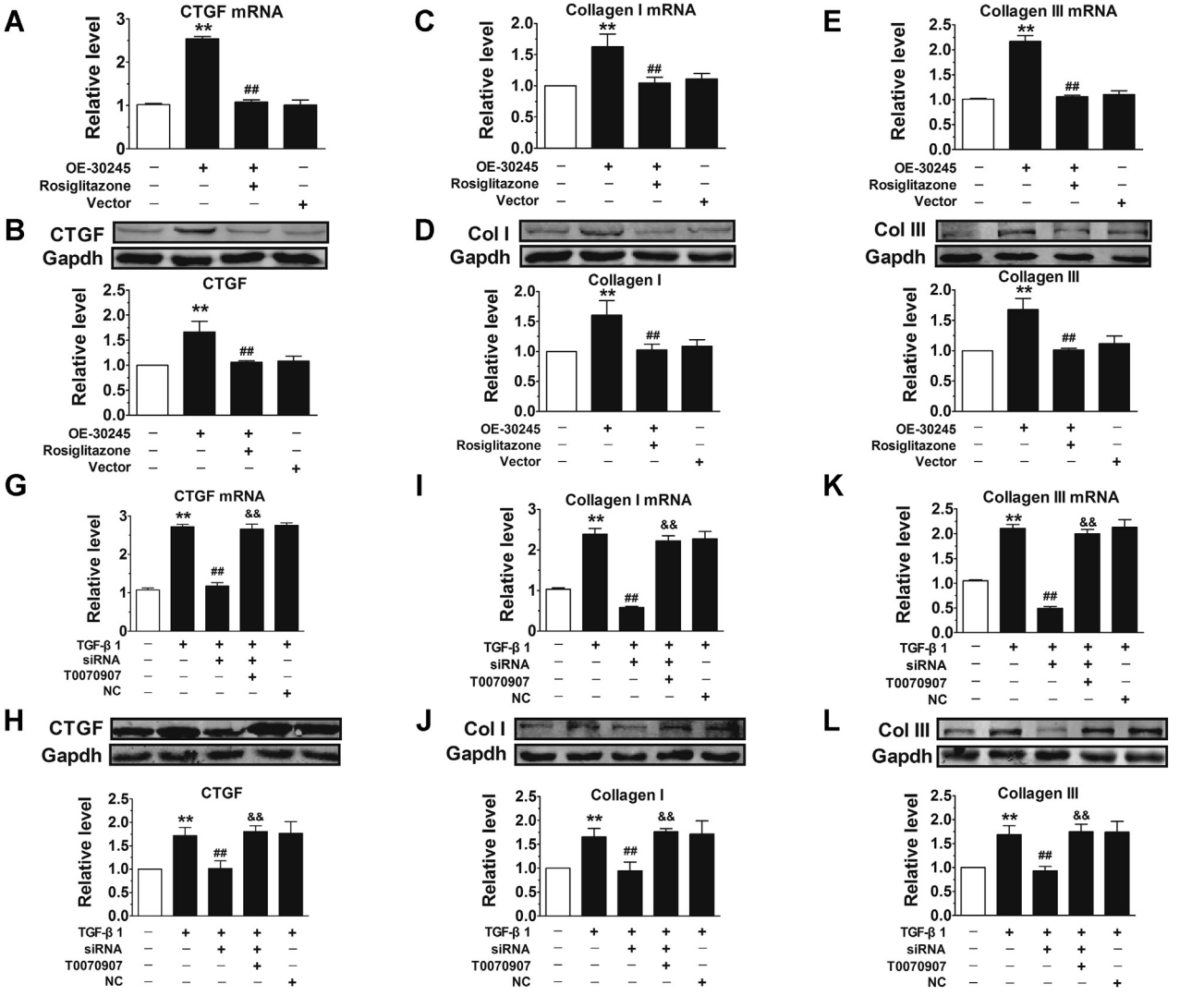

Figure 5. LncR-30245 increases CTGF and collagen synthesis by inhibiting PPAR-γ in CFs. (**A, B**) PPAR-γ agonist rosiglitazone (5 μM) inhibited lncR-30245—induced CTGF mRNA by qRT-PCR and protein expression by Western blot in CFs. $^{**}P < 0.01$ vs Ctl; $^{\#\#}P < 0.01$ vs OE-30245; n = 5 batches of cells in each group. (**C-F**) Rosiglitazone inhibited lncR-30245—induced collagen I and III at both mRNA and protein levels in CFs. $^{**}P < 0.01$ vs Ctl; $^{\#\#}P < 0.01$ vs OE-30245; n = 5 batches of cells in each group. (**G, H**) PPAR-γ antagonist T0070907 (10 nM) reversed the inhibitory effects of lncR-30245 siRNA on TGF-β1—induced CTGF mRNA and protein expression in CFs. $^{**}P < 0.01$ vs Ctl; $^{\#\#}P < 0.01$ vs TGF- β1; $^{\&\&}P < 0.01$ vs TGF-β1 + siRNA; n = 5 batches of cells in each group. (**I-L**) T0070907 abolished the inhibitory effects of lncR-30245 siRNA on TGF-β1—induced collagen I and III at both mRNA and protein expression levels in CFs. $^{**}P < 0.01$ vs Ctl; $^{\#\#}P < 0.01$ vs TGF-β1; $^{\&\&}P < 0.01$ vs TGF-β1 + siRNA; n = 5 batches of cells in each group. CTGF, connective tissue growth factor; siRNA, small interfering RNA; TGF, transforming growth factor.

data suggest that lncR-30245 acts as an important regulator of cardiac fibrosis via mediating PPAR-γ/CTGF pathway.

LncRNAs have recently been reported to participate in a number of pathophysiological processes of cardiovascular diseases, including cardiac fibrosis.[27-29] Wisp2 super-enhancer—associated RNA (Wisper), a CF-enriched lncRNA, regulates CF proliferation, migration, and survival, and silencing of Wisper attenuates MI-induced fibrosis and cardiac dysfunctions in vivo.[19] Knockdown of lncRNA H19 enhances the antifibrotic effects of miR-455 and attenuates CTGF expression and fibrosis-associated protein synthesis.[10] LncRNA Meg3 (maternally expressed gene 3) silencing hinders the induction of matrix metalloproteinase-2 through inhibiting P53 activity and decreases myocardial fibrosis and ameliorated diastolic dysfunction in the murine hypertrophic heart.[30] Our previous results showed that knockdown of lncRNA MI-associated transcript abrogates cardiac fibrogenesis by directly increasing the miR-24 level, which targets Furin, a component of the TGF-β1 signalling pathway.[20] In this study, we identified lncR-30245 as a new profibrotic lncRNA, and the finding could advance our understanding of the cellular functionalities of lncRNAs. Moreover, our findings showed that the profibrotic actions elicited by TGF-β1 could be reversed by silencing lncR-30245. We demonstrated that lncR-30245 silencing alleviates cardiac fibrosis and improves cardiac function after MI. These data suggest that lncR-30245 is a possible molecular target for

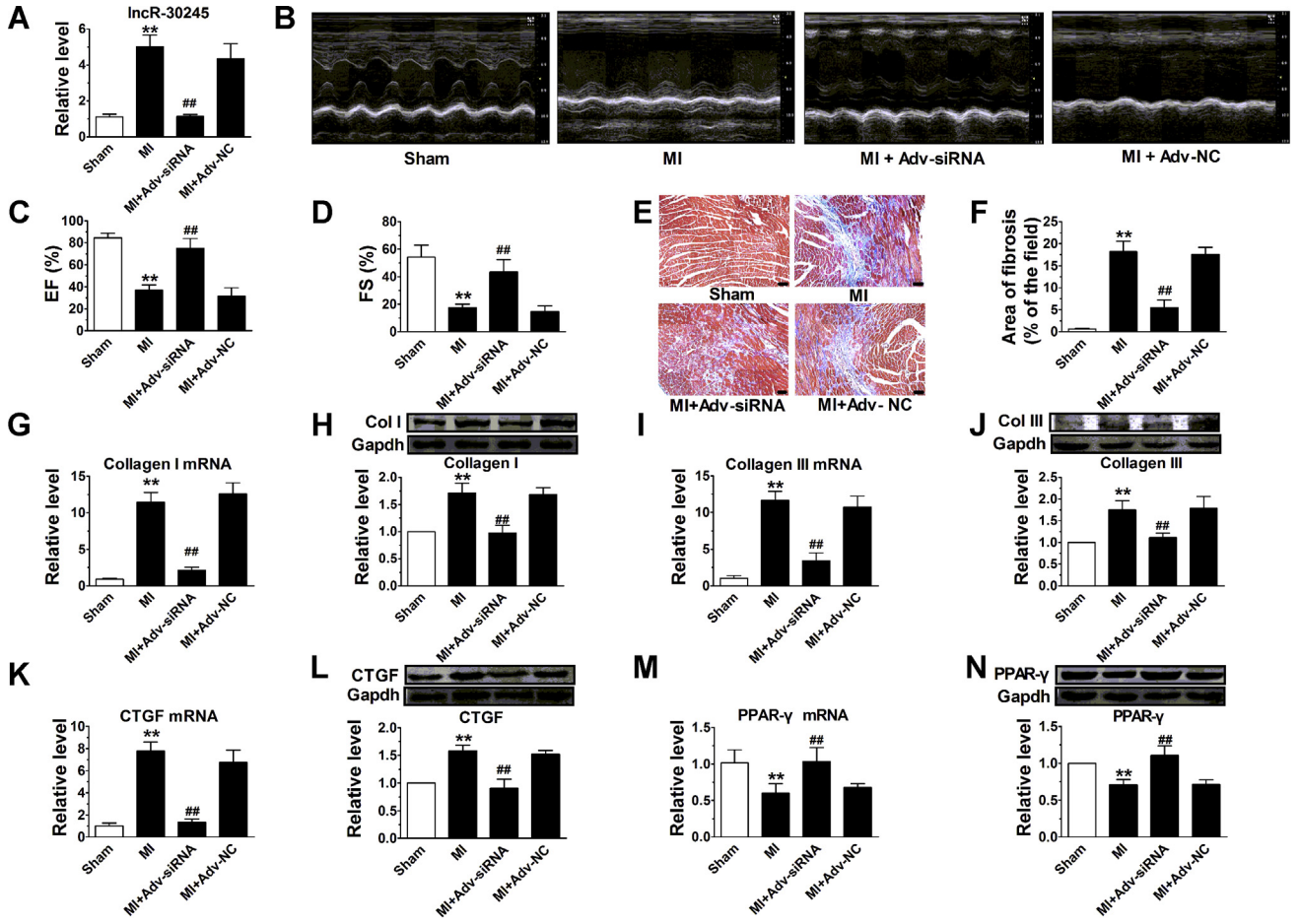

Figure 6. LncR-30245 knockdown improves cardiac function and inhibits cardiac fibrosis in MI mice. (**A**) The expression level of lncR-30245 after lncR-30245 siRNA administration in MI mice determined by qRT-PCR. n = 8 mice in each group. (**B**) Representative echocardiographic images (M-mode) in mice with different treatments. n = 12 mice in each group. (**C, D**) LncR-30245 siRNA administration increased the reduced ejection fraction and fractional shortening in MI mice. n = 12 mice in each group. (**E**) Representative images of Masson's trichrome staining of heart tissue sections, with interstitial fibrosis in blue. Scale bars, 50 μm. n = 8 mice in each group. (**F**) Ratio of collagen surface area to the myocardial surface area expressed as percentage of fibrosis. n = 8 mice in each group. (**G-L**) LncR-30245 siRNA administration attenuated MI-induced collagen I, collagen III, and CTGF mRNA by qRT-PCR and protein expression by Western blot in MI mice. n = 8 mice in each group. (**M, N**) LncR-30245 siRNA administration restored MI-reduced PPAR-γ mRNA and protein expression in MI mice. n = 8 mice in each group. $^{**}P < 0.01$ vs sham; $^{\#\#}P < 0.01$ vs MI. EF, ejection fraction; FS, fractional shortening; MI, myocardial infarction; NC, negative control; siRNA, small interfering RNA.

the interference of the pathophysiological processes associated with cardiac fibrosis.

Our results showed that lncR-30245 silencing alleviates cardiac fibrosis and improves cardiac function after MI through inhibiting CTGF expression. Because lncR-30245 is also expressed in other cell types, including cardiomyocytes, endothelial cells, and smooth muscle cells, lncR-30245 may play a regulatory role in the process of cardiac hypertrophy, ischemic heart disease, and heart failure. In the present study, however, we cannot rule out the beneficial effects of lncR-30245 inhibition on infarcted hearts from other types of cells in the heart.

ECM synthesis is controlled by a multitude of factors. CTGF is a potent regulator of collagenous protein synthesis in CFs.[31] Increased CTGF expression has been shown in human ischemic heart tissue and found to correlate with genes involved in ECM remodelling.[32] CTGF is upregulated in the cardiomyocytes after serum response factor deletion in the adult mouse heart, leading to dilated cardiomyopathies and cardiac fibrosis.[33] Our results show that TGF-β1 upregulated the expression of both lncR-30245 and CTGF in CFs, and overexpression of lncR-30245 also increased the expression of CTGF, whereas knockdown of lncR-30245 attenuated TGF-β1–induced increase of CTGF expression. These findings clearly indicated the participation of CTGF in lncR-30245–regulated cardiac fibrosis.

PPAR-γ plays an important role in ECM deposition as an upstream regulator of CTGF.[34] Activation of PPAR-γ has been reported to repress expression of CTGF and exerts a protective effect on cardiac injury.[23] Loss of function of PPAR-γ develops severe cardiac fibrosis with increased expression of profibrotic genes collagen I and III.[22] Pharmacological activation and overexpression of PPAR-γ block TGF-β–induced collagen accumulation in CFs.[35] PPAR-γ activator reduces ECM deposition and cardiac fibrosis, whereas PPAR-γ antagonist T0070907 reverses these

changes.[36] We demonstrated that PPAR-γ agonist rosiglitazone abolished the increased mRNA and protein expression of CTGF and collagen induced by lncR-30245 in CFs, whereas PPAR-γ antagonist T0070907 abrogated the inhibitory effects of lncR-30245 siRNA on TGF-β1−induced CTGF expression and collagen production. These data suggest that lncR-30245 promoted cardiac fibrogenesis through suppressing PPAR-γ, leading to enhanced CTGF expression.

Study limitations

It should be noted that the present study did not elucidate how lncR-30245 regulates PPAR-γ. It is possible that it acts on PPAR-γ indirectly, because PPAR-γ is a nuclear receptor, whereas lncR-30245 was found mainly in the cytoplasm. LncR-30245 is a cytoplasmic lncRNA and located within the third intron of Man2al on chromosome 17 (strand: +, chr17:64,626,521-64,628,713). There are 2 adjacent protein-coding genes, Fer and Pja2, located upstream of lncR-30245. Overexpression or knockdown of lncR-30245 in CFs did not influence the expression of its host gene Man2a1 and adjacent coding genes Fer and Pja2 (Supplemental Fig. S1). It can be speculated that *cis*-regulation is not involved in lncR-30245 biological function.

Conclusions

Our study demonstrates that lncR-30245 is a critical profibrotic lncRNA that promotes fibroblast proliferation, collagen synthesis, and myocardial fibrosis, and the mechanism involves the suppression of PPAR-γ and consequent upregulation of CTGF. Knockdown of lncR-30245 represents a novel strategy for the intervention of cardiac fibrosis.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

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