

MicroRNA Let-7i Negatively Regulates Cardiac Inflammation and Fibrosis

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Abstract—Angiotensin II stimulates fibroblast proliferation and substantially alters gene expression patterns leading to cardiac remodeling, but the mechanisms for such differences are unknown. MicroRNAs are a novel mechanism for gene expression regulation. Herein, we tested the miRNA and mRNA expression patterns in mouse heart using microarray assay and investigated their role in angiotensin II-induced cardiac remodeling. We found that let-7i was dynamically downregulated in angiotensin II-infused heart at day 3 and 7 and had the most targets that were mainly associated with cardiac inflammation and fibrosis. Overexpression or knockdown of let-7i in cultured cardiac fibroblasts demonstrated that let-7i played an inhibitory effect on the expression of its targets interleukin-6 and collagens. Furthermore, delivery of let-7i to mouse significantly inhibited angiotensin II-induced cardiac inflammation and fibrosis in a dose-dependent manner. Conversely, knockdown of let-7i aggravated this effect. Together, our results clearly demonstrate that let-7i acts as a novel negative regulator of angiotensin II-induced cardiac inflammation and fibrosis by suppressing the expression of interleukin-6 and multiple collagens in the heart and may represent a new potential therapeutic target for treating hypertensive cardiac fibrosis. (*Hypertension*. 2015;66:776–785. DOI: 10.1161/HYPERTENSIONAHA.115.05548.)

• [Online Data Supplement](#)

Key Words: angiotensin II ■ collagen ■ inflammation ■ interleukin-6 ■ microRNAs

Cardiac fibrosis is an important component of heart diseases, which is characterized by excessive accumulation of extracellular matrix in the cardiac interstitium and perivascular space. Among the extracellular matrix proteins, collagens constitute $\leq 85\%$. Cardiac fibroblasts are the major source of collagens in myocardium. In response to hypertrophic stimuli, fibroblasts undergo proliferation and secrete excessive extracellular matrix proteins, triggering cardiac fibrosis.^{1,2} Angiotensin II (Ang II), a main effector hormone of the renin-angiotensin system, via its type 1 receptor stimulates fibroblasts proliferation and production of various cytokines and synthesis of collagens.^{1–3} Previous investigations have shown that Ang II exerts these effects at least partially through activation of nuclear factor- κ B, transforming growth factor- β 1 and oxidative stress signaling pathways.^{4,5} However, the underlying mechanisms are not yet fully understood.

MicroRNAs (miRNAs) are a class of small noncoding RNAs that regulate gene expression by mRNA degradation or translational repression.⁶ Recently, miRNAs have emerged as key

regulators of cardiac development and diseases. Several miRNAs, such as miRNA-1/133, miRNA-30, miRNA-29, and miRNA-21, had been implicated in cardiac remodeling.^{7–10} Furthermore, unique miRNA expression profiles had been detected in human cardiomyopathy and pressure overloaded cardiac hypertrophy, and many miRNAs were dysregulated in the heart, indicating the involvement of miRNAs in cardiac diseases.^{7,11–17}

It is well established that Ang II-mediated cardiac remodeling is a multigene disorder that involves a global change in the gene expression profile. miRNA analysis provides a novel mechanism for this alteration. In this study, we used a parallel microarray-based approach to investigate whether a change in the miRNA expression profile plays an important role in regulating the gene expression pattern in mouse heart after Ang II infusion. Our results indicated that let-7i (let-7i-5p) was significantly downregulated in the heart at day 3 and 7 post Ang II infusion. Furthermore, increased let-7i was sufficient to attenuate Ang II-induced cardiac inflammation and

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fibrosis through directly reducing the expression of interleukin-6 (IL-6) and collagens, and knockdown of let-7i enhanced these effects. Our results clearly demonstrate that let-7i acts as a key regulator of Ang II-induced cardiac inflammation and fibrosis.

Methods

Detailed description of Methods is available in the online-only Data Supplement.

Results

MiRNA Profiles During Ang II-Induced Cardiac Remodeling

To investigate the potential involvement of miRNAs in Ang II-induced cardiac remodeling, C57BL/6 wild-type mouse were infused with Ang II for ≤ 7 days. Our results showed that systolic blood pressure was significantly elevated with increased times (Figure S1A in the online-only Data Supplement). Moreover, Ang II infusion for 3 days only markedly increased the accumulation of proinflammatory cells and Mac-2-positive macrophages, collagen deposition, but had no effect on the ratio of heart weight/body weight, cardiomyocyte cross-sectional area, and ejection fraction compared with saline group (Figure S1B–S1F). However, Ang II infusion for 7 days significantly increased the degree of cardiac remodeling, including the heart weight/body weight ratio, inflammation, fibrosis, hypertrophy, and cardiac contractile function (Figure S1B–S1F).

To identify miRNAs that are differentially expressed during Ang II-induced cardiac remodeling, we performed miRNA microarray on RNA from the hearts at day 3 and 7 after Ang II or saline infusion. The miRNA microarray data are available at the Gene Expression Omnibus (GEO) website under accession number GSE69559: <http://www.ncbi.nlm.nih.gov/geo/>. A hierarchical clustering of dysregulated miRNAs was shown for each time point (Figure 1A). Microarray data revealed that as early as 3 days post Ang II infusion, with no significant change in cardiac structural remodeling, 5 miRNAs were significantly downregulated, whereas 9 miRNAs were upregulated. After 7 days, cardiac remodeling was obvious. Nineteen miRNAs were markedly downregulated, whereas 3 miRNAs were upregulated.

To confirm the microarray data for the observed miRNAs, we performed real-time quantitative polymerase chain reaction (qPCR) analysis on the RNAs isolated from the same hearts at day 3 and 7 of Ang II or saline infusion. As shown in Figure 1B and 1C, the expression patterns of 9 selected miRNAs, including miR-1900, miR-208b-3p, miR-146a-5p, miR-378-3p, miR-191-5p, miR-1839-3p, miR-16-5p, miR-133a-5p and miR-30b-5p, were in agreement with the results from microarray (Figure 1A).

Integrated Analysis Revealed the Important Role of Let-7i in Ang II-Induced Cardiac Fibrosis

To determine the direct targets of miRNAs in the heart, we next performed mRNA microarray to detect the gene expression profiles in Ang II-infused hearts at day 3 and 7. A hierarchical clustering analysis showed a clear separation between

the Ang II and saline infusion groups (Figure S2A). Moreover, we found that total 1912 and 1835 genes were differentially expressed at day 3 and 7 after Ang II infusion, respectively. The gene expression data are available at the GEO website under accession number GSE59437: <http://www.ncbi.nlm.nih.gov/geo/>. Linear correlation analysis further showed a significant correlation between qPCR and microarray results (Figure S2B). We next performed gene ontology analysis to identify the gene ontology significantly regulated by Ang II according to both *P* value and false discovery rate < 0.05 . After Ang II infusion, the high enrichment gene ontology mainly included the regulation of cell proliferation, inflammatory response, collagen fibril organization, regulation of apoptosis, and regulation of cardiac muscle hypertrophy (Figure S2C). Thus, these data suggest that Ang II infusion within 7 days predominantly regulate genes related to cell proliferation, inflammatory response, collagen fibril organization, and apoptosis.

To predict putative miRNA targets and association networks between miRNAs and mRNAs, we performed paired expression profiles of miRNAs and mRNAs to identify functional miRNA–targets relationships with a high precision (Figure 2) and found that let-7i in the downregulated miRNAs had the most target mRNAs of 15 (degree 15) at day 3 and 19 (degree 19) at day 7, respectively (Figure 2). Notably, let-7i regulated some important targets, including IL-6, collagens, transforming growth factor- β receptor 1, insulin-like growth factor 1, and caspase-3, which are known to regulate inflammation, fibrosis, hypertrophy, and apoptosis, suggesting that let-7i may be of great importance to the cardiac remodeling.

Expression of Let-7i and Its Targets in the Heart and Cultured Cardiac Cells

To confirm the expression patterns of let-7i and its target genes in the heart, we performed qPCR analysis with the same RNA from the hearts. Consistent with the results from the miRNA and mRNA microarray, we found that let-7i expression was significantly downregulated, whereas the expression levels of let-7i targets, including IL-6, collagen type I alpha 2 (Col1a2), collagen type III alpha 1 (Col3a1), collagen type IV alpha 1 (Col4a1), and collagen type V alpha 2 (Col5a2), were significantly upregulated in Ang II-infused hearts at day 3 and 7 compared with saline group (Figure 3A and 3B). However, no significant difference in the expression of other targets (including transforming growth factor- β receptor 1, caspase-3, and insulin-like growth factor 1) was observed between 2 groups at day 3 and 7 (date not shown). Thus, these results suggest that let-7i selectively regulates inflammation- and fibrosis-related genes in the heart after Ang II infusion.

We further analyzed the other members of let-7 family except let-7i in our microarray data and found that let-7g was also markedly downregulated in Ang II-treated heart at 7 days compared with saline (Figure S3A), and these results were confirmed by qPCR analysis (Figure S3B). To identify which cardiac-resident cells, including cardiac fibroblasts and cardiomyocytes, mainly express let-7i and its targets, qPCR analysis were performed. We found that let-7i mainly expressed in cultured neonatal rat fibroblasts under basal condition (Figure 3C). After Ang II stimulation, let-7i expression was

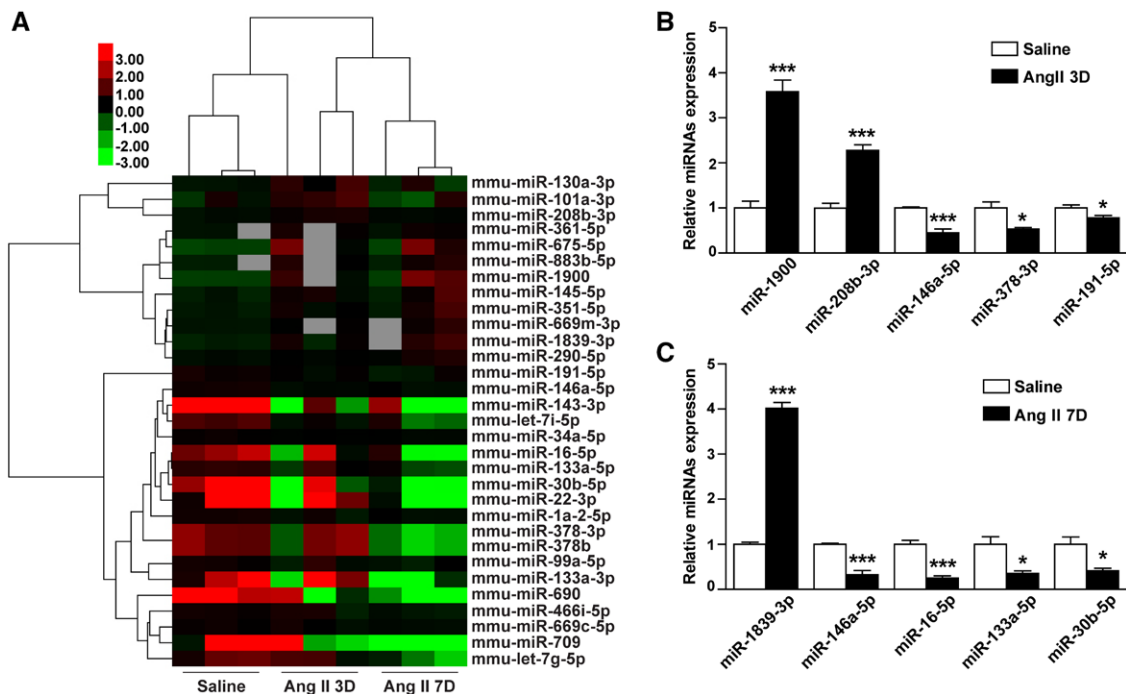


Figure 1. MicroRNA (miRNA) microarray and validation. C57BL/6 wild-type mice were subjected to continuous angiotensin II (Ang II; 1500 ng/kg per min) or saline infusion for 3 and 7 days. **A**, The cluster of miRNA profiles in saline and Ang II infusion groups. Both downregulated and upregulated miRNAs were identified in the heart. The expression of miRNAs was hierarchically clustered on the y axis, and samples were hierarchically clustered on the x axis. The legend on the right indicates the miRNAs. The relative expression of miRNAs was depicted according to the color scale shown on the top of left. Red indicates upregulation and green indicates downregulation. **B** and **C**, Validation of miRNA microarray data by quantitative polymerase chain reaction analysis ($n=8$ per group). The relative expression of miRNAs was normalized to the expression of the internal control (U6). Data are expressed as mean \pm SEM. * $P<0.05$; *** $P<0.001$ vs saline.

significantly decreased but its target genes, including IL-6, Col1a2, Col3a1, Col4a1, and Col5a2, were markedly upregulated in fibroblasts (Figure 3D and 3E). The expression of other let-7 family members was also detected by qPCR analysis, and only let-7g was markedly downregulated but was higher than let-7i in Ang II-treated fibroblasts (Figure S3C). Moreover, in Ang II-treated cardiomyocytes, the hypertrophic markers, such as atrial natriuretic factor, brain natriuretic peptide, and β -myosin heavy chain, were significantly increased compared with control (Figure S3D), but no statistically significant difference in the expression of let-7 family members between 2 groups was observed (Figure S3E). Collectively, these findings suggest that Ang II predominantly regulates the expression of let-7i and its targets in cardiac fibroblasts.

Let-7i Negatively Regulated the Expression of IL-6 and Collagens in Cardiac Fibroblasts

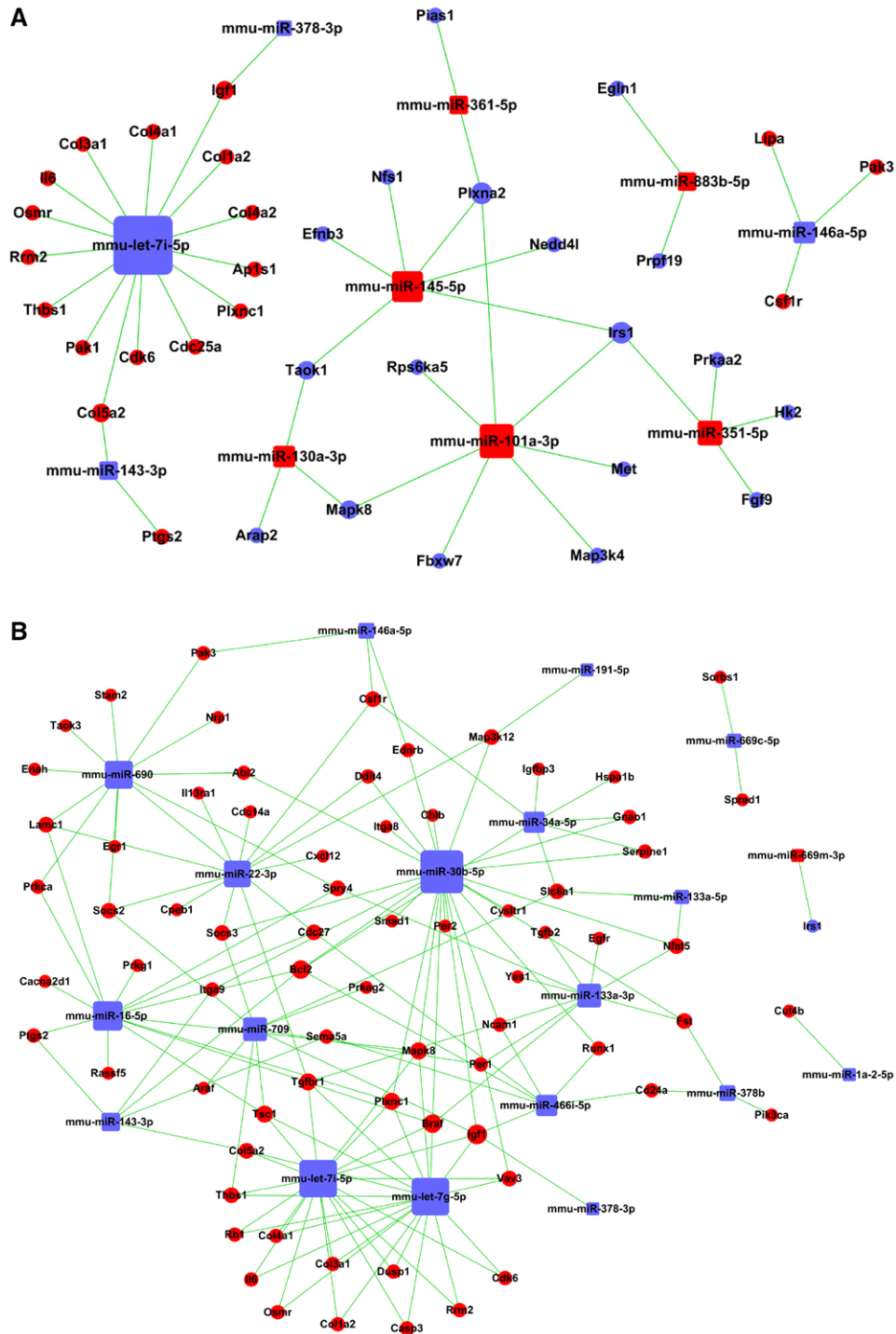
To investigate the action of let-7i on its putative targets, cardiac fibroblasts were transfected with let-7i mimic or inhibitor and then treated with or without Ang II. Transfection efficiency was monitored using fluorescent-labeled nonspecific mimic or inhibitor (data not shown). After Ang II treatment for 24 hours, fibroblasts transfected with let-7i mimic significantly decreased the expression of IL-6, Col1a2, Col3a1, Col4a1, and Col5a2 mRNA when compared with negative control (Figure 4A). In contrast, let-7i inhibitor had an opposite effect (Figure 4B). To further test whether let-7i regulates the migration and proliferation of fibroblasts, we performed scrape-wound healing and 5-Bromo-2-deoxyUridine (BrdU)

incorporation assay. Transfection of fibroblasts with let-7i mimic or inhibitor had no significant effect on Ang II-induced migration and proliferation in cardiac fibroblasts (Figure S4). Moreover, transfection of let-7i mimic or inhibitor did not alter cardiomyocyte size after Ang II stimulation (Figure S5).

To demonstrate whether let-7i directly interacts with a specific target sequence localized in the IL-6 and collagens 3' untranslated region (3'UTR), we searched the homology between let-7i and its targets, including IL-6, Col1a2, Col3a1, Col4a1, and Col5a2, using TargetScan database. We found that IL-6 and collagens (Col1a2, Col3a1, Col4a1, and Col5a2) had conservative let-7i seed sequences in their 3'UTR (Figure 4C). Next, we constructed 2 reporter vectors containing the wild-type or the mutant IL-6 3'UTR or collagen 3'UTR and performed the dual luciferase assay. The relative luciferase activities were significantly decreased in cotransfection of wild-type IL-6 or Col1a2 3'UTR with let-7i mimic compared with negative control. However, this inhibitory effect of let-7i was markedly blunted by transfection of mutant IL-6 or Col1a2 3'UTR, which disrupted the interaction between let-7i and IL-6 or Col1a2 3'UTR (Figure 4D). Collectively, these results confirmed that let-7i can directly regulate its target genes IL-6 and collagens in cardiac fibroblasts.

Overexpression of Let-7i Reduced Ang II-Induced Cardiac Inflammation and Fibrosis

To better understand the function of let-7i in cardiac inflammation and fibrosis in vivo, let-7i agomir (a chemically



modified RNA oligonucleotide) or negative control was administered intravenously to mice on day 1 before Ang II infusion and days 1, 3, 5 after Ang II infusion. Injection of high-dose let-7i agomir (20 nmol) significantly reduced Ang II-induced infiltration of inflammatory cells including Mac-2-positive macrophages in the heart (Figure 5A) and markedly attenuated cardiac fibrosis when compared with negative control group (Figure 5B). qPCR analysis further revealed

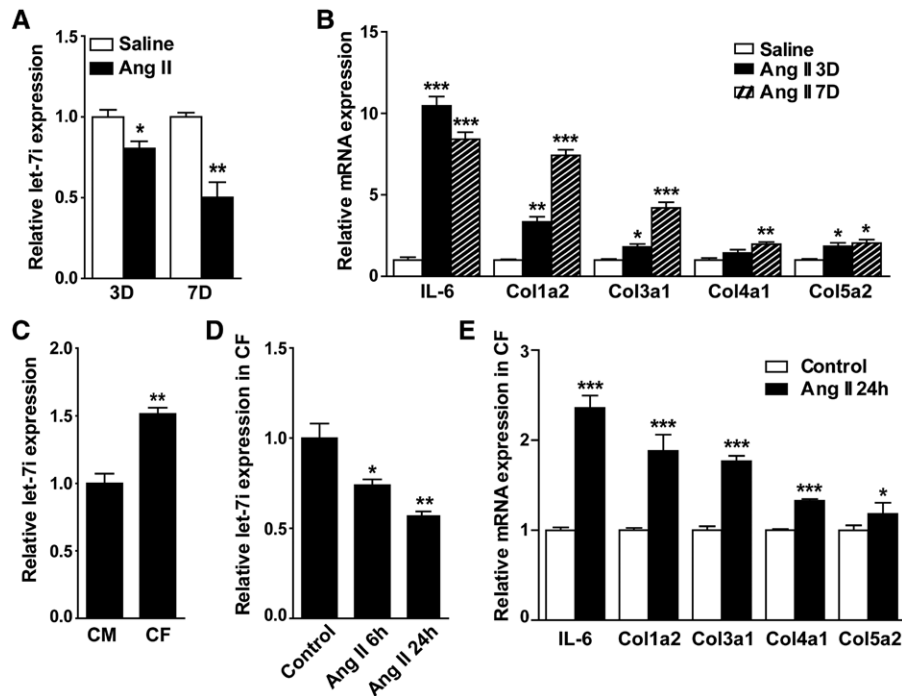


Figure 3. The expression of let-7i and its targets. **A** and **B**, Validation of expression of let-7i and its targets in angiotensin II (Ang II)-infused hearts and saline by quantitative polymerase chain reaction (qPCR) analysis ($n=8$ per group). **C**, Expression of let-7i detected by qPCR analysis in cardiomyocytes (CMs) and cardiac fibroblasts (CFs) isolated from neonatal rat ($n=5$ per group). **D**, Let-7i expression detected by qPCR analysis in CFs after Ang II (100 nmol/L) treatment ($n=5$ per group). **E**, The expression of let-7i's targets examined by qPCR analysis in CFs after Ang II (100 nmol/L) treatment ($n=5$ per group). Data are expressed as mean \pm SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs saline or control.

as well as cardiomyocyte apoptosis 7 days after Ang II infusion (data not shown). All our results demonstrate that overexpression of let-7i significantly inhibits cardiac inflammation and fibrosis induced by Ang II infusion.

Knockdown of Let-7i by Antagomir Aggravated Ang II-Induced Cardiac Inflammation and Fibrosis

To further investigate loss function of let-7i in Ang II-mediated cardiac remodeling in vivo, mice were injected intravenously with a chemically modified antisense RNA oligonucleotide (termed an antagomir) targeted to let-7i. Seven days after Ang II infusion, echocardiographic analysis showed no significant difference in cardiac hypertrophic parameters and the left ventricle diastolic dysfunction as indicated by E/A ratio was observed between let-7i antagomir and negative control-treated mice (Table S3). Moreover, the blood pressure also had no significance between 2 groups (Figure S7B). However, depletion of let-7i via antagomir significantly increased cardiac inflammation, including Mac-2-positive macrophages and aggravated fibrosis, as well as the expression of its targets IL-6 and collagens when compared with negative controls after Ang II infusion (Figure 6A–6C). Collectively, these data indicate that knockdown of let-7i markedly increases Ang II-induced effects.

Discussion

Here, we profiled miRNA and mRNA expression patterns in Ang II-induced cardiac remodeling and found that let-7i was downregulated at day 3 and 7 after Ang II-infusion. Notably, let-7i had the most targets that were mainly associated with

cardiac inflammation and fibrosis. Furthermore, we demonstrated a key role for let-7i in directly regulating IL-6 and collagens expression in cardiac fibroblasts. Importantly, increased let-7i expression in mice heart significantly attenuated Ang II-induced inflammation and cardiac fibrosis via inhibiting IL-6 and collagens. In contrast, knockdown of let-7i aggravated these effects (Figure 6D). Together, these results suggest that let-7i plays a critical role in Ang II-induced cardiac inflammation and fibrosis.

Cardiac fibrosis is an end-stage pathological manifestation of various cardiovascular diseases. Fibroblasts are the main sources of extracellular matrix after their transition into myofibroblasts.¹⁸ Ang II stimulation activates fibroblast transforming growth factor- β and IL-6 signaling pathways, which are the major profibrogenic mediators of cardiac fibrosis.³ However, the molecular mechanisms that regulate this process remain to be explored. Recently, the role of miRNAs in cardiac function and diseases has received great attention. Dysregulation of miRNAs by several mechanisms has been implicated in cardiac remodeling, for example, miR-1, miR-23a, miR-133a, miR-126, miR-143/145, and miR-208 have been demonstrated to involve in the cardiac development and hypertrophy,^{11–13,17,19,20} whereas miR-133, miR-30, miR-21, and miR-29 are involved in cardiac fibrosis.^{7–9} Recently, miRNA microarray have identified 18 miRNAs and miRNA families that are the most strongly expressed in the normal heart and cardiovascular diseases, and the let-7 family accounts for $\approx 14\%$ of all miRNAs expressed in the murine heart,²¹ suggesting that let-7 family may play a central role in the heart diseases.

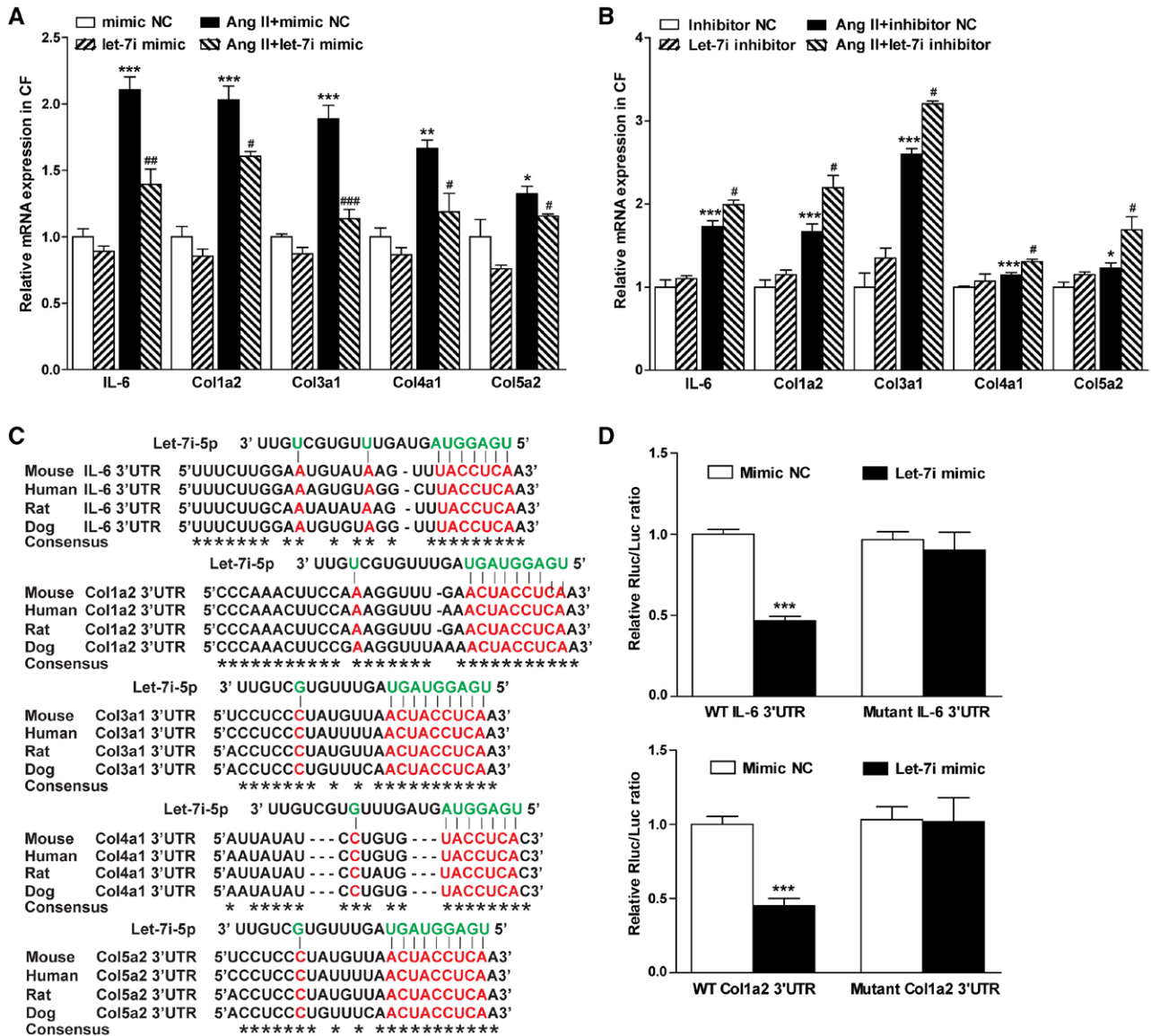


Figure 4. Interleukin-6 (IL-6) and collagens are direct targets of let-7i. After transfection for 24 hours, cardiac fibroblasts (CFs) were stimulated with angiotensin II (Ang II; 100 nmol/L) for additional 24 hours. **A**, After transfected with let-7i mimic and negative control (NC; 100 nmol/L), the expression of IL-6 and collagens was detected by quantitative polymerase chain reaction (qPCR) analysis (n=5 per group). **B**, After transfected with let-7i inhibitor and NC (150 nmol/L), the expression of IL-6 and collagens was detected by qPCR analysis (n=5 per group). **C**, The predicted duplex of let-7i and its targets sites in the 3' untranslated region (3'UTR) of IL-6 and collagens. **D**, Dual luciferase assay was performed in 293T cells transfected with luciferase reporter construct alone or cotransfected with let-7i mimic or NC. Firefly luciferase construct containing mutant target site of the IL-6 and col1a2 3'UTR is generated and transfected as indicated. Firefly luciferase activity was normalized to renilla luciferase activity for each sample (n=3 per group). Data are expressed as mean±SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs NC. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ vs Ang II+NC. WT indicates wild-type.

The human let-7 family contains 13 members located in 9 chromosomes, including let-7a-1/a-2/a-3/b/c/d/e/f-1/f-2/g/i, mir-98, and miR-202. Previous studies focus on the role of let-7 in regulation of tumors.²² Recently, the expression patterns and the roles of let-7 have been reported in cardiovascular system. Let-7 family is highly expressed in several major types of cardiovascular cells, including endothelial cells, vascular smooth muscle cells, and cardiomyocytes, and it is dysregulated in several cardiovascular diseases.²³ Importantly, both in vivo and in vitro studies have demonstrated the important roles of let-7 family in heart development and diverse cardiovascular diseases. For example, let-7b and 7c

were upregulated but let-7d was downregulated after 14 days after transverse aortic constriction by microarray assay.¹³ MiR-98 was upregulated in thioredoxin 1 transgene mice and inhibits cardiac hypertrophy partly through downregulation of cyclin D2 after Ang II infusion.²⁴ Let-7i decreased in dilated cardiomyopathy and was related to poor clinical outcomes in patients with dilated cardiomyopathy.²⁵ Moreover, in patients with coronary artery disease, let-7i had a negative correlation with toll-like receptor 4 levels contributing to the beneficial effects of atorvastatin on coronary artery disease.²⁶ A recent report demonstrated that let-7i, let-7b, and let-7e were significantly affected by postconditioning.²⁷ Let-7c and

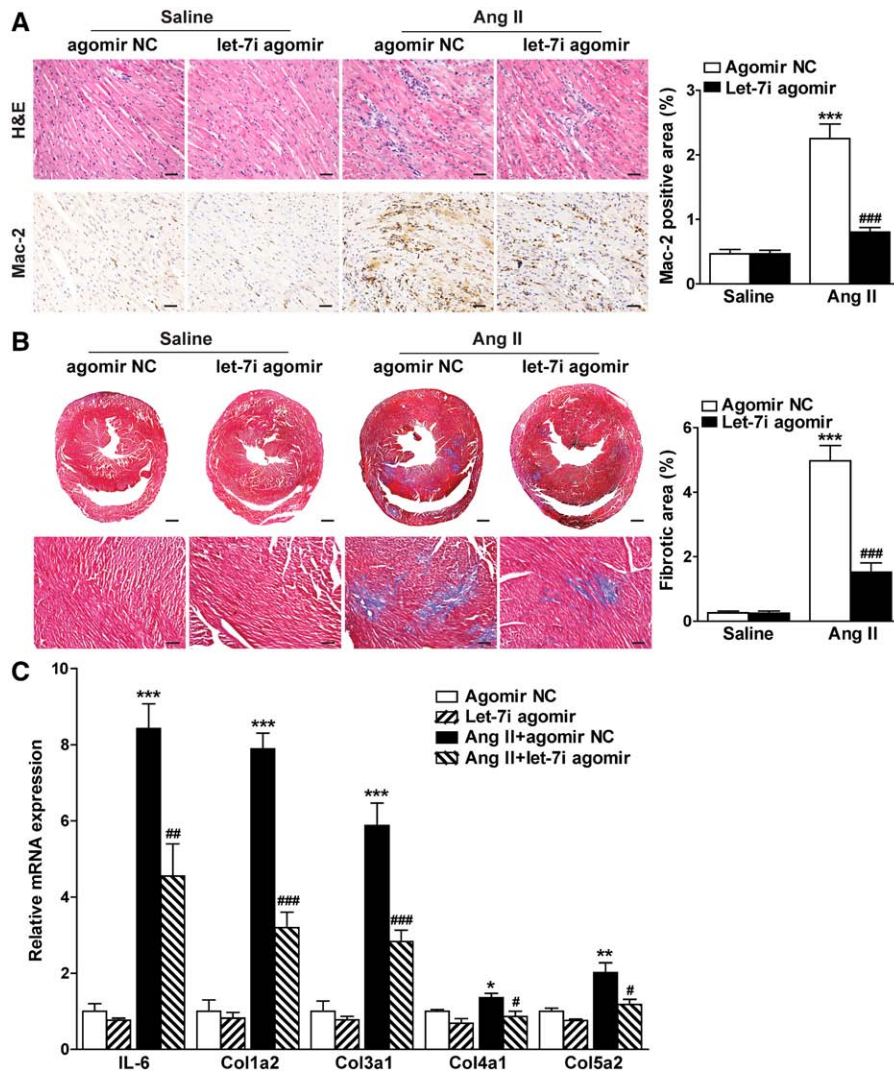


Figure 5. Enhanced expression of let-7i reduces angiotensin II (Ang II)-induced cardiac inflammation and fibrosis. Let-7i agomir and negative control (NC) were injected intravenously on day 1 (20 nmol) before Ang II infusion and days 1, 3, 5 (20 nmol) after Ang II infusion. **A**, Representative images of hematoxylin and eosin (H&E) and immunohistochemical staining of Mac-2 in the heart sections (left, scale bars: 50 μ m). Quantification of Mac-2-positive areas (right, $n=6$ per group). **B**, Representative images of Masson trichrome staining (blue) of heart sections (top, scale bars: 1 mm; bottom, scale bars: 100 μ m). Quantification of fibrotic areas (right, $n=6$ per group). **C**, The expression of interleukin-6 (IL-6) and collagens was detected by quantitative polymerase chain reaction ($n=6$ per group). Data are expressed as mean \pm SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs NC. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ vs Ang II+NC.

7f were upregulated in myocardial ischemic injury or ischemic cardiomyopathy.^{28,29} Let-7c and 7g were reported to be markedly increased during endothelial-to-mesenchymal transition.³⁰ The circulating let-7b was also reported to be a biomarker of acute myocardial infarction.³¹ However, the role of let-7i in regulating cardiac fibrosis remains unclear. In this study, our data revealed that the expression of let-7i is dynamically downregulated at days 3 and 7 after Ang II infusion (Figure 3A). Let-7i was mainly expressed in fibroblasts (Figure 3C). Importantly, overexpression of let-7i significantly reduced Ang II-induced inflammation and cardiac fibrosis (Figure 5A and 5B; Figure S6A and S6B). Conversely, knockdown of let-7i markedly aggravated these effects (Figure 6A and 6B). Together, these results indicate the important role of let-7i in modulating cardiac inflammation and fibrosis.

Identification of the direct targets of miRNAs is a major challenge in the investigation of their function because many putative targets display little or no detectable regulation. Although there are many computational algorithms for predicting miRNA targets, they vary widely with regard to specific targets identified. Thus, more complex algorithms using joint expressions of miRNAs and mRNAs may be useful to predict miRNA targets.^{32,33} Currently, >10 direct targets for let-7 family have been discovered in the heart and other tissues, including Ras, c-Myc, high mobility group A2, argonaute 1, toll-like receptor 4, Bcl-x1, tissue inhibitor of metalloproteinases-1, c-Met, and insulin-like growth factor-1 receptor.^{23,34–36} To dissect the molecular basis underlying this let-7i-associated alteration of cardiac remodeling, we searched for potential let-7i targets by performing miRNA–mRNA interaction networks and identified IL-6 and collagen family as the direct targets

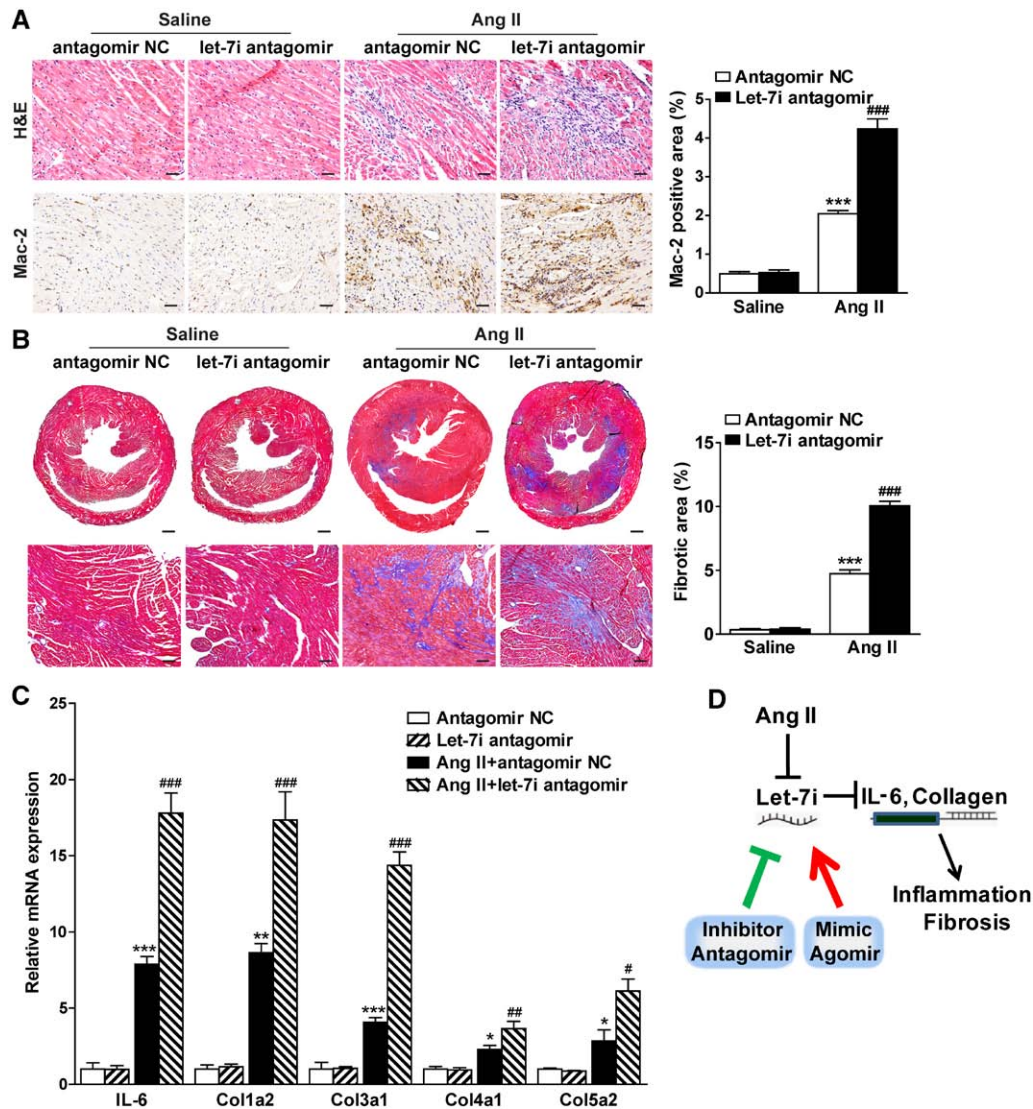


Figure 6. Knockdown of let-7i aggravates angiotensin II (Ang II)-induced cardiac inflammation and fibrosis. Let-7i antagomir and negative control (NC) were administered on day 1 (100nmol) before Ang II infusion and day 3 (100 nmol) after Ang II infusion. **A**, Representative images of hematoxylin and eosin (H&E) and immunohistochemical staining of Mac-2 in the heart sections (**left**, scale bars: 50 μ m). Quantification of Mac-2-positive areas (**right**, $n=5$ per group). **B**, Representative images of Masson trichrome staining (blue) of heart sections (**top**, scale bars: 1 mm; **bottom**, scale bars: 100 μ m). Quantification of fibrotic areas (**right**, $n=5$ per group). **C**, The expression of interleukin-6 (IL-6) and collagens were detected by quantitative polymerase chain reaction analysis ($n=5$ per group). **D**, Schematic model of let-7i's role in regulating cardiac inflammation and fibrosis. Ang II stimulation or hypertension resulted in downregulation of let-7i and consequent upregulation of its targets IL-6 and collagens in cardiac fibroblasts leading to cardiac inflammation and fibrosis. Data are expressed as mean \pm SEM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs NC. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ vs Ang II+NC.

of let-7i in our cell- and animal-based models, along with the previously reported study about cancer,^{37,38} and both IL-6 and collagens are known to play critical roles in cardiac inflammation and fibrosis.³ Moreover, these targets of let-7i were confirmed by qPCR analysis and reporter assay in vitro cultured cardiac fibroblasts (Figure 4) and in vivo animals (Figures 5 and 6; Figure S6). Therefore, inhibition of expression of IL-6 and collagens by let-7i could be one potential mechanism for reduced cardiac inflammation and fibrosis.

Perspectives

For the first time to our knowledge, we used a parallel microarray assay to examine the global miRNA and mRNA expression profiles of murine hearts after Ang II infusion and

demonstrated that let-7i expression was reduced in the heart and cultured fibroblasts after Ang II stimulation. This highlights the potential importance of let-7i in the progression of cardiac inflammation and fibrosis and possible targets (either the miRNAs themselves or their mRNA targets) for future therapeutic intervention. Additional studies will be needed to determine the effect of let-7i on cardiac inflammation and fibrosis in transgenic or knockout model and to evaluate the potential of let-7i overexpression as a therapeutic strategy for cardiac and other fibrotic diseases.

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Disclosures

None.

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Novelty and Significance

What Is New?

- We for the first time tested the microRNA and mRNA expression patterns in the angiotensin II (Ang II)–induced cardiac remodeling using microarray assay.
- MicroRNA let-7i was downregulated during Ang II–induced cardiac remodeling.
- MicroRNA let-7i negatively regulated Ang II–induced cardiac inflammation and fibrosis via directly inhibiting its target genes interleukin-6 and collagen expression.

What Is Relevant?

- Fibroblast microRNA let-7i attenuated Ang II–induced interleukin-6 and collagen expression.

- Increased expression of let-7i attenuates Ang II–induced inflammation and cardiac fibrosis, whereas knockdown of let-7i enhanced this effect.
- These data provide novel evidence for further understanding the effect of let-7 family on cardiac inflammation and fibrosis, and implications for the development of strategies for the treatment of cardiac remodeling.

Summary

Increasing let-7i level in cardiac fibroblasts may be a potential therapeutic strategy for the treatment of cardiac fibrosis and heart failure in patients with hypertensive heart diseases.