EXPERIMENTAL STUDY

MiR-324-3p Regulates Fibroblast Proliferation via Targeting TGF-β1 in Atrial Fibrillation

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Summary
Atrial fibrillation (AF), one of the common clinical arrhythmias, lacks effective treatment manners. Cardiac fibroblasts play an essential role in myocardial fibrosis and cardiac remodeling, which are involved in AF progression. Reportedly, MicroRNAs (miRNAs) regulate the myocardial fibrosis in AF. However, whether miR-324-3p involves myocardial fibrosis in AF and the tentative molecular mechanisms of miR-324-3p regulating cardiac fibroblasts during AF remains unknown. In the present study, miR-324-3p was found to be decreased in patients with AF and AF rat model. Next, we investigated the effect of miR-324-3p on myocardial fibroblast proliferation through miR-324-3p overexpression and found that miR-324-3p inhibited fibroblast proliferation in vitro. Furthermore, we found that miR-324-3p directly targeted transforming growth factor β1 in fibroblast, which may be involved in the development of myocardial fibrosis during AF. Meanwhile, miR-324-3p mimics treatment suppressed the PI3K/AKT signaling pathway in fibroblast. These results demonstrated a molecular mechanism of miR-324-3p regulating fibroblast proliferation in vitro, which might provide a novel potential treatment manner in AF in clinic.

Key words: Arrhythmias, Fibrosis, PI3K/AKT signaling, Rat model
ing pathway. Furthermore, miR-29 was revealed to be involved in the increase of collagen matrix and fibrosis in AF by blocking the promoting effect of transforming growth factor β1 (TGF-β1) on the secretion of collagen fibers and extracellular matrix proteins. Additionally, evidences reported that miR-30, miR-133, and miR-590 were involved in AF development through the regulation of fibrosis. Conversely, the previous study has revealed the decreased miR-324-3p expression in AF. In the present study, through genome-wide miRNAs sequencing, we also found the decreased miR-324-3p expression in patients with AF. However, whether miR-324-3p involves myocardial fibrosis in AF and the tentative molecular mechanisms of miRNA regulating myocardial fibrosis during AF remains unknown. In the present study, we investigated the effect of miR-324-3p on the function of myocardial fibroblast through miR-324-3p overexpression in vitro. Moreover, the interaction of miR-324-3p and downstream signaling genes was further explored in fibroblasts. This study demonstrated a molecular mechanism of miR-324-3p regulating fibroblast proliferation in vitro, which might provide a novel potential treatment manner in AF in clinic.

Methods

Patients and samples: The present study was approved by the Committee for the Conduct of Human Ethics of the First Affiliated Hospital of Bengbu Medical College (Approval No: 2019KY023), and all patients signed informed consent forms. Samples from 20 patients who exhibited clinical characteristics of AF and 20 normal healthy controls were collected. The diagnosis of AF was conducted based on medical records and electrocardiograms.

Isolation of exosomes: Exosomes were isolated from AF plasma. Briefly, the plasma was obtained and centrifuged to remove cellular debris. The precipitates were mixed with total exosome isolation reagent (Invitrogen, USA) overnight at 4°C. After centrifuging at 10,000 x g for 1 hour, the pellet was then carefully resuspended in 200 μL of PBS and stored at −80°C.

Transmission electron microscopy (TEM): The image of exosomes was recorded by a TEM (HT7700, HITACHI). Samples were prepared by drop drying a water-diluted suspension of the studied samples. Images were processed by TEM imaging software (HITACHI).

Total RNA isolation and sequencing: Small RNA library preparation and sequencing were conducted with Illumina sequencing technology (BGI, Shenzhen, China). Briefly, the small RNA (sRNA) population was isolated by separating 10 μg of total RNA using denaturing polyacrylamide gel electrophoresis and excising the portion of the gel corresponding to the appropriate size (15-30 nt) based on standard oligonucleotide markers. Briefly, total RNAs of six samples (two groups with three replications) were extracted using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol; 18-30 nt sRNAs were purified from a 15% denaturing polyacrylamide gel and then ligated with 5’ and 3′ adaptors. After being reverse-transcribed, sRNAs were amplified by PCR and then deep sequenced on a BGI HiSeq platform (Shenzhen, China).

AF rat models: The healthy male Sprague Dawley (SD) rats (200-220 g) were acquired from the experimental animal center of Second Military Medical University. The study was approved by the animal care committee at Bengbu Medical College (No. 20190192). All animal experiments were conducted according to local protocols concerning the care and use of laboratory animals and in strict obedience with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health in the USA. These rats were randomly divided into two groups, namely, control rats (n = 4) and AF rats (n = 4). The rats were anesthetized by intraperitoneal injection with 7% chloral hydrate with a dose of 0.5 mL/100 g. The AF rats were administered with a calcium chloride-acetycholine (CaCl2-Ach) mixture (CaCl2, 10 mg/mL; Ach, 66 μg/mL) via tail vein injection (1 mL/kg) once per day for three consecutive days. The control rats were administered with 1 mL/kg of saline by tail vein. Cardiac electrophysiological and atrial effective refractory period (AERP) were analyzed in AF and control rats.

Measurement of the duration of AF: At the first, second, third, fourth, and fifth days after the administration of CaCl2-Ach, the duration of AF was detected. The measuring electrode was inserted into the right atria through the right jugular vein for programmed electrical stimulation. The duration from AF onset to AF termination was recorded as the duration of AF.

Measurement of AERP: At the fifth day after the administration of CaCl2-Ach, the rats were anesthetized by intraperitoneal injection with 7% chloral hydrate with a dose of 0.5 mL/100 g, and then, the heart was acquired and washed. The auricle was separated and threaded. After stabilization for 40 minutes, the threshold of evoked action potential was measured by programmed stimulation. The AERP was measured by extrastimulus pacing. The stimulation parameters were as follows: frequency, 10 Hz; wave width, 3 ms; and 1.5 times threshold voltage. The double-pulse interval decreased gradually from 50 ms and decreased in 1 ms steps until the atrium capture failure. The longest interval that failed to capture was defined as the AERP.

Masson’s trichrome staining: Atrial tissue was acquired, fixed with 4% paraformaldehyde, and paraffin embedded. The sections were stained with Masson’s trichrome and visualized under an optical microscope (Olympus, Tokyo, Japan). The results were analyzed with Image Pro-Plus 6.0 software.

Isolation and culture of cardiac fibroblasts: The atria was acquired and cut into pieces after the rats were sacrificed. The tissue samples were then digested with 0.1% collagenase II (Sigma-Aldrich, St. Louis, USA) solution for 1 hour at 37°C to obtain the single cell. The cells were washed, and the fibroblasts were isolated by removing cardiac myocytes using the differential adhesion method. Fibroblasts were cultured in high-glucose DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco) at 37°C in an atmosphere of 5% CO2.
GenePharma (Shanghai, China). Fibroblasts were treated with three groups: control (fibroblasts transfected with miR-324-3p negative control), and miR-324-3p mimics (fibroblasts transfected with miR-324-3p mimics). The cell transfection was conducted using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer’s instruction.

Cell viability: The effects of miR-324-3p and NC control on fibroblasts proliferation were evaluated using a cell counting kit-8 (CCK-8) (Dojindo, Kumamoto, Japan). The fibroblasts were trypsinized and suspended at a final concentration of 1 × 10^5 cells/well and cultured in 96-well plates. After 0, 24, and 48 hours, 10 µL of the CCK-8 solution was added to each well. The cells were incubated for another 4 hours at 37°C. The OD value at 450 nm was measured using an ELISA microplate reader (ELX 800, BioTek, USA).

Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR): Trizol Reagent (Invitrogen) was used to extract RNA from fibroblasts. An ABI PRISM 7500 real-time PCR System and SYBR Green Master Mix (Roche, Shanghai, China) were used to conduct polymerase chain reactions. GAPDH and U6 were used as the internal references for mRNA and miRNA, respectively. PCR primers were as follows: miR-324-3p forward: 5'-CGGCCGACTGGCCCAAGCTGC-3', reverse: 5'-CTCAACTGTTGTCGTTGACGTCG-3'; TGF-β1 forward: 5'-CAAAGACATCAGACAGTAA-3', reverse: 5'-GGTGTGAGCCTTCTCAGG-3'; U6 forward: 5'-GTCGCCGACAGCATACATAC-3', reverse: 5'-GGTTGCAGACCATATAATAATCT-3'; Postn forward: 5'-CTCTTGCAATTTCGGTGTCAT-3', reverse: 5'-CAGCTCTCTGTCACACGCAT-3'; Acta2 forward: 5'-TTACTGGGAGCCCTTTCCAGG-3'; U6 forward: 5'-GAGCTGTGTTAGAAAGAC CA-3', reverse: 5'-CTATCGAGTTTACCAGGCTTGG-3', reverse: 5'-TACGTGTCCTGTTAGACGA T-3'.

Western blot: The treated cells were analyzed, and the protein was extracted. After quantification, the protein was transferred into nitrocellulose membranes, blocked, and incubated in a specific primary antibody at 4°C overnight. The membranes were incubated with goat sera at 37°C for 1 hour and 4°C overnight. Finally, the membranes were washed and incubated with the secondary antibody for 1 hour at room temperature. The protein bands were visualized using a chemiluminescence antibody for 1 hour at room temperature. Finally, the incubated in a specific primary antibody at 4°C overnight.

Immunofluorescence assay: The specific biomarker of TGF-β1 and p-AKT expression in fibroblasts was determined by immunofluorescence assay. Cells were washed with PBS, fixed with 4% paraformaldehyde for 15 minutes and then permeabilized in 0.1% Triton X-100 for 30 minutes. After being blocked with goat sera at 37°C for 1 hour, the cells were treated with anti-TGF-β1 (Santa Cruz Biotechnology, Dallas, TX, USA) and anti-p-AKT (Cell Signaling, Danvers, MA, USA) at 4°C overnight. After being washed, the bound antibodies were detected with FITC green-conjugated secondary antibodies, and the results were acquired under a fluorescent microscope.

Immunohistochemical (IHC) analysis: After 4 µm paraffin-embedded sections were heated and deparaffinized, the samples were used to expose binding sites for antibodies against TGF-β1. After washing the sample with PBS, the second antibody was added. The signal was amplified and visualized with 3'-diaminobenzidine chromogen (DAB) and then counterstained with hematoxylin.

Statistical analysis: The data were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as the mean ± SD of at least three independent experiments. The independent sample t test was used for comparing groups for statistical differences. Statistical significance was defined as P < 0.05.

Results

MiR-324-3p expression was decreased in patients with AF: Exosomes from patients with AF and control were isolated and observed by TEM (Figure 1A). Through the RNA sequencing analysis of exosomes from patients with AF and control, 646 miRNAs were acquired, in which 171 miRNAs were down-regulated and 158 miRNAs were up-regulated. The differential expression of miRNAs was displayed by a heat map (Figure 1B). The result showed that miR-324-3p expression was down-regulated in patients with AF compared with the control. To further testify the abnormal expression in AF, exosomes from 20 patients with AF and 20 healthy controls were collected, and the result revealed that miR-324-3p expression levels were significantly decreased in patients with AF compared with the control (Figure 1C).

MiR-324-3p was down-regulated in AF rat models: The AF rat models were successfully established. As shown in Figure 2A, the duration of AF in two groups was gradually prolonged. Compared with that in the control group, the duration of AF in AF groups was remarkably enhanced on the fourth and fifth days. Furthermore, compared with that in the control group, the AERP was significantly prolonged. Compared with the control rats (Figure 2C and D). A previous study has revealed the decreased miR-324-3p expression in AF. To investigate the effect of miR-324-3p on AF, the clinical AF samples and AF rat models were obtained to testify miR-324-3p expression. Consistently, in the rats of AF, the levels of miR-324-3p were showed to obviously reduce compared with those in the control rats (Figure 2E). TGF-β1 was associated with AF progression. Additionally, immunohistochemical (IHC) analysis showed higher TGF-β1 positive staining in AF atrial tissues than in control tissues (Figure 2F). The relative expression lev-
The differential miR-324-3p expression was determined in patients with AF and healthy control. A: Represented TEM image of exosomes. Scale bar: 50 nm. B: The heat map of differentially expressed miRNAs, the red indicates high expression, and the blue indicates low expression. C: miR-324-3p expression in patients with AF (n = 20) and the healthy control (n = 20). **P < 0.01. N indicates normal healthy control; P indicates patients with AF.

els of Acta2 and Postn were determined in AF rats at 24 and 72 hours post-treatment. The results revealed that the relative expression levels of Acta2 and Postn were significantly increased in AF rats compared with the control rats at both 24 and 72 hours post-treatment (Figure 2G and H). Moreover, in AF rats at 72 hours post-treatment, the Acta2 and Postn relative expression levels were remarkably increased compared with this in the group of AF rats at 24 hours post-treatment (Figure 2G and H).

**MiR-324-3p inhibited fibroblast proliferation and reduced TGF-β1 expression levels:** To determine the effect of abnormal miR-324-3p expression on fibroblast of AF, miR-324-3p was up-regulated by transfection with miR-324-3p mimics. The overexpression efficiency was determined in fibroblast by treatment with miR-324-3p mimics (Figure 3A). Interestingly, miR-324-3p mimics transfection led to a significant decrease in the protein levels of the activated form of TGF-β1 (Figure 3B and C). Moreover, the TGF-β1 mRNA relative expression was significantly reduced in the presence of miR-324-3p mimics when compared with that in the control (Figure 3D). Importantly, miR-324-3p mimics treatment slightly inhibited the cell proliferation at 24 hours, and significantly suppressed the cell proliferation at 48 hours (Figure 3F). Additionally, loss of TGF-β1 expression was found in the presence of miR-324-3p mimics (Figure 3E).

**MiR-324-3p regulated fibroblast proliferation by targeting TGF-β1:** To explore the detailed mechanism of miR-324-3p regulating fibroblast proliferation, the tentative interaction was predicted by the target prediction program. A previous study has shown that TGF-β1 is involved in promoting the progress of AF. Here, we found that TGF-β1 was predicted to interact with miR-324-3p from the target analysis results (Figure 4A). To investigate the relationship between miR-324-3p and TGF-β1, luciferase reporter assays were conducted in fibroblast following treatment with miR-324-3p mimics and wt/Mut of the human TGF-β1 luciferase reporters. The luciferase activity of TGF-β1 WT reporters was significantly reduced in the presence of miR-324-3p mimics compared with that in mimics control, whereas not significantly changed in TGF-β1 Mut reporters (Figure 4B). To investigate whether TGF-β1 was involved in miR-324-3p regulating fibroblast proliferation, fibroblast was treated with the TGF-β1 inhibitor SB431542. The prohibited effect of SB431542 on the TGF-β1 protein expression level has been shown in Figure 4C. The cell proliferation was significantly enhanced by miR-324-3p inhibitor treatment compared with that in the control group, whereas SB431542 treatment dramatically decreased the cell proliferation for 48 hours in fibroblast compared with the miR-324-3p inhibitor treatment group (Figure 4D).

**MiR-324-3p regulated PI3K/AKT signaling pathway via targeting TGF-β1:** To further investigate the mecha-
Figure 2. MiR-324-3p was down-regulated in AF rat model. A: Duration of atrial fibrillation in rats at different time points. B: The changes of AERP in the control and AF groups. C: Representative Masson’s trichrome staining of atrial tissues from rats. Scale bar: 25 μm. D: Percentage of Masson’s trichrome staining area showing fibrosis from control and AF rats (n = 4 for each group). E: Q-PCR analysis examined miR-324-3p expression in AF rats (n = 4) and control rats (n = 4). F: TGF-β1 expression levels were determined in atrial tissue of AF rats by immunohistochemical (IHC) assay (×200). Black arrow represented fibroblasts, and red arrow represented cardiomyocytes. Scale bar: 50 μm. G: The relative expression levels of Acta2 were determined in AF rats at 24 and 72 hours post-treatment. H: The relative expression levels of Postn were determined in AF rats at 24 and 72 hours post-treatment. *P < 0.05, **P < 0.01 versus control. *P < 0.05 versus the group at 24 hours post-treatment.
MiR-324-3p inhibited fibroblast proliferation and reduced TGF-β1 expression levels. A: miR-324-3p expression was determined following treatment with miR-324-3p mimics. B, C: The protein expression levels and quantifications of the mature TGF-β1 were determined following treatment with miR-324-3p mimics. D: The relative expression levels of TGF-β1 were determined following treatment with miR-324-3p mimics. E: TGF-β1 was detected by immunofluorescence assay. Scale bar: 20 μm. F: The cell proliferation was estimated by miR-324-3p overexpression for 0, 24, and 48 hours in fibroblast. *P < 0.05, **P < 0.01.

The mechanism of miR-324-3p regulating fibroblast proliferation, the Collagen and PI3K/AKT pathway-related proteins were determined following transfection with miR-324-3p mimics. As shown in Figure 5A, Collagen-I, Collagen-III, and p-AKT protein levels were down-regulated in the presence of miR-324-3p mimics, whereas AKT protein levels were not obviously changed between the miR-324-3p mimics-treated group and the control group. Subsequently, the quantification result revealed that the relative protein expression levels of Collagen-I and Collagen-III were markedly decreased after treatment with miR-324-3p mimics compared with those in the control group (Figure 5B). Meanwhile, the fibroblast in the miR-324-3p mimics group had significantly reduced p-AKT protein expression.
Figure 4. MiR-324-3p regulated fibroblast proliferation by targeting TGF-β1. A: TGF-β1 was predicted to interact with miR-324-3p from target prediction program. B: Fibroblast was co-transfected with miR-324-3p mimics and WT/ Mut of TGF-β1 luciferase reporter. Luciferase reporter assays were used to detect the luciferase activity. C: The prohibited effect of TGF-β1 inhibitors, SB431542 on TGF-β1 protein expression level. D: The cell proliferation was evaluated following treatment with SB431542 and/or miR-324-3p inhibitors for 48 hours in fibroblast. **P < 0.01 versus control, ##P < 0.01 versus miR-324-3p inhibitors.

Discussion

Recent studies have shown that miR-324-3p is up-regulated in exosomes from vascular smooth muscle cells associated with coronary artery calcification. Moreover, miR-324-5p was reported to attenuate mitochondrial fission, cardiomyocyte apoptosis, and myocardial infarction by suppressing Mtrf1 translation in vitro. Furthermore, Daz, et al. demonstrated that miR-324-3p overexpression promoted dysregulation of genes expression involved in cell death and apoptosis, cell stress, and metabolism, which contributed to urocortin protection against myocardial ischemia-reperfusion injury. Additionally, the latest study revealed that miR-324-3p was down-regulated in patients with AF-associated rheumatic mitral valve disease. These studies have demonstrated that miR-324-3p plays an essential role in heart-related diseases. Conversely, Macconi, et al. showed that miR-324-3p contributed to the development of fibrosis in progressive nephropathy by targeting prolyl endopeptidase (Prep). However, the effect and molecular mechanism of miR-324-3p in myocardial fibrosis and AF remain unclear.

In the present study, through genome-wide miRNAs sequencing, we found miR-324-3p was down-regulated in patients with AF. In the AF rat model, miR-324-3p expression levels were obviously reduced in comparison to the control rats, which was consistent with the result in AF patients. A large number of studies have proved that myocardial fibroblast proliferation and migration are the essential features of myocardial fibrosis. Therefore, we isolated the cardiac fibroblasts to explore the effect of miR-324-3p in myocardial fibrosis from the atrial tissue of AF rats. A previous study has shown that miR-324-3p suppresses nasopharyngeal carcinoma cell migration and invasion by targeting WNT2B. Another report revealed that miR-324-3p promoted cell growth, migration, and invasion through the activation of the Wnt/β-catenin pathway in human colon cancer cells. In this study, we found that miR-324-3p overexpression slightly prohibited the cell proliferation for 24 hours and significantly suppressed the cell proliferation for 48 hours in fibroblast, which suggested that miR-324-3p could suppress cell proliferation in fibroblast of AF rats.

TGF-β1 is a key factor of myocardial fibrosis, which can induce myocardial fibroblast proliferation, collagen secretion, and transdifferentiation of myocardial fibroblast into myofibroblasts. Effectively inhibiting the proliferation of myocardial fibroblasts induced by TGF-β1 is of great significance to suppress myocardial fibrosis and reduce the mortality of cardiovascular diseases. Previous studies have demonstrated that TGF-β1 can induce cardiac
fibrosis via various signaling pathways in rats. Recent studies have shown that TGF-β1 was involved in promoting the progress of AF. Interestingly, we found that miR-324-3p overexpression led to a decrease in the mRNA and protein levels of TGF-β1 in fibroblasts. Further study revealed miR-324-3p directly targeted TGF-β1 in fibroblast. Moreover, SB431542, the TGF-β1 inhibitor, dramatically decreased the cell proliferation, which was enhanced by miR-324-3p inhibitors in fibroblast. These results indicated that miR-324-3p suppressed fibroblast proliferation via targeting TGF-β1.

**Conclusions**

In conclusion, we investigated the effect of miR-324-3p on the function of myocardial fibroblast isolated from AF rats. Moreover, we found the interaction of miR-324-3p and TGF-β1 in fibroblasts, which were involved in the change of myocardial fibrosis in AF. Meanwhile, miR-324-3p mimics could suppress the PI3K/AKT signaling pathway in fibroblasts. The present study demonstrated a molecular mechanism of miR-324-3p regulating fibroblast proliferation by targeting TGF-β1 in vitro, which might provide a novel potential treatment manner in AF in clinic.

**Disclosure**

**Conflicts of interest:** The authors have declared that no competing interests exist.

**References**

1. Akkaya E, Berkowitz A, Zaltsberg S, et al. Second-generation cryoballoon ablation as a first-line treatment of symptomatic atrial fibrillation: two-year outcome and predictors of recurrence after a single procedure. Int J Cardiol 2018; 259: 76-81.
2. Kumar P, Kiser AC, Gehi AK. Hybrid treatment of atrial fibrillation. Prog Cardiovasc Dis 2015; 58: 213-20.
3. Margulescu AD, Mont L. Persistent atrial fibrillation vs paroxysmal atrial fibrillation: differences in management. Expert Rev Cardiovasc Ther 2017; 15: 601-18.
4. Nattel S. Molecular and cellular mechanisms of atrial fibrillation in atrial fibrillation. JACC Clin Electrophysiol 2017; 3: 425-35.
5. Yoo S, Aistrop G, Shiferaw Y, et al. Oxidative stress creates a unique, CaMKII-mediated substrate for atrial fibrillation in heart failure. JCI Insight 2018; 3.
6. Han L, Li J. Canonical transient receptor potential 3 channels in atrial fibrillation. Eur J Pharmacol 2018; 837: 1-7.
7. Liu L, Gan S, Li B, Ge X, Yu H, Zhou H. Fisetin alleviates atrial inflammation, remodeling, and vulnerability to atrial fibrillation after myocardial infarction. Int Heart J 2019; 60: 1398-406.

8. Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration. Cell Tissue Res 2016; 365: 563-81.

9. Centurión OA, Alderete JF, Torres JM, Garcia LB, Scavenius KE, Mino LM. Myocardial fibrosis as a pathway of prediction of ventricular arrhythmias and sudden cardiac death in patients with nonischemic dilated cardiomyopathy. Crit Pathw Cardiol 2019; 18: 89-97.

10. Haemers P, Hamdi H, Guedj K, et al. Atrial fibrillation is associated with the fibrictive remodelling of adipose tissue in the subepicardium of human and sheep atria. Eur Heart J 2017; 38: 53-61.

11. Li L, Zhao Q, Kong W. Extracellular matrix remodeling and cardiac fibrosis. Matrix Biol 2018; 68-69: 490-506.

12. Pessoa FG, Mady C, Fonseca KCB, et al. Myocardial fibrosis contributes to mechanical stress induced cardiac fibroblast proliferation and extracellular matrix remodeling associated with atrial fibrillation. Int Heart J 2019; 60: 944-57.

13. Park S, Ranjbarvaziri S, Lay FD, et al. Genetic regulation of fibroblast activation and proliferation in cardiac fibrosis. Circulation 2018; 138: 1224-35.

14. Shinde AV, Frangogiannis NG. Fibroblasts in myocardial infarction: a role in inflammation and repair. J Mol Cell Cardiol 2014; 70: 74-82.

15. Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2012; 129: 949-84.

16. Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-5.

17. Krol J, Loedige I, Filipowicz W. MicroRNA in cardiovascular biology and disease. Adv Clin Exp Med 2017; 26: 865-74.

18. Cardin S, Guasch E, Luo X, et al. Role for MicroRNA-21 in atrial profibrillatory fibrotic remodeling associated with experimental postinfarction heart failure. Circ Arrhythm Electrophysiol 2012; 5: 1027-35.

19. Feinberg MW, Moore KJ. MicroRNA regulation of atherosclerosis. Circ Res 2016; 118: 703-20.

20. Wojciechowska A, Braniewska A, Kozar-Kamińska K. MicroRNA in cardiovascular biology and disease. Adv Clin Exp Med 2017; 26: 865-74.

21. Gao Q, Xu L, Yang Q, Guan TJ. MicroRNA-21 contributes to high glucose-induced fibrosis in peritoneal mesothelial cells in rat models by activation of the Ras-MAPK signaling pathway via Sprouty-1. J Cell Physiol 2019; 234: 5915-25.

22. Zhang Y, Huang XR, Wei LH, Chang AC, C M, Yu C, Lan HY. miR-29b as a therapeutic agent for angiotensin II-induced cardiac fibrosis by targeting TGF-beta/Smad3 signaling. Mol Ther J Am Soc Gene Ther 2014; 22: 974-85.

23. Yuan CT, Li XX, Cheng QJ, Wang YH, Wang JH, Liu CL. MiR-30a regulates the atrial fibration-induced myocardial fibrosis by targeting snail 1. Int J Clin Exp Pathol 2015; 8: 15527-36.

24. Shan H, Zhang Y, Lu Y, et al. Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines. Cardiovasc Res 2009; 83: 465-72.

25. Yan Y, Shi R, Yu X, Sun C, Zang W, Tian H. Identification of atrial fibrillation-associated microRNAs in left and right atria of rheumatic mitral valve disease patients. Genes Genet Syst 2019; 94: 23-34.

26. Pan W, Liang J, Tang H, et al. Differentially expressed microRNA profiles in exosomes from vascular smooth muscle cells associated with coronary artery calcification. Int J Biochem Cell Biol 2020; 118: 105645.

27. Wang K, Zhang DL, Long B, et al. NFAT4-dependent miR-324-5p regulates mitochondrial morphology and cardiomyocyte cell death by targeting Mfr1. Cell Death Dis 2015; 6: e2007.

28. Díaz I, Calderón-Sánchez E, Toro RD, et al. miR-125a, miR-139 and miR-324 contribute to urocortin protection against myocardial ischemia-reperfusion injury. Sci Rep 2017; 7: 8989.

29. Maccioni D, Tomasoni S, Romagnani P, et al. MicroRNA-324-3p promotes renal fibrosis and is a target of ACE inhibition. J Am Soc Nephrol 2012; 23: 1496-505.

30. Shibamoto M, Higo T, Naito AT, et al. Activation of DNA damage response and cellular senescence in cardiac fibroblasts limit cardiac fibrosis after myocardial infarction. Int Heart J 2019; 60: 944-57.

31. Lin C, Wei D, Xin D, Pan J, Huang M. Ellagic acid inhibits proliferation and migration of cardiac fibroblasts by downregulating expression of HDAC1. J Toxicol Sci 2019; 44: 425-33.

32. Liu C, Li G, Yang N, et al. miR-324-3p suppresses migration and invasion by targeting WNT2B in nasopharyngeal carcinoma. Cancer Cell Int 2017; 17: 2.

33. Yan D, Liu W, Liu Y, Luo M. LINC00261 suppresses human colon cancer progression via sponging miR-324-3p and inactivating the Wnt/beta-catenin pathway. J Cell Physiol 2019; 234: 22648-56.

34. Zhang Y, Lu Y, Ong’achwa MJ, et al. Resveratrol inhibits the TGF-beta1-Induced proliferation of cardiac fibroblasts and collagen secretion by downregulating miR-17 in rat. BioMed Res Int 2018; 2018: 8730593.

35. Zhou XL, Fang YH, Wan L, et al. Notch signaling inhibits cardiac fibroblast to myofibroblast transformation by antagonizing TGF-beta1/Smad3 signaling. J Cell Physiol 2019; 234: 8834-45.

36. Lei B, Hitomi H, Mori T, et al. Effect of efodipine on TGF-beta1-induced cardiac fibrosis through Smad2-dependent pathway in rat cardiac fibroblasts. J Pharmacol Sci 2011; 117: 98-105.

37. Fix C, Carver-Molina A, Chakrabarti M, Azhar M, Carver W. Effects of the isothiocyanate sulforaphane on TGF-beta1-induced cardiac fibroblast activation and extracellular matrix interactions. J Cell Physiol 2019; 234: 13931-41.

38. Song J, Zhu Y, Li J, et al. Pellinol-mediated TGF-beta1 synthesis contributes to mechanical stress induced cardiac fibroblast activation. J Mol Cell Cardiol 2015; 79: 145-56.

39. He R, Zhang J, Luo D, et al. Upregulation of transient receptor potential canonical Type 3 channel via AT1R/TGF-beta/Smad2/3 induces atrial fibrosis in aging and spontaneously hypertensive rats. Oxid Med Cell Longev 2019; 2019: 4025496.

40. Yu RB, Li K, Wang G, Gao GM, Du JX. MiR-23 enhances cardiac fibroblast proliferation and suppresses fibroblast apoptosis via targeting TGF-beta in atrial fibrillation. Eur Rev Med Pharmacol Sci 2019; 23: 4419-24.