

Original Article

MiR-30a regulates the atrial fibrillation-induced myocardial fibrosis by targeting snail 1

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Abstract: Objective: Our study aims at assessing the association between miR-30a along with its target gene snail 1 and atrial fibrillation (AF)-induced myocardial fibrosis. Methods: Ang II was used to up-regulate cardiac fibroblasts fibrosis *in vitro*, and then the cardiac fibroblasts were divided into the mimics group (mimics miR-30a), inhibitors group (inhibitors miR-30a), NC group (transfected miR-30a, negative control) and blank control group (non-transfected cells). Two-group (sham operated group and rapid pacing group) AF rabbit models were constructed according to whether rapid pacing was presented in the subject. Then the establishment of rabbit models was examined using histopathology after Masson staining. The mRNA and protein expression levels of snail 1 and periostin in cardiac fibroblasts and myocardial tissues were detected using the method of RT-PCR and Western blot, respectively. Results: *In vitro*, our experiment showed that overexpression of miR-30a in cardiac fibroblasts contribute to a significant decrease in the average expression level of snail 1 and periostin ($P < 0.05$) whereas inhibition of miR-30a significantly increased the average expression level of snail 1 and periostin ($P < 0.05$). *In vivo*, the average expression level of miR-30a significantly decreased in myocardial tissues with an increased degree of myocardial fibrosis, while the snail 1 and periostin expression level significantly increased during a certain period of time ($P < 0.05$). Conclusion: Our results suggest that miR-30a target snail 1 protein may be related to AF-induced myocardial fibrosis. The average expression levels of snail 1 increased significantly in both myocardial cells and tissues, while miR-30a could inhibit the expression of snail 1. Thus, we speculate that miR-30a and snail 1 may be potential therapeutic targets for curing AF-induced myocardial fibrosis.

Keywords: miR-30a, snail 1, atrial fibrillation, cardiac fibrosis, periostin, RT-PCR, Western blot, Ang II

Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinics [1, 2] and it has significantly high morbidity and mortality [2, 3]. The optimal therapy for AF is still unclear although a few treatment options have been developed. In general, AF can be classified into four different stages: initial, paroxysmal, persistent and permanent stage and its common clinical symptoms include palpitations, chest pain, dyspnoea, fatigue, dizziness and syncope [4]. Atrial fibrosis is a common clinical characteristic of AF and its magnitude related to AF significantly differed in a variety of experimental studies [5, 6]. Regional conduction abnormalities potentially caused by interstitial atrial fibrosis may increase the vulnerability to AF [7].

A recent study has further confirmed that myocardial fibrosis can contribute to the development of both atrial fibrosis and AF [7]. Furthermore, the increased fibrosis will increase the recurrent risk of AF [6].

MicroRNAs (miRNAs) is a kind of post-transcriptional suppressor with small RNA molecules of about 22-61 nucleotides, which showed inhibitory activity against mRNA and a single miRNA may regulate various protein-coding genes. Accumulating evidence has suggested a critical role of miRNAs in the pathogenesis of AF, mainly through atrial remodeling [8-11]. Particularly, miR-30a involves in regulating cardiac and pulmonary fibrosis [12] and acts as a tumor suppressor in various types of cancer [13, 14]. Snail was firstly described as an essential fac-

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Table 1. Primer sequence of luciferase reporter

	Sequence
Snail 1 3'UTR	Sense: 5'-ATTCTAGACGAGGCTCCCTCTCCTCTCCA-3' Antisense: 5'-GCTCTAGAAATATCAATAAACTGTACATAT-3'
Snail 1 3'UTR mutation	Sense: 5'-CTGGGAGGAAGATGAAGCCATTTTAAAGGTA-3' Antisense: 5'-TACCTTTAAAAATGGCTTCATCTCCTCCAG-3'

Table 2. Primer sequence and program of RT-PCR

Gene		Sequence
Snail 1	Sense	5'-CAAGGAATACCTCAGCCTGG-3'
	Antisense	5'-ATTCACATCCAGCACATCCA-3'
Periostin	Sense	5'-GACTGCTTCAGGGAGACACA-3'
	Antisense	5'-TGATCGTCTTCTAGGCCCTT-3'
GADPH	Sense	5'-TGGTATCGTGAAGGACTCAT-3'
	Antisense	5'-GTGGGTGTCGCTGTTGAAGTC-3'
miR-30a	Sense	5'-CATCCTCGACTGGAAG-3'
	Antisense	5'-AACTGGTGTGCTGGAG-3'

Table 3. Result of luciferase report

Luciferase activity	WT	Mut	Vector
NC	1.00±0.10	1.00±0.17	1.00±0.14
miR-30a	0.36±0.02*	1.07±0.18	1.14±0.14

* $P < 0.05$ versus NC group.

tor for the formation of mesoderm [15-17]. Subsequently, a total of three members have been identified in the Snail family: Snail 1 (Snail), Snail 2 (Slug) and Snail 3 (Smuc) [17, 18]. Snail family members encode transcriptional repressors and they share a similar structure in vertebrate. In recent years, studies have shown that miRNA-30a could target Snail 1 through post-translation modifications [12].

Previous reports suggest that miR-30a and its targeted protein snail 1 may participate in the development of AF. However, it is still unknown whether miR-30a and its targeted protein snail 1 are involved in the AF-induced myocardial fibrosis and the molecular mechanisms of AF have not been thoroughly delineated. Therefore, it is important to illuminate the molecular and cellular mechanisms of miR-30a and snail 1 in AF-induced myocardial fibrosis. In this study, we intend to determine whether the expression of miR-30a and snail 1 is related to AF-induced myocardial fibrosis and to explore the role of miR-30a and snail 1 in preventing and curing AF-induced myocardial fibrosis.

Materials and methods

Cell culture

Myocardial fibroblasts were isolated from Sprague-Dawley rats, as described previously [19]. Cells were characterized using the immuno histochemical of vimentin. Then cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and incubated at 37°C in a humidified incubator with 5% CO₂.

On experiment, cells were cultured in the presence of

Ang II. In order to decide the optimal condition of Ang II for cell culture, cells were treated with different Ang II concentrations (including 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵ mol/L) for 24 h and MTT confirmed that 10⁻⁶ mol/L was the optimal concentration. Then cells were treated by different length of time (including 6 h, 12 h, 24 h, 48 h) with 10⁻⁶ mol/L Ang II and checked with MTT to confirm a better control [20].

Cells were divided into four groups for the purpose of generating a stable miR-30a-expressing or anti-miR-30a-expressing cell line. Except for the control group, cells were stably transfected with miR-30a mimics vector, miR-30a inhibitors vector and negative control vector using the Lipofectamine LTX kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Cells were treated with 10⁻⁶ mol/L Ang II in 24 h before they were transfected.

Luciferase reporter

The fragment of snail 1 3'UTR sequence was cloned into pGL3 vector at the XbaI-digested site [21]. Then the mutation of snail 1 3'UTR

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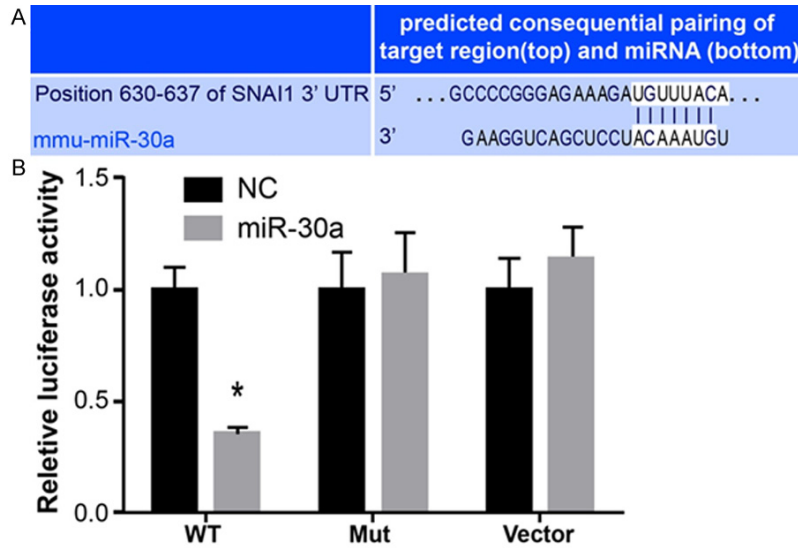


Figure 1. Result of luciferase report. A: Putative targets were predicted by TargetScan; B: The luciferase reporter assay of Snail 1 3'UTR reporter in myocardial fibroblasts at 48 h after transfection (* $P < 0.05$ versus the NC group).

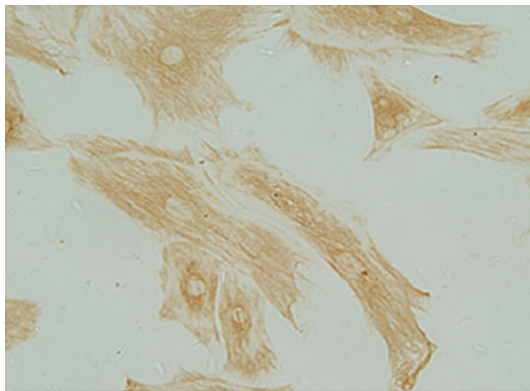


Figure 2. Immuno-histochemical of vimentin (yellow) in myocardial fibroblasts, $\times 400$.

was established by replacing four nucleotides in binding site of miR-30a using the Quik Change site-directed mutagenesis kit (stratagene). All primers used for PCR were shown in **Table 1** in which the red sequence indicated the mutated site. Myocardial fibroblasts were plated in 48-well plates which were cotransfected with 200 ng pGL3-control luciferase reporter, 10 ng of the pRL-TK vector, miR-30a or negative control vector using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. The luciferase assay was performed at 48 h after transfection.

Animal model

All animal experiments were performed under the protocol approved by the Institutional Animal Care and Use Committee of Seoul National University Hospital and complied with the National Institutes of Health Guide.

80 male New Zealand rabbits weighing 2 to 2.5 kg were purchased from the Laboratory Animal Center of Chinese Medical University. A rabbit model of rapid cardiac pacing was induced, as previously described [22]. Rabbits were randomly divided into the sham group (n=40 in group

A) and the model group (n=40 in group B). Cardiac tissue were collected for analysis at 3, 7, 14 and 28 days after operation. Then rabbits were further divided into 8 groups including group A1-A4 and B1-B4 (n=10 in each groups). All rabbits were put pacing electrodes in left atrium epicardial surgically, rabbits in group B were paced with 900 min^{-1} on the 4 days after surgery for the period of 4 weeks.

Tissue preparation and masson's trichrome staining

On day 3, 7, 14, 28 after surgery, rabbits were sacrificed by the injection of xylazine hydrochloride, the left atrial for each rabbit was caught and the left atrial mass was calculated.

Histologically, paraffin sections from the myocardial tissue were stained with a Masson's Trichrome kit (Sigma) [23]. The images were obtained using a photomicroscope together with a DP50 camera and each section was measured at three random sites.

Real-time PCR

Total RNA and miRNA were isolated from myocardial fibroblasts or myocardial tissue using-Trizol Reagent (Invitrogen). Expression of miRNA was detected by the TaqMan microRNA assay (CA) according to manufacturer's instructions.

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Table 4. The influence of different density of Ang II for cell activity

	control	10 ⁻⁸ mol/L	10 ⁻⁷ mol/L	10 ⁻⁶ mol/L	10 ⁻⁵ mol/L
OD value	0.117±0.007	0.132±0.007	0.147±0.007*	0.174±0.005*#、○	0.189±0.007*#、○

*P < 0.05 versus control group; #P < 0.05 versus 10⁻⁸ mol/L group; ○P < 0.05 versus 10⁻⁷ mol/L group.

Table 5. The influence of different time of Ang II for cell activity

	0 h	6 h	12 h	24 h	48 h
OD value	0.102±0.013	0.120±0.003	0.134±0.007*	0.155±0.015*#	0.168±0.011*#、○

*P < 0.05 versus 0 h group; #P < 0.05 versus 6 h group; ○P < 0.05 versus 12 h group.

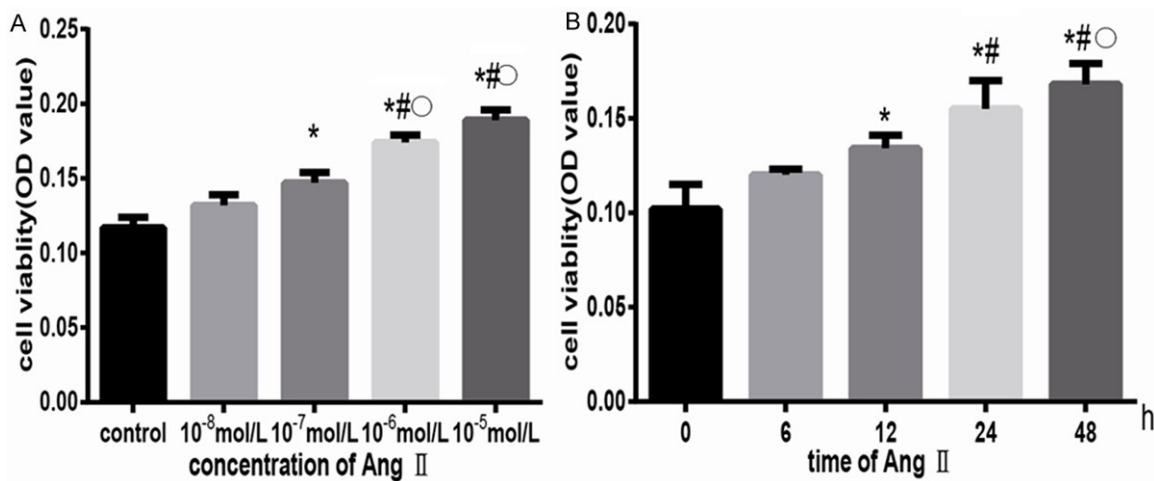


Figure 3. The cell activity is up-regulated by Ang II in myocardial fibroblasts. A: *P < 0.05 versus the control group; #P < 0.05 versus the 10⁻⁸ mol/L group; ○P < 0.05 versus the 10⁻⁷ mol/L group. B: *P < 0.05 versus the 0 h group; #P < 0.05 versus the 6 h group; ○P < 0.05 versus the 12 h group.

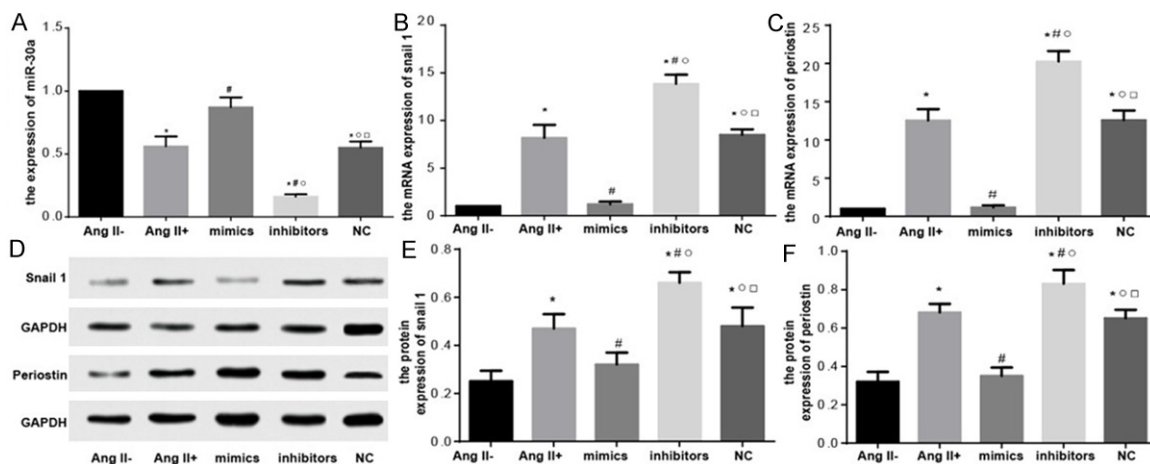


Figure 4. Ang II and the transfection of miR-30a affect the expression level of miR-30a, snail 1 and periostin in myocardial fibroblasts. A-C: Quantitative data of mRNA level of miR-30a, snail-1 and periostin in cells with different controls. D: Western blot analysis of snail 1 and GAPDH in myocardial fibroblasts. E, F: protein level of snail-1 and periostin in cells with different controls (normalized to GAPDH). Data was presented using means ± SD for the three independent experiments. *P < 0.05 versus the Ang II- group, #P < 0.05 versus the Ang II+group, ○P < 0.05 versus the mimics group, □P < 0.05 versus the inhibitor group.

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Table 6. The mRNA expression level of miR-30a, snail 1 and periostin in myocardial fibroblasts

mRNA expression level	miR-30a	Snail 1	Periostin
Ang II ⁻	1.00±0.00	1.00±0.00	1.00±0.00
Ang II ⁺	0.56±0.05*	8.15±1.39*	12.52±1.51*
Ang II ⁺ , miR-30a mimics ⁺ (mimics group)	0.87±0.08 [#]	1.19±0.28 [#]	1.16±0.29 [#]
Ang II ⁺ , miR-30a inhibitors ⁺ (inhibitor group)	0.16±0.02 ^{*,#}	13.83±0.99 ^{*,#}	20.23±1.39 ^{*,#}
Ang II ⁺ , empty vector ⁺ (NC group)	0.55±0.05 ^{*,○,□}	8.47±0.60 ^{*,○,□}	12.57±1.30 ^{*,○,□}

**P* < 0.05 versus Ang II⁻ group, #*P* < 0.05 versus Ang II⁺ group, ○*P* < 0.05 versus mimics group, □*P* < 0.05 versus inhibitor group.

Table 7. The protein expression level of snail 1 and periostin in myocardial fibroblasts

Protein expression level	Snail 1	Periostin
Ang II ⁻	0.25±0.044	0.32±0.052
Ang II ⁺	0.47±0.060*	0.68±0.046*
Ang II ⁺ , miR-30a mimics ⁺ (mimics group)	0.32±0.050 [#]	0.35±0.044 [#]
Ang II ⁺ , miR-30a inhibitors ⁺ (inhibitor group)	0.66±0.045 ^{*,#}	0.83±0.072 ^{*,#}
Ang II ⁺ , empty vector ⁺ (NC)	0.48±0.077 ^{*,○,□}	0.65±0.045 ^{*,○,□}

**P* < 0.05 versus Ang II⁻ group, #*P* < 0.05 versus Ang II⁺ group, ○*P* < 0.05 versus mimics group, □*P* < 0.05 versus inhibitor group.

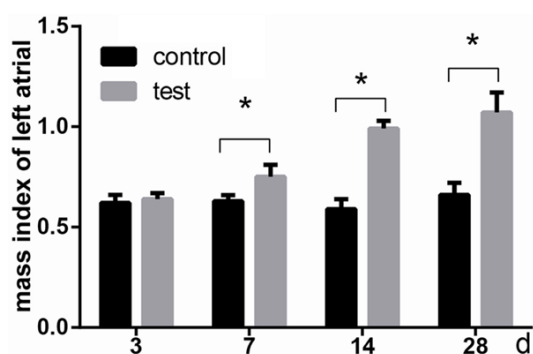


Figure 5. The left atrial mass value of rabbits in the model group is significantly higher compared with those of the control group from day 7 to day 28 (**P* < 0.05 versus the control group).

For other mRNA, total RNA was reverse transcribed to cDNA by RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD) and subsequently amplified by PCR, all primers were shown in **Table 2**. GAPDH was used as control and three independent experiments were performed.

Western blot analysis

Cells and tissues were lysed by radio immunoprecipitation assay buffer. Total protein was subjected to SDS-PAGE and transferred to a nitrocellulose membrane. Protein expression was analyzed by Western blot with primary anti-

body against snail 1 (Cell Signaling Technology, Danvers, MA) or periostin (Southern Biotech, Birmingham, AL) and then incubated with an appropriate secondary antibody. After washing, the protein was visualized using Super Signal Western Picochemiluminescent substrate (Pierce, Rockford, IL), and

signals were detected by an Odyssey Detection System (Li-COR, Lincoln, NE). The relative protein levels of Snail 1 and periostin were normalized to GAPDH, and then the ratio was compared with that of the control group.

Statistical analysis

Measurement data were expressed as means ± SD. The unpaired t test or one-way analysis variance was used to compare the differences between groups. *P* values of less than 0.05 were considered as statistically significant. All statistical analyses were performed by SPSS 17.0 (SPSS, Chicago, IL).

Results

Result of luciferase report

As shown in **Table 3** and **Figure 1**, compared with those of the mutant snail 1 3'UTR reporter and contrast vector, over expression of miR-30a in myocardial fibroblasts attenuated the activity level of wild-type Snail 1 3'UTR reporter to 35%. As a result of this, we demonstrated that miR-30a could target snail 1 by binding to its 3'UTR in myocardial fibroblasts.

Cell culture and Ang II-treated cells

In optical microscope (×400), myocardial fibroblasts presented triangle, shuttle or polygon

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Table 8. The left atrial mass value of rabbits in control group and model group

Group	n	Left atrial mass value (g/kg)			
		3 d	7 d	14 d	28 d
Control group	10	0.62±0.04	0.63±0.03	0.59±0.05	0.66±0.06
Model group	10	0.64±0.03	0.75±0.06*	0.99±0.04*	1.07±0.10*

**P* < 0.05 versus control group.

patterns. The results of immunohistochemical experiment indicated that vimentin protein is stained yellow and distributed evenly in of cell plasma with a purity of 95% (Figure 2).

The result of MTT in Ang II-treated cells showed that Ang II can up-regulate the cell activity of myocardial fibroblasts in a dose- and time-dependent manner (Tables 4, 5; Figure 3). In this experiment, 10⁻⁶ mol/L Ang II for 48 h resulted in a remarkable loss of the cobblestone-like growing appearance and a gain of elongated fibroblast-like morphological features. A fibrosis model of cardiac fibroblasts was established in this group.

Over expression of miR-30a attenuates Ang II-induced the expression of snail 1 and periostin

As shown in Figure 4 and Table 6, cells treated with Ang II were significantly associated with an up-regulation of snail 1 and periostin at both the mRNA and protein levels, but a down-regulation of miR-30a. MiR-30a significantly blocked Ang II, the transfect of miR-30a mimics can decrease the level of snail 1 and periostin expressions. By contrast, miR-30a inhibitors vector can induce the expression of snail 1 and periostin (Tables 6, 7; Figure 4).

Left atrial mass and masson's trichrome staining

Compared with the control group, there was no significant difference in the value of left atrial mass between the control and the model group on day 3. However, the difference was significant from day 7 to day 28 (Figure 5; Table 8).

As shown in Figure 6, compared with the control group, fibrous tissue was strongly stained (blue) in cardiac tissue of model rabbits, which suggested that rapid pacing was involved in the disease process. The GV value of fibrous tissue in model group did not reach statistical signifi-

cance at day 3, but it was significantly higher than that of the control group over 7 to 28 days (Table 9; Figure 7).

The expression of miR-30a, snail 1 and periostin in model rabbits

As shown in Table 10 and Figure 8, rapid pacing in model rabbits resulted in significantly lower average miR-30a expression levels over 7 to 28 days. Real-time PCR showed that the model animals had a distinct up-regulation of mRNA expression levels of snail 1 and periostin over 7 to 28 days compared with the expression level on day 3. The mRNA expression reached the highest level on day 14 and then down-regulated between day 14 and day 28. Results from western plot suggested that the protein expression levels of snail 1 and periostin was similar to the result of their mRNA. The only difference was that the expression level of periostin protein remained the highest on day 28 (Tables 10, 11; Figure 8).

Discussion

MicroRNAs play crucial roles in atrial fibrillation. For instance, Xiao et al. validated a total of 136 differentially expressed miRNA in AF by combining miRNA microarray and miRNA blot, including 50 upregulated and 86 downregulated miRNAs [24]. Further research has suggested the evidence that miRNA participated in atrial structural remodeling especially in myocardial fibrosis. Down-regulation of miRNA-21 could suppress the atrial fibrillation through its target genes [10]. The over-expression of miRNA-133 and miRNA-590 promoted myocardial fibrosis and participated in AF susceptibility matrix formation by inhibiting target genes TGF-β₁ and TGF-βR II [25]. The mechanisms of miRNA regulating AF-induced myocardial fibrosis are still unclear. Here, we found that miR-30a is down-regulated in the atrial fibrosis caused by AF and showed that the over-expression of miR-30a might suppress myocardial fibrosis.

In the myocardial fibrosis cells stimulated by Ang II in rats [26, 27], the expression level of miR-30a was down-regulated while the expression level of snail 1 and periostin were up-regulated. The analysis of TargetScan system showed that miR-30a could target snail 1

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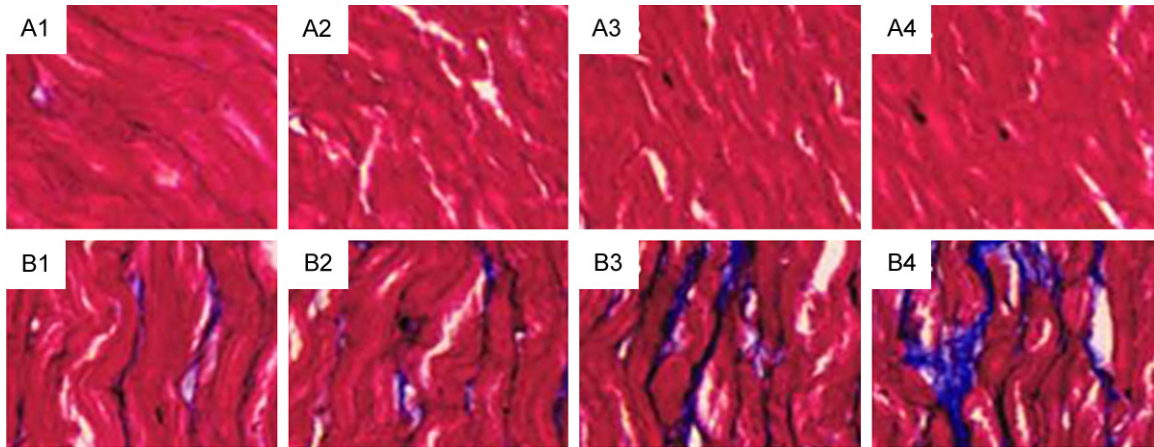


Figure 6. Masson staining of fibrous tissue in atrial of rabbits. A-H: Atrial tissue of rabbits in each group, fibrous tissue was stained with blue, $\times 200$. (A-D: the sham operated group, E-H: the rapid pacing group). There was no obvious fibrous tissue presented in the sham operated group between day 3 and day 28 whereas the fibrous tissue area increased substantially from day 3 to day 28 in the rapid pacing group.

Table 9. GV value of atrial tissue in Masson stained rabbits

Group	n	GV value of atrial tissue			
		3 d	7 d	14 d	28 d
Control group	10	0.45 \pm 0.04	0.46 \pm 0.05	0.44 \pm 0.06	0.45 \pm 0.04
Model group	10	0.47 \pm 0.04	0.82 \pm 0.04*	1.15 \pm 0.05*	1.47 \pm 0.07*

* $P < 0.05$ versus control group.

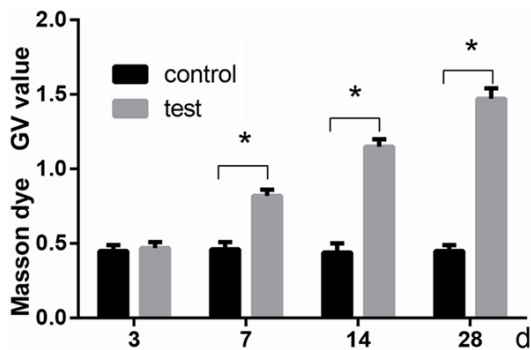


Figure 7. The average GV value of atrial tissue in the model group is significantly higher than that in the control group from day 7 to day 28 day after operation (* $P < 0.05$ versus control group).

through binding to its 3'-URT region. Results of dual-luciferase reporter assay also suggested that miR-30a could directly target snail 1 gene. Transfected with miR-30a mimic to cardiac fibroblasts, the up-regulation of miR-30a can significantly decrease the expression level of snail 1. Moreover, the expression level of periostin also significantly decreased which is closely correlated with myocardial fibrosis. A few rabbit animal experiments based on the

rapid atrial pacing model [28, 29] revealed that the expression level of miR-30a in the surgery group was significantly down-regulated, while snail 1 and periostin expression levels significantly increased accompanied with the aggra-

vation of myocardial fibrosis in cardiac tissues. Therefore, the present study suggested a close link between miR-30a, snail 1 and myocardial fibrosis. On the basis of our results, it appears that miR-30a suppresses snail 1 in myocardial fibroblasts, which then results in a down-regulated expression of periostin via some unknown mechanisms. On the other hand, the over-expression of miR-30a level causes a down-regulation of snail 1 protein due to an enhanced translational inhibition by miR-30a. Hence, the decreased snail 1 expression level results in periostin production removal and fibrosis generation.

The significance of miR-30a in lung fibrosis was demonstrated by Mao et al. [30] who revealed that miR-30a up-regulation could represses AECs-II apoptosis through decreasing mitochondrial fission depending on inhibiting Drp-1 expression and translocation, and they can be novel therapeutic targets for lung fibrosis. Besides that, Zhou et al. discovered the down-regulation of miR-30a in peritoneal tissues of progressive fibrosis in patients and a rat model of peritoneal dialysis. They also identified snail

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Table 10. The mRNA expression level of miR-30a, snail 1 and periostin in operated rabbits

mRNA expression level of miR-30a, snail 1 and periostin		3 d	7 d	14 d	28 d
Model group	miR-30a	1.01±0.18	0.53±0.14*	0.57±0.17*	0.54±0.16*
	Snail 1	0.98±0.24	17.44±2.00*	26.84±2.97* [#]	5.91±1.95* ^{#,○}
	Periostin	0.97±0.58	35.17±4.82*	57.93±7.64* [#]	17.93±4.13* ^{#,○}

**P* < 0.05 versus 3 d group, #*P* < 0.05 versus 7 d group, ○*P* < 0.05 versus 14 d group.

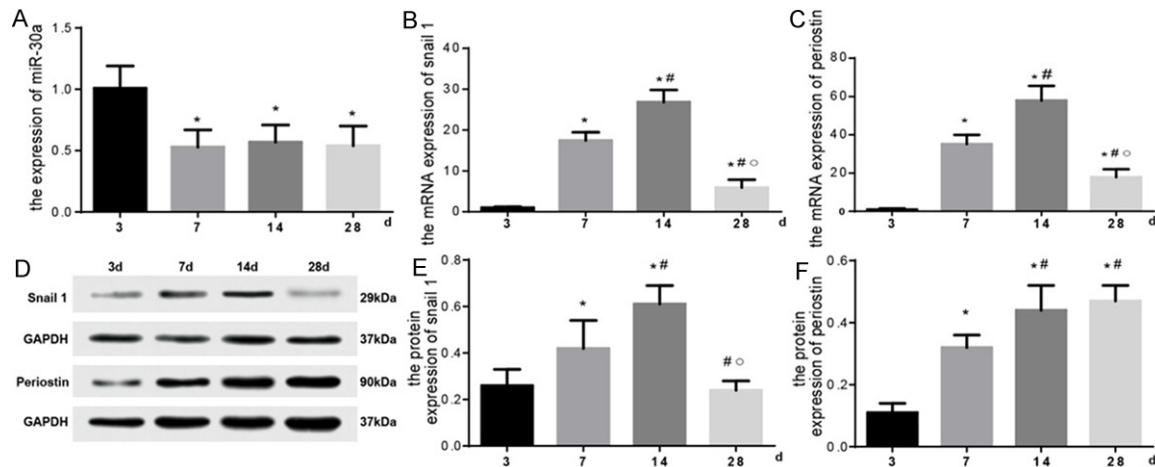


Figure 8. Rapid pacing operations affect the expression level of miR-30a, snail 1 and periostin in model rabbits. A-C: Quantitative data of mRNA level of miR-30a, snail-1 and periostin in model rabbits. D: Western blot analysis of snail 1 and GAPDH in atrial tissue of model rabbits. E, F: Quantitative data protein level of snail-1 and periostin in model rabbits (normalized to GAPDH). **P* < 0.05 versus the 3 d group, #*P* < 0.05 versus the 7 d group, ○*P* < 0.05 versus the 14 d group.

Table 11. The protein expression level of snail 1 and periostin in operated rabbits

Protein expression level of snail 1 and periostin		3 d	7 d	14 d	28 d
Model group	Snail 1	0.26±0.07	0.42±0.12*	0.61±0.08* [#]	0.24±0.04* ^{#,○}
	Periostin	0.11±0.03	0.32±0.04*	0.44±0.08* [#]	0.47±0.05* [#]

**P* < 0.05 versus 3 d group, #*P* < 0.05 versus 7 d group, ○*P* < 0.05 versus 14 d group.

1 as a target of miR-30a and demonstrated that snail 1 was repressed by miR-30a in TGF-β1 induced peritoneal fibrosis. Additionally, MiR-30a up-regulation was associated with the down-regulation of snail 1, epithelial-mesenchymal transition, and inhibited peritoneal fibrosis [21].

Our study found that miR-30a decreased significantly in cardiac fibrosis cells and tissues, while up-regulation of miR-30a could inhibit the cardiac fibrosis by targeting snail 1. As a result of this, miR-30a could be considered as a negative regulator for cardiac fibrosis. Snail family including snail 1, snail 2 and snail 3 is an important set of regulators for fibrosis in various

organs such as kidney [31, 32], liver [33] and lung [34]. However, the function of snail related to the pathogenesis of cardiac fibrosis was unknown. We found that snail 1 was significantly over-expressed in cardiac fibroblasts and cardiac tissues, as well as the periostin expression level which could reveal the extent of myocardial fibrosis [35-37]. When the up-regulated miR-30a inhibited the expression level of snail 1, then significant decrease in the periostin expression level was found in cardiac fibroblasts. Thus, snail 1 might be associated with myocardial fibrosis and our results were consistent with those obtained from previous studies [38-40]. As a result of this, targeting snail 1 by miR-30a might be a novel approach for treating

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cardiac fibrosis. Although we have demonstrated the critical role of miR-30a and snai1 in myocardial fibrosis, functions of other miRNAs have not been identified and the influence of their specific molecular mechanisms on myocardial fibrosis are still unclear.

In conclusion, the present study demonstrated that miR-30a was down-regulated in myocardial fibrosis cells and cardiac tissues as suggested by the rabbit model. MiR-30a can negatively regulate AF-induced myocardial fibrosis by targeting snai1 in vitro and over-expression of miR-30a may be considered as an effective therapeutic method for myocardial fibrosis.

Disclosure of conflict of interest

None.

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