Circular RNA hsa_circ_0000118 promotes atrial fibrosis by regulating the microRNA-34a-5p/Smad4 axis

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Abstract. The regulatory functions and underlying mechanisms of circular RNAs (circRNAs/circs) in atrial fibrillation (AF) are largely unknown. The present study aimed to assess the prognostic roles and potential biological functions of hsa_circ_0000118 in structural remodeling in AF. Gain- and loss-of-function cell models were established in mouse cardiac fibroblasts. Cell Counting Kit-8 and Transwell assays were used to analyze the proliferation and migration of cardiac fibroblasts. To evaluate the underlying mechanisms, RNA immunoprecipitation and luciferase reporter assays were performed. hsa_circ_0000118 was demonstrated to be significantly upregulated in atrial tissues of patients with valvular heart disease with AF compared to those without AF. Elevated plasma levels of hsa_circ_0000118 were independently associated with poor prognosis in patients with AF. *In vitro*, hsa_circ_0000118 promoted collagen I and collagen III expression, and the migration of cardiac fibroblasts. Functional assays demonstrated that hsa_circ_0000118 acted as a competing endogenous RNA of microRNA (miR)-34a-5p to reduce the suppressive effect of miR-34a-5p on its target Smad4 in cardiac fibroblasts. In conclusion, hsa_circ_0000118 may serve as a non-invasive prognostic biomarker for AF. The newly identified roles of hsa_circ_0000118 in AF provide a mechanistic understanding of structural remodeling in AF and pave the way towards novel therapies.

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Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice worldwide, and is primarily attributed to aging, obesity, alcohol use, hypertension, diabetes, obstructive sleep apnea, valvular heart disease and heart failure (1). Due to the increasing morbidity and mortality rates associated with AF, it has become a serious public health problem worldwide. According to the 2019 Global Burden of Disease Study, there were ~59.7 million cases of AF worldwide (2), resulting in ~315,000 disease-associated mortalities and 8.39 million disability-adjusted life-years in 2019 (3).

Research suggests that triggering (abnormal pacing or ectopic electrical activity) and maintenance (reentry, single or multiple wavelet hypothesis) disorders promote the occurrence and development of AF, and atrial remodeling is the pathological basis (4). Atrial remodeling is characterized by electrical and structural remodeling, which manifest as shortened action potential duration and atrial fibrosis, respectively (5). Although previous research has explored the molecular mechanism underlying atrial remodeling, the pathogenesis of AF is not fully understood, and the efficacy of current therapies for AF is suboptimal (6). Several non-coding RNAs have been reported to serve important roles in structural remodeling, electrical remodeling and autonomic nerve remodeling in AF, which has opened up novel prospects for the development of therapies and improving the prognosis of patients with AF (7-9).

Circular RNAs (circRNAs/circs) are single-stranded, covalently closed non-coding RNAs without 5'end caps or 3'poly(A) tails. This molecular characteristic makes them more stable than linear RNAs and ideal biomarkers for diseases (10).

Although the diagnostic, prognostic and potential therapeutic values of circRNAs in other diseases, such as cancer (11), acute ischemic stroke (12) and non-alcoholic fatty liver disease (13), have been intensively researched over the past decade, their regulatory role in AF remains to be explored. Previous studies have reported that circRNA expression is dysregulated in patients with AF (14,15). Furthermore, several circRNAs have been reported to be involved in atrial fibrosis. For example, circRNA_010567 and circRNA_000203 have been reported to promote cardiac fibrosis by sponging microRNA (miRNA/miR)-141 and miR-26b-5p, respectively (16,17). However, there is still a lack of understanding in terms of the regulatory function and underlying mechanism of circRNAs in AF.

Our previous study used RNA sequencing to assess differences in circRNA expression profiles between 7 patients with AF and 7 patients without AF. The results revealed that 280 circRNAs were differentially expressed with a fold change of >1.5 (18). As non-valvular AF is the most common type of AF (96.1% in a national cross-sectional epidemiological study of China from July 2020 to September 2021) (19), the present study aimed to assess the prognostic value of the candidate circRNAs in non-valvular AF. However, as the availability of atrial tissues from patients with non-valvular AF is limited (few patients with non-valvular AF patients ever undergo surgery, making their cardiac tissue rare for researchers), candidate dysregulated circRNAs in valvular AF were first determined and, subsequently, their potential as a prognostic biomarker in non-valvular AF was assessed.

The present study aimed to evaluate the prognostic role of hsa_circ_0000118, a upregulated circRNA and plasma-stable circRNA, in non-valvular AF, and to explore its role in cardiac fibroblasts and potential action mechanisms. The present study may hold significance for screening circRNAs with clinical prognostic value in AF, and elucidating its function and regulatory mechanism in atrial structural remodeling.

Materials and methods

Subjects and sample collection. The present study was a retrospective study. Patients with valvular heart disease with and without AF were consecutively recruited at Southwest Hospital of Army Medical University (Chongqing, China) between January 2014 and December 2017. In our previous study, a total of 7 pairs of atrial tissues from age- and sex-matched patients with and without AF were subjected to Hiseq/Proton RNA sequencing, as described previously (18). A total of 34 pairs of age- and sex-matched atrial tissues from patients with and without AF (average age, 49.2 years; male: 28.6%) were used to validate candidate circRNA expression by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) between July 2021 and August 2021 (18). Briefly, the inclusion criteria were as follows: i) Diagnosis of valvular heart disease; and ii) recent diagnosis of AF (AF group) or no AF (non-AF group). AF was diagnosed according to the guidelines for the management of AF from the European Society of Cardiology (20). The exclusion criteria were as follows: i) Presence of other arrhythmias; and ii) comorbid cardiovascular disease, including coronary heart disease, congenital heart disease or myocardial bridge. All atrial tissues were obtained during valve replacement or valvuloplasty, and were frozen in liquid nitrogen at -196˚C immediately after resection.

To assess the possibilities of the candidate dysregulated circRNA as a biomarker in non-valvular AF, venous blood collected from 221 continuously recruited newly diagnosed patients with non-valvular AF (average age: 68.3 years; male: 52.5%) at Southwest Hospital of Army Medical University between January 2014 and December 2017 was used. The following inclusion criteria were used: i) Recent diagnosis of non-valvular AF [AF was diagnosed according to the guidelines for the management of AF from the European Society of Cardiology (20)]; and ii) age ≥18 years. Furthermore, the exclusion criteria for patients with non-valvular AF included: i) Diagnosis of structural heart disease; ii) moderate-to-severe mitral stenosis; iii) malignant tumor; iv) prosthetic valve replacement; v) sepsis; vi) hyperthyroidism; vii) history of drug abuse; and viii) undergoing ablation. Venous blood was collected from patients with AF within 24 h of admission without any treatment, and the anticoagulant ethylenediaminetetraacetic acid was added. Subsequently, the collected blood was centrifuged at 400 x g for 15 min at 4˚C within 2 h after drawing. The upper plasma fraction was aspirated and divided into 500 μl aliquots into cryopreservation tubes for long-term storage in a -80˚C refrigerator. The expression levels of the 12 dysregulated circRNAs in 29 plasma samples (mean age, 70.3 years, male: 58.6%) and those of hsa_circ_0000118 in 192 plasma samples (average age: 68.0 years, male: 51.6%) were examined between September 2021 and October 2021.

The baseline clinical characteristics of patients were collected from electronic medical records according to the designed questionnaire, which included age, sex, lifestyle, medical history, and examination results. The primary end points of the present study were stroke and all-cause death, and these data were collected retrospectively by a trained investigator based on medical records and via telephone. Stroke was defined as an ischemic event diagnosed by CT or magnetic resonance imaging with clinical symptoms such as neurological dysfunction lasting for >24 h. All-cause death was defined as death from any cause. If the patient died, the cause and time of death were documented.

Written informed consent was obtained from all included patients. The study protocol, including tissue and blood sample utilization procedures, was approved by the Ethics Committee of Southwest Hospital, Army Medical University (approval no. KY2020231; Chongqing, China). All procedures involving human participants were performed in accordance with The Declaration of Helsinki.

circRNA selection. In the present study, the candidate circRNAs were first selected based on previous RNA sequencing (18), and the screening criteria for circRNAs were as follows: i) Fold change of >2, with P<0.05 and a false discovery rate of <0.05; ii) counts were >0 in at least 8 atrial tissue samples for sequencing; and iii) the host genes of the circRNAs were associated with possible mechanisms of AF, such as apoptosis, fibrosis, energy metabolism and inflammation (21), which were identified through circbank (circbank.cn/) and GeneCards (genecards.org/). Subsequently, circRNAs that met the inclusion criteria were further verified to be stable in the plasma of 29 patients with non-valvular AF by RT-qPCR (Fig. S1).

The candidate circRNA (hsa_circ_0000118) that was stably expressed in all plasma samples was selected for further assessment of its expression in 34 pairs of atrial tissues from patients with and without AF, and the association between its expression in plasma samples from 192 patients with AF and prognosis.

Cell culture. The mouse cardiac fibroblast cell line (cat. no. 340098) was purchased from BeNa Culture Collection; Beijing Beina Chunglian Institute of Biotechnology. The cells were cultured in RPMI-1640 medium (HyClone; Cytiva) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin solution (Beyotime Institute of Biotechnology). All cells were cultured at 37˚C in a 5% CO_2 incubator.

Plasmid construction and cell transfection. The mouse homologous circRNA of hsa_circ_0000118, mmu_circ_0010297, was identified using the circBase database (circbase.org/) and amplified by PCR and subcloned into the pLC 5-ciR vector to form the pLC 5-ciR-C10297 overexpression vector by Guangzhou Gisai Biotechnology Co., Ltd. The empty plasmid vector pLC 5-ciR was used as a control. Sanger sequencing was used to demonstrate whether the plasmid was constructed successfully. Furthermore, five small interfering RNAs (siRNAs/sis) were synthesized for mmu_circ_0010297 silencing, along with a non-targeting siRNA negative control (siNC), and miR-34a-5p mimics were prepared for overexpression, together with a non-targeting mimic NC (Shanghai GenePharma Co., Ltd.). The sequences of the siRNAs and miR-34a-5p mimics are listed in Table SI. The plasmid (2 μg), siRNAs, mimics (2.5 μg), or NC (all 2.5 μg) were transfected for 6 h at room temperature into cells using Lipofectamine™ 2000 (Invitrogen™; Thermo Fisher Scientific, Inc.) in 6-well plates according to the manufacturer's instructions. RNA was extracted after 48 h of transfection and protein was extracted after 72 h.

RT-qPCR. Total RNA was extracted from tissues, plasma and cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The Primer Script™ RT Reagent kit with gDNA Eraser (Takara Biotechnology Co., Ltd.) was used for cDNA synthesis for circRNAs and mRNAs, while stem-loop RT-qPCR using the miRNA RT Reagent Kit (Sangon Biotech Co., Ltd.) was used for miRNAs. RT was performed as follows: 2 min at 42˚C followed by holding at 4˚C, and 15 min at 37˚C followed by 5 sec at 85˚C and final holding at 4˚C for the second step, while the stem-loop method utilizes a protocol of 30 min at 16˚C, 30 min at 37˚C, 5 min at 85˚C and subsequent maintenance at 4˚C. Subsequently, cDNA was amplified using SYBR® Premix Ex Taq™ (Takara Biotechnology Co., Ltd.). The thermocycling conditions consisted of an initial step at 95˚C for 30 sec, followed by 40 cycles of denaturation at 95˚C for 5 sec and annealing/extension at 60˚C for 30 sec. All experiments were performed according to the manufacturer's instructions. The primer sequences used in the experiments are listed in Tables SII and SIII. The relative expression of genes was calculated using the $2^{-\Delta\Delta Cq}$ method (22). β-actin was used as the internal reference for circRNAs and mRNAs, while U6 was used as the internal reference for miRNAs.

RNase R treatment. circRNA expression was assessed using quantification of circRNAs and their linear parent genes in RNA samples treated with and without RNase R enzyme. RNase R enzyme is an exonuclease that can digest linear RNA molecules without degrading circRNAs due to their covalently closed circular structure (23). The same batch of total RNA was equally divided into two groups, in which one group was treated with 8 U RNase R, 8 ng RNA and 2 μl 10X RNase R buffer, and the other group was not treated with RNase R. In both groups, RNase R-free water was added to 20 μl, and samples were digested at 37˚C for 30 min. Subsequently, the stability of both hsa_circ_0000118 (circRNA) and MANIA2 (linear mRNA) was evaluated by RT-qPCR as aforementioned.

Cell Counting Kit-8 (CCK-8) assay. Cardiac fibroblast cells were seeded at a density of 3,000 cells per well into 96-well cell culture plates. Cell proliferation was detected on days 1, 2, 3, 4 and 5 after transfection using CCK-8 reagent (Dojindo Laboratories, Inc.). A mixture of 100 μl complete medium and 10 μl CCK-8 reagent was added to each well, and the absorbance of each sample was measured at a wavelength of 450 nm after incubation for 2 h in an incubator. The growth curves of cardiac fibroblasts were drawn based on the average absorbance of the three wells at each time point.

Transwell cell migration assay. After 48 h of transfection, cardiac fibroblasts were resuspended and diluted to $1x10^5$ cells/ml using FBS-free medium (RPMI-1640 culture medium, HyClone; Cytiva). A total of 100 μl cell suspension (containing 10,000 cells) was inoculated into the upper chamber of an insert (8-μm pore size; MilliporeSigma), and 500 μl medium (1640 culture medium, HyClone; Cytiva) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added to the lower chamber. After incubation at 37˚C for 12 h, cells that migrated were fixed with 100% anhydrous methanol for 30 min at room temperature, stained with 0.1% crystal violet for 15 min at room temperature and washed in distilled water. Subsequently, cell migration was assessed under a fluorescence microscope (ECLIPSE 55I; Nikon) after drying. Cells were manually counted in five fields of view, selected from four quadrants and the central area of the chamber.

Western blotting. Total protein was extracted from cells using RIPA (Beyotime Institute of Biotechnology) containing 1/10 volume of 100 mM PMSF (Beyotime Institute of Biotechnology). The protein concentration was detected using a bicinchoninic acid protein concentration determination kit (Beyotime Institute of Biotechnology). A total of 30 μg denatured protein was loaded and separated on SDS-PAGE gels (8%), which were prepared according to the molecular weight of the protein. Subsequently, the proteins were transferred to a PVDF membrane (Bio-Rad Laboratories, Inc.), which was blocked with 5% skimmed milk on a shaker at room temperature for 1 h. Subsequently, the PVDF membrane was placed in diluted primary antibodies (1:1,000) overnight at 4˚C. The primary antibodies used in the present study included: Collagen type I α1 chain (Col1a1; cat. no. ab270993; Abcam), collagen type III α1 chain (Col3a1; A0817; ABclonal Biotech Co., Ltd.), Smad4 (A5657; ABclonal Biotech Co., Ltd.) and β-actin (ab8227; Abcam). The next day, after three 15-min washes in 20 mM

PBS with 0.1% Tween 20 (PBST), the PVDF membrane was incubated with secondary antibodies (HRP-conjugated Goat anti-Rabbit IgG; 1:2,000; cat. no. AS014; ABclonal Biotech Co., Ltd.) for 1 h at room temperature and then washed three times with PBST before detection. Equal volumes of Developer A and B (Super ECL Plus Western Blotting Substrate, Bioground Biotech Co., Ltd.) were mixed to prepare the working solution. The washed PVDF membrane was saturated with this solution and detected in a gel imaging system (Bio-Rad). The densitometric analysis was performed using ImageJ (version 1.54f; National Institutes of Health).

Luciferase reporter assay. Using miRanda (cbio.mskcc.org/miRNA2003/miranda.html), miRDB (mirdb.org/) and RNA22 (https://cm.jefferson.edu/rna22/) databases to screen the potential candidate target miRNAs of mmu_circ_0010297. Wild-type mmu_circ_0010297 sequences that were predicted to bind miR-34a-5p and the corresponding mutant mmu_circ_0010297 sequences were constructed and inserted into the pmirGLO Dual-Luciferase vector (Promega Corporation) by Shanghai GenePharma Co., Ltd. (Fig. S2). The constructed plasmids were co-transfected with miR-34a-5p mimics or negative control mimics into mouse cardiac fibroblasts using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). A Dual-Luciferase reporter gene detection kit (cat. number: RG027, Beyotime Institute of Biotechnology) was used to detect the Firefly and *Renilla* luciferase activities after transfection for 48 h. The Firefly luciferase activity was normalized to the Renilla luciferase activity for each sample.

RNA immunoprecipitation (RIP). The EZ-Magna RIP Kit (MilliporeSigma) was employed to perform RIP according to the manufacturer's protocol. Briefly, 500 μl cell lysates (cell lysis buffer for Western and IP, Beyotime Institute of Biotechnology) of cardiac fibroblasts were incubated with 50 μl protein A/G beads coupled with anti-IgG or anti-argonaute 2 (5 μg, Ago2, ab186733; Abcam) overnight at 4˚C. The beads were collected using a magnetic stand and washed six times with RIP Wash Buffer (MilliporeSigma). The RNA extraction was performed by adding 150 μl of prepared Proteinase K Buffer to the washed beads, followed by incubation at 55 ˚C for 30 min with vortexing every 5 min. After magnetic separation, the supernatant was transferred to a new tube and mixed with 250 μl of RIP Wash Buffer. Then, 400 μl of RNA extraction buffer (composed of phenol : chloroform : isoamyl alcohol in a ratio of 125 : 24 : 1) was added, and the mixture was vortexed and centrifuged at 19,100 x g for 10 min at room temperature. The upper aqueous phase (350 μl) was collected, combined with 400 μl chloroform, vortexed, and centrifuged again under the same conditions. Subsequently, 300 μl aqueous layer was transferred to a new tube, and RNA was precipitated by adding 50 μl Salt Solution I (MilliporeSigma), 15 μl of Salt Solution II (MilliporeSigma), 5 μl of Precipitation Enhancer (MilliporeSigma), and 850 μl of anhydrous ethanol, followed by overnight storage at -80 ˚C. The sample was centrifuged at 19,100 x g for 30 min at 4 ˚C, washed with 1 ml 80% ethanol, and centrifuged at 19,100 x g for 15 min at 4˚C before air-drying. The immunoprecipitated RNA was finally dissolved in 10 μl nuclease-free water and was subjected to RT-qPCR analysis as described before.

Statistical analysis. All experiments were performed independently with ≥3 biological replicates. Continuous variables are presented as the mean ± standard deviation or median (interquartile range), and comparisons between two groups were made using the unpaired t-test if the data were normally distributed or the Mann-Whitney U test if the data were not normally distributed. Normality was assessed using the Shapiro-Wilk test. One-way ANOVA was used for comparisons among three groups if the data were normally distributed, and the Sidak's post hoc test was used for correction for multiple comparisons. The Kruskal Wallis test was used for comparison among three groups if the data were not normally distributed, and Dunn's post hoc test was used for multiple comparisons. The results of the CCK-8 assay were analyzed by two-way ANOVA, and the comparison between groups was corrected using the Sidak method. Categorical variables are presented as n (%).

For the prognostic analysis, the relative expression levels of hsa_circ_0000118 were transformed into binary variables, and X-tile software version 3.6.1 (Yale School of Medicine) was used to determine the best cut-off value. Survival analysis was performed using the Kaplan-Meier method with log-rank test for comparison. The multivariate Cox proportional hazards model was used to analyze the association between hsa_circ_0000118 and stroke and death outcomes by adjusting for potential confounding variables with $P<0.1$ in the univariate Cox proportional hazards model.

A two-sided $P<0.05$ was considered to indicate a statistically significant difference. All analyses were performed using SPSS 19.0 (IBM Corp.), R 3.5.2 (The R Foundation for Statistical Computing) and GraphPad Prism 9.0 (Dotmatics).

Results

Molecular characteristics of hsa_circ_0000118. A total of 12 dysregulated circRNAs (six upregulated and six downregulated) were selected based on previous RNA sequencing results, according to the predefined screening criteria. To identify the circRNAs that could be the potential biomarkers for AF prognosis, the expression levels of these 12 candidate circRNAs were examined in plasma samples from 29 patients with AF. hsa_circ_0000118 was the only stably expressed circRNA in all 29 plasma samples (Table SIV; Fig. S1). Subsequently, hsa_circ_0000118 expression was validated in atrial tissues from 34 patients with AF and 34 patients without AF using RT-qPCR and the outward primer of hsa_circ_0000118 (Table SII). Consistent with the sequencing results, it was demonstrated that hsa_circ_0000118 expression was significantly upregulated in atrial tissue of valvular heart disease patients with AF compared with those without AF (Fig. 1A). Therefore, hsa_circ_0000118 was selected as the candidate circRNA to assess its predictive effect in AF prognosis and its underlying molecular mechanism.

Using circbank, it was demonstrated that hsa_circ_000018 was derived from exons 2-5 of the mannosidase α class 1A member 2 (MAN1A2) gene and was located on the sense strand of chromosome 1 (data not shown). The resistance of hsa_circ_0000118 to digestion by the RNase R exonuclease confirmed that it was circular (Fig. 1B). Furthermore, a mouse homologous circRNA (mmu_circ_0010297) of hsa_circ_0000118 was identified using the circBase database (Fig. S3).

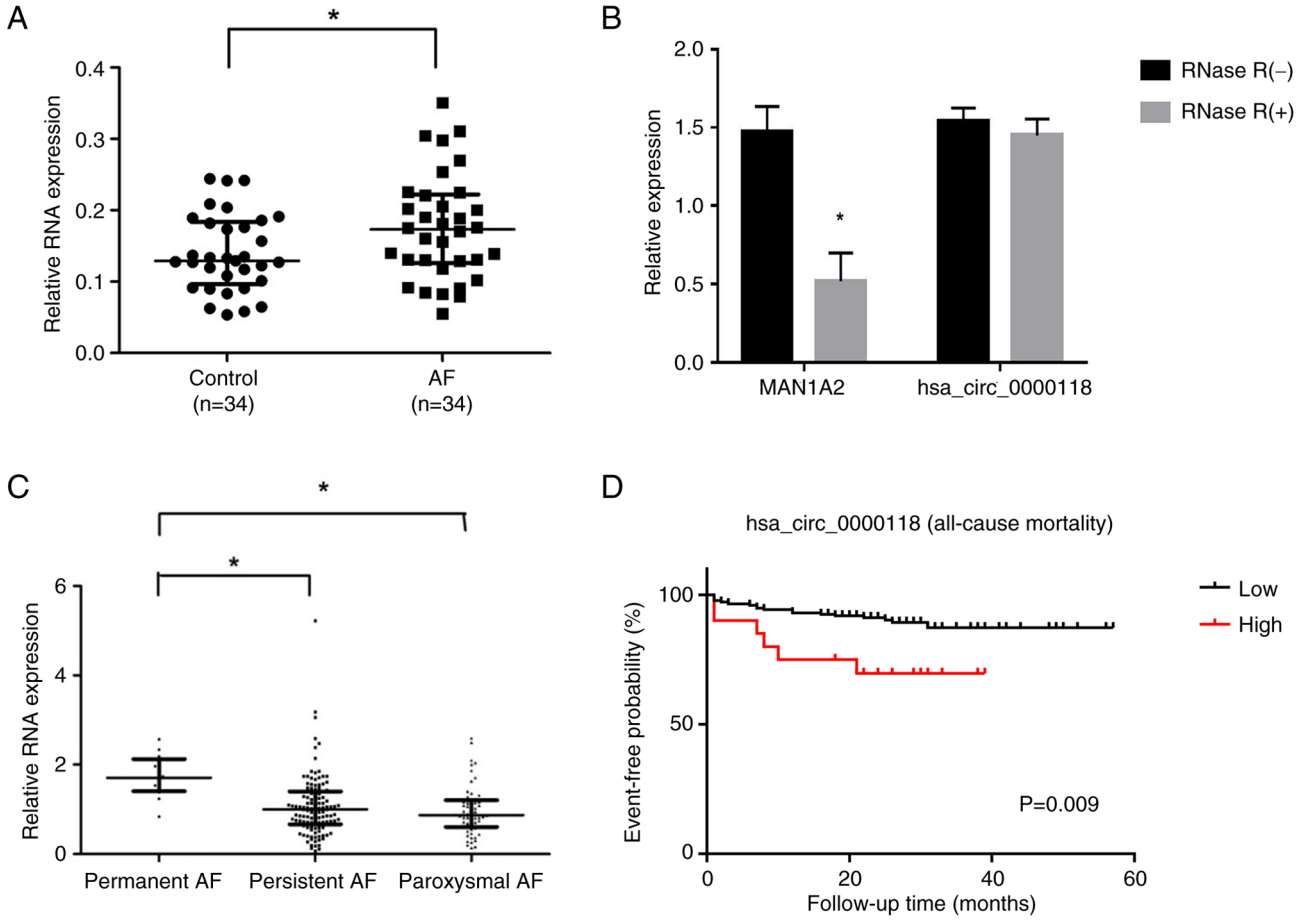

Figure 1. Expression of hsa_circ_0000118 in AF. (A) Expression levels of hsa_circ_0000118 in 34 pairs of age- and sex-matched atrial tissues from patients with and without AF (unpaired t-test). (B) hsa_circ_0000118 and MAN1A2 expression with and without RNase R treatment (unpaired t-test). (C) hsa_circ_0000118 expression in different types of AF (paroxysmal AF, n=60; persistent AF, n=120; and permanent AF, n=12; one-way ANOVA followed by the Sidak method). (D) Kaplan-Meier event-free survival curves for relative hsa_circ_0000118 expression in plasma. *P<0.05. AF, atrial fibrillation; circ, circular RNA; MAN1A2, mannosidase α class 1A member 2.

Upregulation of hsa_circ_0000118 expression is associated with poor prognosis in AF. To assess the clinical value of hsa_circ_0000118 and its potential as a prognostic biomarker, the association between its plasma levels and clinical stages and prognosis of AF was analyzed in a cohort study. A total of 192 patients with newly diagnosed AF were enrolled, with a median age of 68 years. A total of 51.6% of the patients were male, and 15 were lost during follow-up (Table I). Among the 192 patients with AF, 60 (31.3%) had paroxysmal AF, 120 (62.5%) had persistent AF and 12 (6.3%) had permanent AF. The plasma levels of hsa_circ_0000118 were highest in patients with permanent AF, who had significantly higher levels than patients with persistent and paroxysmal AF (Fig. 1C). During a median follow-up of 26 months, the incidence rates of stroke and all-cause death were 5.93 and 5.76 per 100 person-years, respectively (data not shown). Patients with higher levels of hsa_circ_0000118 had a significantly higher mortality rate than those with lower levels (P=0.009; Fig. 1D). Multivariate Cox proportional hazards regression analysis did not demonstrate that hsa_circ_0000118 was associated with the risk of stroke [hazard ratio (HR), 0.69; 95% CI, 0.28-1.68; P=0.412], whereas elevated levels of hsa_circ_0000118 were associated with a 5.0-fold increased risk of all-cause death (HR, 5.02; 95% CI, 1.94-12.98; P<0.001; data not shown).

hsa_circ_0000118 promotes the migration of cardiac fibroblasts and increases the expression levels of collagen I and collagen III. As hsa_circ_0000118 was demonstrated to be associated with the prognosis of patients with AF, its effect on atrial structural remodeling was evaluated, of which the main manifestation is cardiac fibrosis (6-8). Furthermore, the effects of mmu_circ_0010297 on proliferation, migration and the expression levels of extracellular matrix-related proteins in cardiac fibroblasts were assessed to investigate the potential role of hsa_circ_0000118 in atrial fibrosis. Gain- and loss-of-function cell models were established in cardiac fibroblasts, and 2/5 siRNAs (si3 and si5) were demonstrated to be effective in knocking down mmu_circ_0010297 expression without markedly affecting the linear form of MAN1A2 (Figs. 2A and S4). Furthermore, it was revealed that overexpression of mmu_circ_0010297 did not affect the proliferation rate of cardiac fibroblasts; however, it significantly promoted their migration compared with the empty vector group (Fig. 2B and C). Subsequently, the RT-qPCR results revealed that overexpression of mmu_circ_0010297 had no marked effect on the mRNA expression levels of Acta2 and Vimentin (Vim) (markers of the transformation of cardiac fibroblasts into cardiac myofibroblasts), but significantly increased the expression levels of collagen I and collagen III (main component

Table I. Baseline demographic and clinical data of 192 patients with AF.

Characteristic	No. (%)
Age, years	
<65	66 (34.4)
65-74	75 (39.1)
≥75	51 (26.6)
Male	99 (51.6)
Education	
Junior high school and below	159 (82.8)
High school and above	33 (17.2)
Family annual per capita income, 10,000 yuan/year	
<2.5	91 (47.4)
≥2.5	101 (52.6)
Smoking	63 (32.8)
Drinking	160 (83.3)
BMI, kg/m^2	
<18.5	14 (7.3)
18.5-23.9	96 (50.0)
≥24	82 (42.7)
Type of AF	
Paroxysmal AF	60 (31.3)
Persistent AF	120 (62.5)
Permanent AF	12 (6.3)
Comorbidities	
Hypertension	107 (55.7)
Diabetes	38 (19.8)
Coronary heart disease	87 (45.3)
Myocardiopathy	21 (10.9)
Heart failure	65 (33.9)
TIA or stroke	28 (14.6)
Vascular disease	7 (3.6)
Drug therapy	
Antiarrhythmic drugs	90 (46.9)
ACEI	65 (33.9)
ARB	19 (9.9)
β blockers	75 (39.1)
Warfarin	64 (33.3)
Statins	98 (51.0)
Electrocardiogram parameters	
Left atrial diameter, mm	
≤37	33 (17.2)
>37	159 (82.8)
LVEF, %	
<50	39 (20.3)
≥50	153 (79.7)
CHA_2DS_2-VASc score ≥2	147 (76.6)

AF, atrial fibrillation; TIA, transient ischemic attack; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; LVEF, left ventricular ejection fraction. CHA_2DS_2-VASc, congestive heart failure, hypertension, age [(2 points), diabetes mellitus (1 point), stroke/transient ischemic attack/thromboembolism (2 points), vascular disease (1 point), age 65-74 years (1 point), sex category (female, 1 point)].

of the extracellular matrix) (Fig. 3A) (24,25). Compared with those in the control group, the protein levels of collagen I and collagen III in the mmu_circ_0010297 overexpression group were also significantly increased (Fig. 3C). By contrast, downregulation of mmu_circ_0010297 did not affect the proliferation rate of the cardiac fibroblasts; however, it significantly decreased their migration rate (Fig. 2D and E). In addition, knockdown of mmu_circ_0010297 resulted in significantly decreased expression levels of type I and III collagen at both the mRNA and protein levels (Fig. 3B and D).

Taken together, the aforementioned findings suggested that mmu_circ_0010297 could increase the migration rate and collagen synthesis of cardiac fibroblasts in atrial fibrosis.

hsa_circ_0000118 participates in fibrosis of cardiac fibroblasts by sponging miR-34-5p to regulate Smad4. The present study assessed whether hsa_circ_0000118 may act as a natural miRNA sponge to prevent miRNAs from binding to their target mRNAs in atrial fibrosis. Possible candidate target miRNAs were screened in the miR databases miRanda, miRDB and RNA22, revealing 10 candidate target miRNAs of mmu_circ_0010297 (data not shown). Subsequently, the present study evaluated whether the expression levels of the 10 candidate target miRNAs were altered when mmu_circ_0010297 was overexpressed or knocked down in cardiac fibroblasts. The results demonstrated that when mmu_circ_0010297 was overexpressed, 7 out of 10 targeted miRNAs were significantly downregulated; however, when mmu_circ_0010297 was knocked down, three miRNAs, miR-34a-5p, miR-6987-5p, and miR-6387, were significantly upregulated. Notably, only miR-34a-5p and miR-6387 showed significant changes following both overexpression and knockdown conditions of mmu_circ_0010297 (Fig. 4A and B). The RIP assay further revealed that, compared with the IgG antibodies, the Ago2 antibodies could coimmunoprecipitate mmu_circ_0010297 and miR-34a-5p, but not miR-6387 (Fig. 4C). Therefore, mimics of miR-34a-5p were designed, which could significantly upregulate the expression levels of miR-34a-5p in mouse cardiac fibroblasts (Fig. S5). The luciferase reporter assay demonstrated that the luciferase activity of wild-type mmu_circ_0010297 was significantly decreased after overexpression of miR-34a-5p, whereas that of mutant mmu_circ_0010297 did not change, which confirmed that miR-34a-5p was the target gene of mmu_circ_0010297 (Fig. 4D).

Previous studies have reported that Smad4, a fibrosis-related gene, is the target of miR-34a-5p (26,27). The present study demonstrated that overexpression of mmu_circ_0010297 significantly increased the expression levels of Smad4 compared with those in the empty vector group, while knockdown of mmu_circ_0010297 had the opposite effect (Fig. 4E). A rescue experiment revealed that although mmu_circ_irc_0010297 overexpression increased Smad4, co-overexpression of miR-34a-5p attenuated this effect, which suggested that mmu_circ_0010297 participated in collagen production by sponging miR-34a-5p to regulate Smad4 (Fig. 4F).

The aforementioned data indicated that mmu_circ_0010297 regulated atrial fibrosis by sponging miR-34a-5p to reverse its inhibitory effect on Smad4, thereby participating in the structural remodeling in AF.

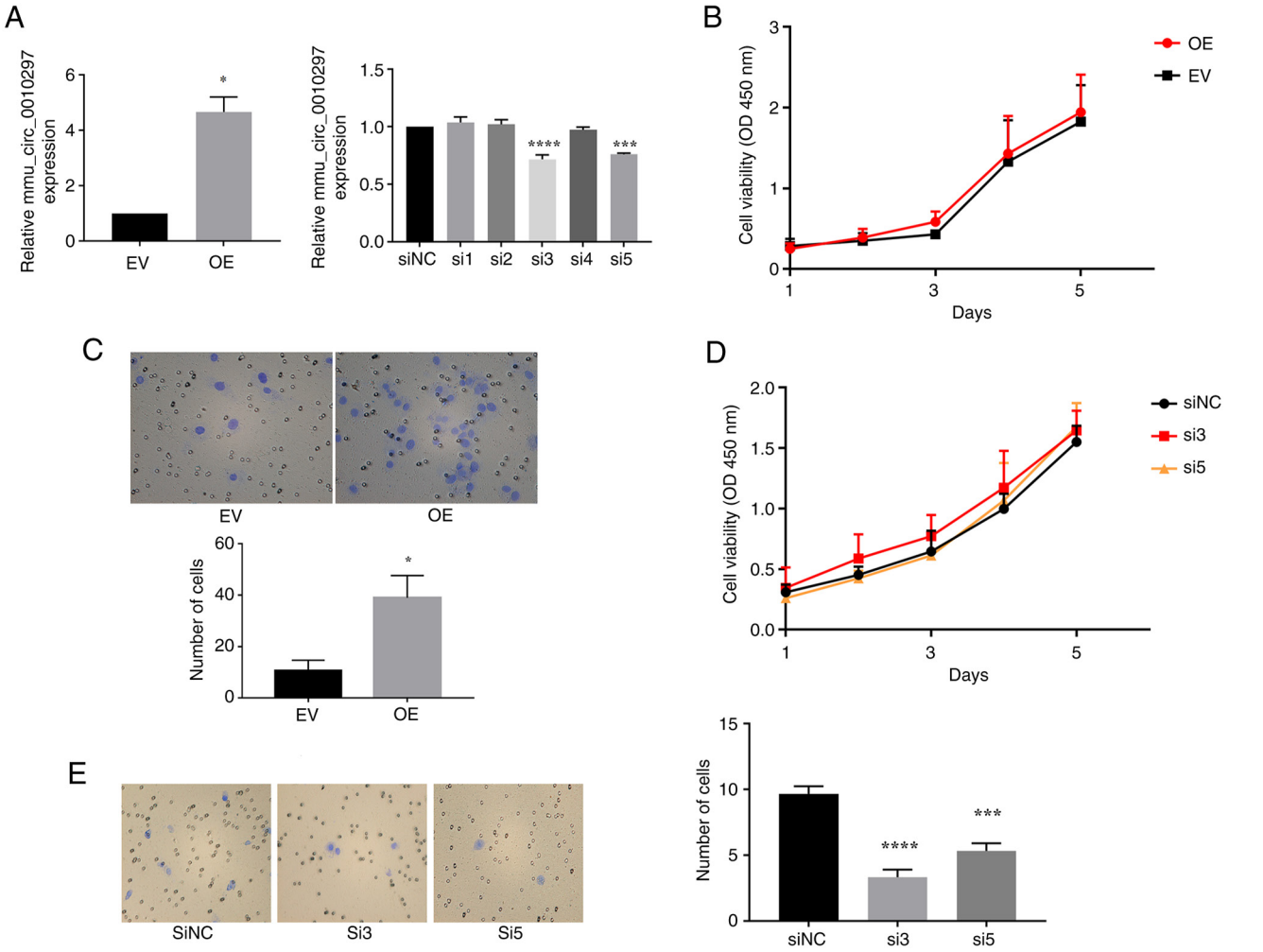

Figure 2. Effect of mmu_circ_0010297 overexpression and knockdown on the proliferation and migration of mouse cardiac fibroblasts. (A) Overexpression and knockdown efficiency of mmu_circ_0010297 in cardiac fibroblasts [unpaired t-test (left) and one-way ANOVA followed by the Sidak method (right)]. (B) Growth curves of cardiac fibroblasts after transfection with mmu_circ_0010297 or EV (two-way ANOVA). (C) Migration of cardiac fibroblasts after transfection with mmu_circ_0010297 or EV (unpaired t-test). (D) Growth curves of cardiac fibroblasts after transfection with mmu_circ_0010297 siRNAs or siNC (two-way ANOVA). (E) Migration of cardiac fibroblasts after transfection with mmu_circ_0010297 siRNAs or siNC (magnification, x100×; one-way ANOVA followed by the Sidak method). *P<0.05, ***P<0.001, ****P<0.0001 vs. EV or siNC). circ, circular RNA; EV, empty vector; NC, negative control; OD, optical density; OE, overexpression; siRNA/si, small interfering RNA.

Discussion

The present study identified that hsa_circ_0000118 expression was significantly upregulated in atrial tissues in AF. High circulating plasma levels of hsa_circ_0000118 were associated with poor prognosis of AF. Mechanistically, hsa_circ_0000118 could promote the migration of cardiac fibroblasts and the expression of collagen I and collagen III by acting as a competing endogenous RNA of miR-34a-5p to relieve its suppressive effect on Smad4 in cardiac fibroblasts.

circRNAs are a class of highly conserved non-coding RNAs with good stability due to their unique closed loop structure, making them ideal biomarkers (10,28). At present, there are few studies on hsa_circ_0000118 or other MAN1A2-derived circRNA isoforms, and most of them focus on tumors (29-32). For example, higher hsa_circ_0000118 (also referred to as 'circMAN1A2' in the study) levels were reported to be associated with poor survival in patients with gastric cancer, and a mechanistic study revealed that hsa_circ_0000118 could suppress T-cell antitumor immunity by inhibiting F-box and WD repeat domain containing 11-mediated splicing factor proline and glutamine rich degradation (29). circMAN1A2 was upregulated in serum samples of patients with malignant tumors such as nasopharyngeal, oral, thyroid, ovarian and lung cancer, indicating that circMAN1A2 had good diagnostic value for malignant tumors (32). However, as most studies did not provide detailed sequence or characterization data for 'circMAN1A2', it cannot be conclusively determined if circMAN1A2 is identical to hsa_circ_0000118 or a distinct MAN1A2 circRNA variant. Future studies should provide specific characteristic data of circRNAs when reporting novel circRNAs. Previous studies on circRNAs in AF have mainly focused on expression profiling and searching for differentially expressed circRNAs (14,15). However, the potential of differentially expressed circRNAs as biomarkers and their function or mechanism in AF lack exploration. Therefore, to the best our knowledge, the present study was the first to reveal the prognostic value, regulatory function and underlying mechanisms of hsa_circ_0000118 in patients with AF, which may provide novel prospects for the treatment and prognosis of AF.

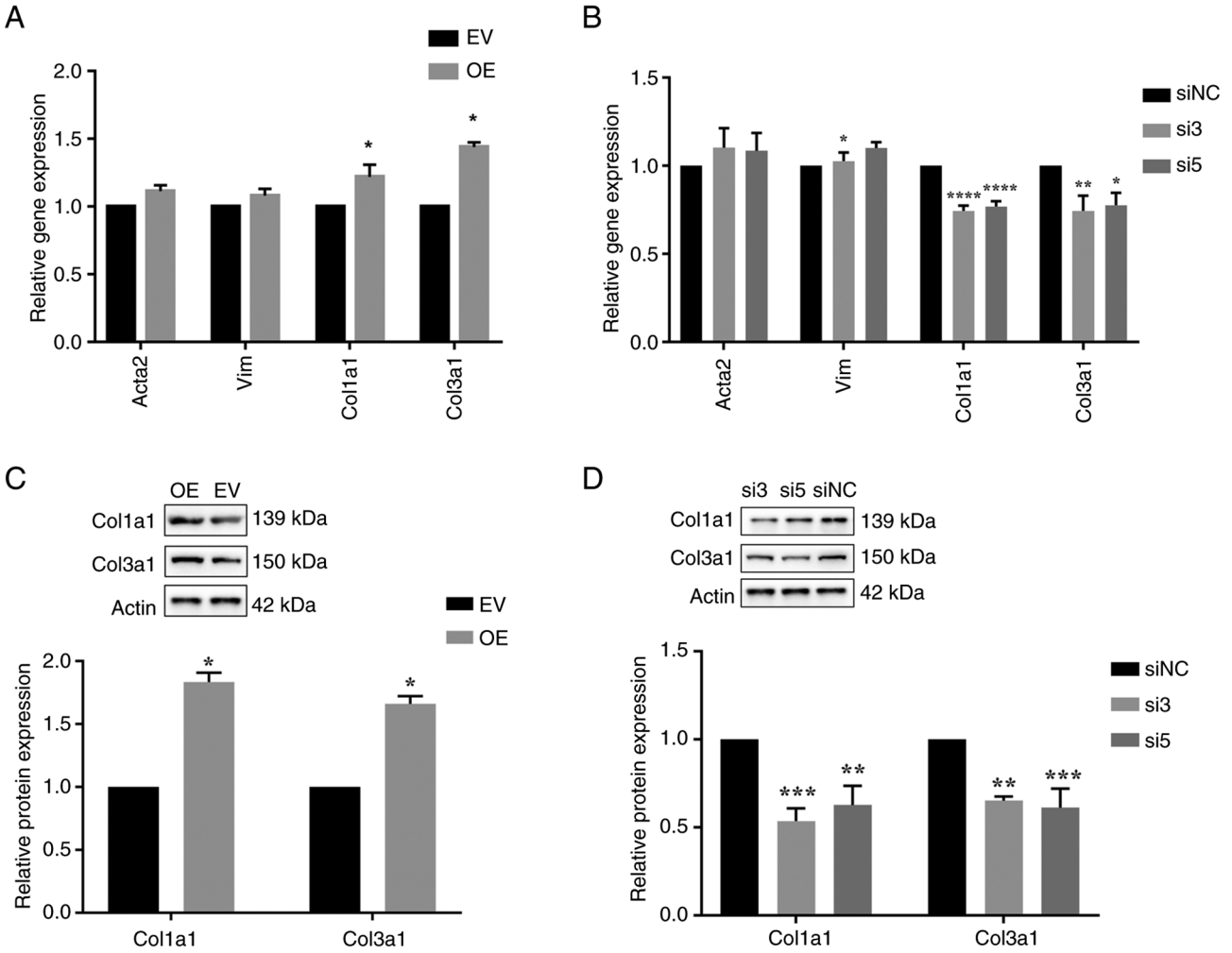

Figure 3. Effect of mmu_circ_0010297 overexpression and knockdown on differentiation into myofibroblasts and collagen expression. (A) Relative Acta2, Vim, Col1a1 and Col3a1 mRNA expression of cardiac fibroblasts after transfection with mmu_circ_0010297 or EV (unpaired t-test). (B) Relative Acta2, Vim, Col1a1 and Col3a1 mRNA expression of cardiac fibroblasts after transfection with mmu_circ_0010297 siRNAs or siNC (one-way ANOVA followed by the Sidak method). (C) Relative Col1a1 and Col3a1 protein expression of cardiac fibroblasts after transfection with mmu_circ_0010297 OE or EV (unpaired t-test). (D) Relative Col1a1 and Col3a1 protein expression of cardiac fibroblasts after transfection with mmu_circ_0010297 siRNAs or siNC (one-way ANOVA followed by the Sidak method). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. EV or siNC. circ, circular RNA; Col1a1, collagen type I α1 chain; Col3a1, collagen type III α1 chain; EV, empty vector; NC, negative control; OE, overexpression; siRNA/si, small interfering RNA; Vim, Vimentin.

Several studies have preliminarily explored the roles of circRNAs in AF (33,34). Gao *et al* (33) reported 92 notably dysregulated circRNAs in the plasma of patients with persistent AF compared with patients with paroxysmal AF, five of which were confirmed using RT-qPCR. The circRNA-miRNA-mRNA network of the five dysregulated circRNAs was further established based on predictions of miRNA response elements and target genes. Kyoto Encyclopedia of Genes and Genomes analysis revealed that the five dysregulated circRNAs were clustered in the mitogen-activated protein kinase and TGF-β signaling pathways. Another study reported that 20 circRNAs were upregulated and three were downregulated in both left and right atrial appendages of patients with AF compared with in patients without AF (34). Furthermore, gene set enrichment analysis suggested that hsa_circ_0000075 and hsa_circ_0082096 were related to AF pathogenesis via the TGF-β signaling pathway (34). Both studies suggested that circRNAs were associated with atrial fibrosis in AF; however, the exact underlying mechanism was not confirmed.

Atrial remodeling is one of the main reasons for the triggering and maintenance of AF, and its prominent manifestation is cardiac fibrosis. Cardiac fibrosis is a multifactor-mediated process characterized by excessive deposition of extracellular matrix proteins, primarily type I and type III collagen (35). Cardiac fibroblasts are a key cell type in the heart that can be activated and differentiated into myofibroblasts during fibrosis, resulting in increased proliferation, migration and collagen production capacity (25,36). The results of the present study demonstrated that overexpression of mmu_circ_0010297 in mouse cardiac fibroblasts did not alter their proliferation rate but increased their migration rate. Furthermore, overexpression of mmu_circ_0010297 did not change the levels of Acta2 and Vim, but increased the expression levels of Col1a1 and Col3a1, suggesting that mmu_circ_0010297 may affect the synthesis of type I and III collagen without influencing the transformation of cardiac fibroblasts into myofibroblasts. Furthermore, the present study demonstrated that mmu_circ_0010297 could regulate Smad4 by targeting miR-34a-5p, thereby regulating cardiac fibrosis and participating in atrial structural remodeling.

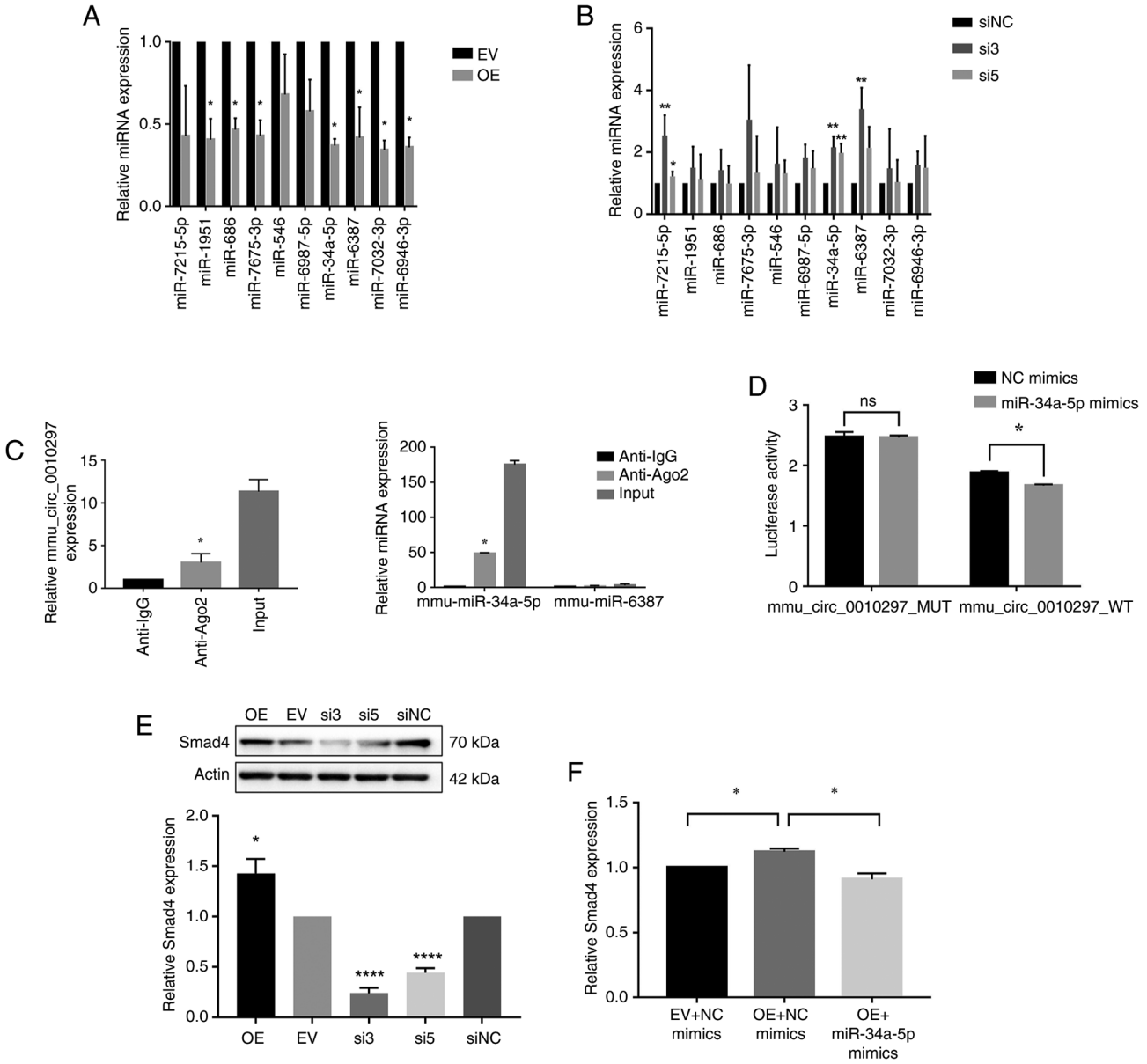

Figure 4. Examination of the specific mechanism of mmu_circ_0010297 in regulating collagen production. Expression of the candidate miRNAs after (A) overexpression of mmu_circ_0010297 (unpaired t-test) or (B) knockdown of mmu_circ_0010297 (one-way ANOVA and the Sidak method). (C) mmu_circ_0010297 and miR-34a-5p levels were determined using reverse transcription-quantitative PCR after Ago2 or IgG RNA immunoprecipitation assays (unpaired t-test, as only the Ago2 and IgG groups were compared). (D) Dual-luciferase reporter assay (unpaired t-test). (E) Regulatory relationship between Smad4 and mmu_circ_0010297. For the comparison of OE and EV, an unpaired t-test was used. For the comparison of si3, si5 and siNC, one-way ANOVA and the Sidak method were used. (F) Relative expression levels of Smad4 in cardiac fibroblasts were determined by RT-qPCR after co-transfection with mmu_circ_0010297 or control EV and miRNA-34a-5p mimics or NC mimics (one-way ANOVA and the Sidak method). *P<0.05, **P<0.01, ****P<0.0001 vs. EV, siNC or Anti-IgG. Ago2, argonaute 2; circ, circular RNA; EV, empty vector; miRNA/miR, microRNA; MUT, mutant; NC, negative control; OE, overexpression; si, small interfering RNA; WT, wild-type; RT-q, reverse transcription-quantitative; ns, not significant.

However, the present study has several limitations. First, the sample size used to test the prognostic effect of hsa_circ_0000118 in AF was relatively small, which may explain why no association with stroke was demonstrated. In addition, as the patients with AF were recruited from one tertiary hospital, selection bias may exist. Furthermore, the association between hsa_circ_0000118 and poor prognosis was observed in patients with non-valvular AF. Therefore, these findings cannot be extended to other types of AF. At the time of the study, commercial human cardiac fibroblasts were not widely available, and direct harvesting of fibroblasts from human heart tissues was limited by the difficulty of obtaining samples; thus, only the effect of the homologous circRNA hsa_circ_0000118 was explored in mouse cardiac fibroblasts, which made the extrapolation of the results of the present study limited by species. Further experiments could employ alternative human cardiac fibroblast cell lines to perform luciferase reporter assays for direct verification of the hsa_circ_0000118-miR-34-5p interaction. Finally, the findings of the present study need to be further validated in animal models of AF.

Despite the limitations, the results of the present study are of importance as they provide a relatively comprehensive exploration of the specific mechanistic role of hsa_circ_0000118 in AF. The closed-loop structure of circRNAs makes them promising

disease biomarkers, and the findings demonstrated the prognostic value of hsa_circ_0000118 in AF. Future studies are needed to further explore how to quickly and accurately measure circRNA expression for its clinical translation. By contrast, circRNA-based vaccines or drugs have been reported to have advantages over miRNA-based vaccines or drugs due to their stability, and the fact that the amount of translation required for rolling-loop translation is lower than for mRNA (37). With the maturity of circRNA research, it is expected that circRNA-based drugs will be developed in the future.

In conclusion, to the best of our knowledge, the present study was the first to demonstrate that high plasma levels of hsa_circ_0000118 were associated with poor prognosis of AF. hsa_circ_0000118 was revealed to promote AF development by increasing the expression levels of collagen I and collagen III, and the migration of cardiac fibroblasts via the miR-34a-5p/Smad4 axis. The findings of the present study contribute to elucidating the role and mechanism of hsa_circ_0000118 in AF development.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YL, LZ and ZZ conceived and designed the study. NW, YZh, YZe, LY, JL, YC, ZY, XC, CL, LW and TC performed experiments. YZh and YZe wrote the manuscript and confirm the authenticity of all the raw data. NW, YZh and YZe performed statistical analysis. NW, TC, YL, LZ and ZZ critically revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all included patients and the study was approved by the Ethics Committee of Southwest Hospital, Army Medical University (approval no. KY2020231; Chongqing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Morin DP, Bernard ML, Madias C, Rogers PA, Thihalolipavan S and Estes NA III: The state of the art: Atrial fibrillation epidemiology, prevention, and treatment. Mayo Clin Proc 91: 1778-1810, 2016.
2. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, *et al*: Global burden of cardiovascular diseases and risk factors, 1990-2019: Update from the GBD 2019 study. J Am Coll Cardiol 76: 2982-3021, 2020.
3. Xu S, Chen Y, Lin R, Huang W, Zhou H, Lin Y and Xu M: Burden of atrial fibrillation and its attributable risk factors from 1990 to 2019: An analysis of the global burden of disease study 2019. Front Cardiovasc Med 9: 997698, 2022.
4. Lau DH, Linz D and Sanders P: New findings in atrial fibrillation mechanisms. Card Electrophysiol Clin 11: 563-571, 2019.
5. Sagris M, Vardas EP, Theofilis P, Antonopoulos AS, Oikonomou E and Tousoulis D: Atrial Fibrillation: Pathogenesis, predisposing factors, and genetics. Int J Mol Sci 23: 6, 2021.
6. Brundel B, Ai X, Hills MT, Kuipers MF, Lip GYH and de Groot NMS: Atrial fibrillation. Nat Rev Dis Primers 8: 21, 2022.
7. Šustr F, Stárek Z, Souček M and Novák J: Non-coding RNAs and cardiac arrhythmias. Adv Exp Med Biol 1229: 287-300, 2020.
8. Zeng Y, Wu N, Zhang Z, Zhong L, Li G and Li Y: Non-coding RNA and arrhythmias: Expression, function, and molecular mechanism. Europace 25: 1296-1308, 2023.
9. Franco D, Aranega A and Dominguez JN: Non-coding RNAs and atrial fibrillation. Adv Exp Med Biol 1229: 311-325, 2020.
10. Huang A, Zheng H, Wu Z, Chen M and Huang Y: Circular RNA-protein interactions: Functions, mechanisms, and identification. Theranostics 10: 3503-3517, 2020.
11. Kristensen LS, Jakobsen T, Hager H and Kjems J: The emerging roles of circRNAs in cancer and oncology. Nat Rev Clin Oncol 19: 188-206, 2022.
12. Zhang X, Wan M, Min X, Chu G, Luo Y, Han Z, Li W, Xu R, Luo J, Li W, *et al*: Circular RNA as biomarkers for acute ischemic stroke: A systematic review and meta-Analysis. CNS Neurosci Ther 29: 2086-2100, 2023.
13. Zeng Q, Liu CH, Wu D, Jiang W, Zhang N and Tang H: LncRNA and circRNA in patients with Non-alcoholic fatty liver disease: A systematic review. Biomolecules 13: 560, 2023.
14. Zhu X, Tang X, Chong H, Cao H, Fan F, Pan J, Wang D and Zhou Q: Expression profiles of circular RNA in human atrial fibrillation with valvular heart diseases. Front Cardiovasc Med 7: 597932, 2020.
15. Zhang Y, Ke X, Liu J, Ma X, Liu Y, Liang D, Wang L, Guo C and Luo Y: Characterization of circRNA-associated ceRNA networks in patients with nonvalvular persistent atrial fibrillation. Mol Med Rep 19: 638-650, 2019.
16. Zhou B and Yu JW: A novel identified circular RNA, circRNA_010567, promotes myocardial fibrosis via suppressing miR-141 by targeting TGF-β1. Biochem Biophys Res Commun 487: 769-775, 2017.
17. Tang CM, Zhang M, Huang L, Hu ZQ, Zhu JN, Xiao Z, Zhang Z, Lin QX, Zheng XL, Yang M, *et al*: CircRNA_000203 enhances the expression of fibrosis-associated genes by derepressing targets of miR-26b-5p, Col1a2 and CTGF, in cardiac fibroblasts. Sci Rep 7: 40342, 2017.
18. Wu N, Li J, Chen X, Xiang Y, Wu L, Li C, Zhang H, Tong S, Zhong L and Li Y: Identification of long non-coding RNA and circular RNA expression profiles in atrial fibrillation. Heart Lung Circ 29: e157-e167, 2020.
19. Shi S, Tang Y, Zhao Q, Yan H, Yu B, Zheng Q, Li Y, Zheng L, Yuan Y, Zhong J, *et al*: Prevalence and risk of atrial fibrillation in China: A national cross-sectional epidemiological study. Lancet Reg Health West Pac 23: 100439, 2022.
20. European Heart Rhythm Association; European Association for Cardio-Thoracic Surgery; Camm AJ, Kirchhof P, Lip GY, Schotten U, Savelieva I, Ernst S, Van Gelder IC, Al-Attar N, *et al*: Guidelines for the management of atrial fibrillation: The task force for the management of atrial fibrillation of the european society of cardiology (ESC). Eur Heart J 31: 2369-2429, 2010.
21. Hu Z, Ding L and Yao Y: Atrial fibrillation: Mechanism and clinical management. Chin Med J (Engl) 136: 2668-2676, 2023.
22. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

23. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, *et al*: Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495: 333-338, 2013.
24. Tarbit E, Singh I, Peart JN and Rose'Meyer RB: Biomarkers for the identification of cardiac fibroblast and myofibroblast cells. Heart Fail Rev 24: 1-15, 2019.
25. Tallquist MD: Cardiac fibroblast diversity. Annu Rev Physiol 82: 63-78, 2020.
26. Qi Y, Zhao A, Yang P, Jin L and Hao C: miR-34a-5p Attenuates EMT through targeting SMAD4 in Silica-induced pulmonary fibrosis. J Cell Mol Med 24: 12219-12224, 2020.
27. Xue F, Yang J, Li Q and Zhou H: Down-regulation of microRNA-34a-5p promotes trophoblast cell migration and invasion via targetting Smad4. Biosci Rep 39: BSR20181631, 2019.
28. Zhou WY, Cai ZR, Liu J, Wang DS, Ju HQ and Xu RH: Circular RNA: Metabolism, functions and interactions with proteins. Mol Cancer 19: 172, 2020.
29. Shen Y, Lin J, Jiang T, Shen X, Li Y, Fu Y, Xu P, Fang L, Chen Z, Huang H, *et al*: GC-derived exosomal circMAN1A2 promotes cancer progression and suppresses T-cell antitumour immunity by inhibiting FBXW11-mediated SFPQ degradation. J Exp Clin Cancer Res 44: 24, 2025.
30. Guo R, Cui X, Li X, Zang W, Chang M, Sun Z, Liu Z, Sun Y, Jia J and Li W: CircMAN1A2 is upregulated by Helicobacter pylori and promotes development of gastric cancer. Cell Death Dis 13: 409, 2022.
31. Dang QQ, Li PH, Wang J, Zhao JY, Zhai SN, Zheng YJ and Yang DK: CircMAN1A2 contributes to nasopharyngeal carcinoma progression via enhancing the ubiquitination of ATMIN through miR-135a-3p/UBR5 axis. Hum Cell 36: 657-675, 2023.
32. Fan CM, Wang JP, Tang YY, Zhao J, He SY, Xiong F, Guo C, Xiang B, Zhou M, Li XL, *et al*: circMAN1A2 could serve as a novel serum biomarker for malignant tumors. Cancer Sci 110: 2180-2188, 2019.
33. Gao Y, Liu Y, Fu Y, Wang Q, Liu Z, Hu R, Yang X and Chen M: The potential regulatory role of hsa_circ_0004104 in the persistency of atrial fibrillation by promoting cardiac fibrosis via TGF-β pathway. BMC Cardiovasc Disord 21: 25, 2021.
34. Zhang PP, Sun J and Li W: Genome-wide profiling reveals atrial Fibrillation-related circular RNAs in atrial appendages. Gene 728: 144286, 2020.
35. Liu M, López de Juan Abad B and Cheng K: Cardiac fibrosis: Myofibroblast-mediated pathological regulation and drug delivery strategies. Adv Drug Deliv Rev 173: 504-519, 2021.
36. Souders CA, Bowers SL and Baudino TA: Cardiac fibroblast: The renaissance cell. Circ Res 105: 1164-1176, 2009.
37. Zhao X, Zhong Y, Wang X, Shen J and An W: Advances in circular RNA and its applications. Int J Med Sci 19: 975-985, 2022.