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The impact of MicroRNA-155 on the effect of cryoablation in paroxysmal atrial fibrillation

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ABSTRACT

Elevated plasma MicroRNA-155 (miR-155) levels are strongly associated with cardiac fibrosis and chronic inflammation processes. However, the relationship between miR-155 and paroxysmal atrial fibrillation (PAF) recurrence following cryoablation remains poorly explored. We aimed to evaluate whether elevated miR-155 is related to long-term AF recurrence following cryoablation. Preoperative miR-155 levels were determined in PAF patients undergoing initial cryoablation. Multivariate-adjusted Cox models were constructed to determine the relationship between miR-155 levels and PAF recurrence. Multivariate logistic regression analyses were performed to determine predictors of PAF recurrence. Of the 66 enrolled patients, 13 patients (19.7%) had recurrence at the 12-month following-up. These patients had significantly higher baseline miR-155 levels than those without PAF recurrence ((AAA ± BBB) vs. (AAA ± BBB), $P < 0.05$). The study results showed that miR-155 expression levels were significantly higher in the experimental group than in the control group. Additionally, logistic regression analysis revealed that miR-155 expression was positively correlated with PAF recurrence after cryoablation. Elevated preoperative miR-155 levels are related to a higher risk of AF recurrence and can independently predict AF recurrence following cryoablation.

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Introduction

As is widely known, the effect of ablation in atrial fibrillation is significantly related to the size of the left atrium, type of atrial fibrillation, and duration of atrial fibrillation (1, 2). The size of the left atrium is actually positively correlated with the degree of atrial fibrosis. Currently, there is a lack of effective methods to evaluate the degree of left atrial fibrosis in clinical practice and commonly used methods include cardiac magnetic resonance and intracavity voltage mapping (3-5). However, it has been proven that MicroRNA-155 (miR-155) plays an important role in cardiac fibrosis and chronic inflammation processes (6). The dynamic changes in its expression level and its relationship with atrial fibrillation, including its impact on the effectiveness of ablation, lack in-depth research.

MicroRNAs (miRNAs) are a type of non-coding small RNA molecule containing approximately 22 nucleotides. They affect gene expression at the post-transcriptional level by binding to the 3'-UTR region of target gene mRNA to inhibit its translation or cause its degradation, playing an important biological function (7). In recent years, miR-155 has been widely expressed in cardiovascular disease states, especially in vascular smooth muscle cells, endothelial cells, cardiac fibroblasts, and cardiomyocytes, attracting increasing attention (8). Previous studies have confirmed that the expression level of miR-155 in myocardial cells and plasma of acute myocardial infarction is significantly increased and that it can reduce myocardial cell apoptosis, reduce the area of myocardial infarction, and improve cardiac contractile function through the miR-155-TP53INP1 signaling pathway, playing a beneficial role.

Materials and Methods

Study population

The study population of this prospective cohort study comprised PAF patients who underwent their initial cryoablation at the Department of Cardiology of Wuhan Renmin Hospital of Wuhan University between February 2018 and February 2020. All patients were followed up for 12 months following ablation. The inclusion criteria were as follows: non-valvular AF; >18 years of age; suitable for cryoablation and voluntary participation in this study; cryoablation treatment for the first time; and signed informed consent. The exclusion criteria were as follows: history or findings of cardiovascular disease, including rheumatic heart diseases, pericarditis, coronary atherosclerotic heart disease, and HF symptoms or systolic dysfunction (left ventricular ejection fraction (LVEF)<50%); severe hepatic and/or renal dysfunction; acute and chronic inflammatory diseases. The study was performed in accordance with the Declaration of Helsinki for Human Research and was approved by the Medical Ethics Committee of the Wuhan Renmin Hospital of Wuhan University.

Sixty-six patients were divided into two groups: The control group (n=53) and Recurrence group (n=13). Patients with successful ablation were assigned to the Control group, while those with recurrence of PAF after ablation were assigned to the Recurrence group.

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Data collection

Data on baseline characteristics of PAF patients at admission were collected from the hospital medical records, including (i) general clinical data: age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, duration of AF, comorbidities and calculation of CHADS2 score, CHA2DS2-VASc score, ablation procedures, and history of medication; (ii) hematological indices (results of fasting blood sample obtained on the latest preoperative morning): hemoglobin, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, fasting plasma glucose, serum creatinine, uric acid, and high-sensitivity C-reactive protein (hs-CRP); echocardiographic parameters.

Blood sampling

Fasting peripheral venous blood samples were collected and processed on the day before ablation. Plasma miR-155 levels were determined strictly following the manufacturer's instructions, with the levels expressed as U/mL.

miR-155 levels measurements

Total RNA was extracted from the plasma using the TRIzol LS reagent kit, and reverse transcription was performed using the RNA reverse transcription kit and qRT-PCR reagent kit (Invitrogen, Carlsbad, CA, USA). The miR-155 sequence was 5'-UUAAUGCUAAUCGUGAUAGGGG-3' (ABI, Foster City, CA, USA), and the β-actin sequence was CACGATGGAGGGGCCGGACTCATC (ABI, Foster City, CA, USA). The OD260/OD280 readings were measured for each sample, and the RNA concentration was determined. Reverse transcription was performed using 500 ng of RNA, and PCR amplification was performed using cDNA as a template. The relative expression levels of each sample were calculated using the $2^{-\Delta\Delta}$CT formula.

Pulmonary vein morphological study

All patients underwent pulmonary vein (PV) scanning using a Siemens 256-slice CT scanner. PV reconstruction was performed using multi-planar reconstruction and volume rendering techniques. Three experienced radiologists measured and analyzed the PV of each of the 40 patients, and the average value was used as the final result. Left atrial appendage (LAA) structure measurement was performed using delayed phase scanning, and multi-phase reconstruction was used to reconstruct the original images.

Morphology study of left atrial appendage

After the pulmonary vein scan, a delayed-phase scan is performed 20 seconds later. The electrocardiogram gating technique is used, with the scanning phase set within 70% of the RR interval. The scanning range extends from the apex of the heart to the middle of the ventricle. The scanning parameters are as follows: tube voltage of 100 Kv, automatic tube current technology, and collimator width of 32 mm × 0.6 mm. After the scan, the original image data is reconstructed into multiple phases to measure the dimensions of the left atrial appendage. Measurements are taken of the short-axis and long-axis diameters of the left atrial appendage after multiplanar reconstruction.

Intraoperative parameter study of cryoablation

After inflating the cryoballoon, the assistant injects a contrast agent to visualize the pulmonary veins, and the absence of contrast leakage indicates good occlusion. At this point, the freezing process begins. The temperature (in degrees Celsius) is recorded at 30, 60, 120, and 180 seconds, along with the time and temperature during pulmonary vein isolation. The time it takes to rewarm to 37°C after stopping the freezing process is also recorded in seconds.

Mean left atrial pressure study

All patients are required to fast from water for 6 hours before the procedure. In the puncture room, after placing the specialized large sheath for cryoablation, a three-way stopcock is connected to perform zero calibration. Prior to freezing, the left atrial systolic pressure (in mmHg) is measured.

Follow-up

After discharge, all patients underwent routine follow-ups at our cardiology clinic where at least a monthly 12-lead ECG and a 24-h Holter monitoring every 3 months were obtained. They were strongly recommended to visit the nearest hospital for an ECG if they felt symptoms that could be attributed to an arrhythmia or noticed any irregularity of their peripheral pulse by routine self-measurement. AF recurrence was defined as any documented atrial tachyarrhythmia (AF, atrial flutter, or atrial tachycardia) episode lasting for at least 30 s after ablation, excluding a 3-month blanking period.

Statistical Analysis

Statistical analysis was performed using Statistic Package for Social Science (SPSS) version 22.0 (IBM, Armonk, NY, USA). The chi-square test and t-test were used to analyze the differences in clinical data between the experimental and control groups. The Mann-Whitney U test was used to compare the expression levels of miR-155 between the two groups. The correlation between miR-155 expression and PAF recurrence was analyzed using logistic regression analysis. A P-value < 0.05 was considered statistically significant.

Results

Patient characteristics

We enrolled 66 PAF patients in the study, and all of them completed follow-ups for the analysis (Table 1). There were no significant differences in baseline characteristics between the two groups.

Comparison of miR-155 levels between the two groups

The level of plasma miR-155 in the Control group before cryoablation was significantly lower than that in the Recurrence group (31.89±2.33 vs. 42.12±3.71, $P < 0.05$). After 12-month follow-up, the level of plasma miR-155 in the Control group was still significantly lower than that in the Recurrence group (26.31±1.44 vs. 41.07±2.16, $P < 0.05$). However, there was a decreasing trend in the experimental group before and after cryoablation (31.89±2.33 vs. 26.31±1.44, $P < 0.05$), while there was no significant change in the control group before and after cryoablation (42.12±3.71 vs. 41.07±2.16, $P > 0.05$) (Figure 1).

Table 1. Characteristics of patients between the Control group and Recurrence group.

Characteristics	Total (n=353)	Recurrence (n=85)	No recurrence (n=268)	P-value
General clinical characteristic				
Age (years)	61.56±9.47	61.87±9.49	61.46±9.47	0.643
Sex				0.306
Male	199(56.5)	52(61.2)	147(54.9)	
Female	154(43.6)	33(38.8)	121(45.1)	
BMI (kg/m2)	24.79±3.09	24.83±3.14	24.77±3.08	0.867
SBP (mmHg)	128.10±15.37	127.94±14.46	128.15±15.68	0.912
DBP (mmHg)	79.18±10.33	79.78±9.93	78.99±10.47	0.541
Type of AF				<0.001
Paroxysmal	257(72.8)	48(56.5)	209(78.0)	
Persistent	96(27.2)	37(43.5)	59(22.0)	
AF duration (months)	25.26±36.68	29.12±33.32	24.03±37.66	0.045
CHADS2 score	0.90±0.93	1.04±1.06	0.86±0.88	0.288
CHA2DS2-VASc score	1.78±1.37	1.89±1.47	1.75±1.34	0.443
Comorbidity				
Hypertension	208(58.9)	53(62.4)	155(57.8)	0.461
Diabetes	34(9.6)	12(14.1)	22(8.2)	0.108
Dyslipidemia	18(5.1)	6(7.1)	12(4.5)	0.509
Stroke/TIA	21(5.9)	6(7.1)	15(5.6)	0.62
Hematological index				
Hemoglobin (g/L)	138.70±16.19	140.24±15.02	138.21±16.54	0.316
FBG (mmol/L)	5.07±0.82	5.13±0.99	5.06±0.76	0.925
Sc (umol/L)	70.20±15.60	73.94±20.37	68.88±13.55	0.163
UA (umol/L)	347.16±88.31	355.63±92.40	344.46±86.97	0.311

Comparison of pulmonary vein morphology between two groups

There were no significant differences in the diameters of both left and right pulmonary veins between the two groups (Figure 2).

Left atrial appendage comparison between two groups

There was no significant difference in left atrial appendage ejection fraction (EF%) between the Control group and the Recurrence group (51.2±3.77 vs. 52.3±2.13, P > 0.05). However, there was a significant difference in the number of lobes between the Control group and the Recurrence group (3.58±1.89 vs. 5.21±0.92, P <0.01). Similarly, there was a significant difference in the left atrial appendage orifice diameter and depth between the Control group and the Recurrence group (diameter: 28.11±4.37 vs. 33.26±3.88; depth: 27.51±2.12 vs. 35.12±2.70, P < 0.05, respectively) (Figure 3).

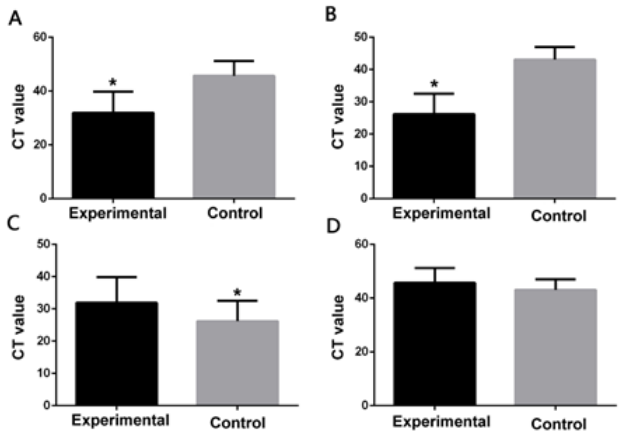

Figure 1. Comparison of plasma miR-155 levels before cryoablation in two groups of patients.

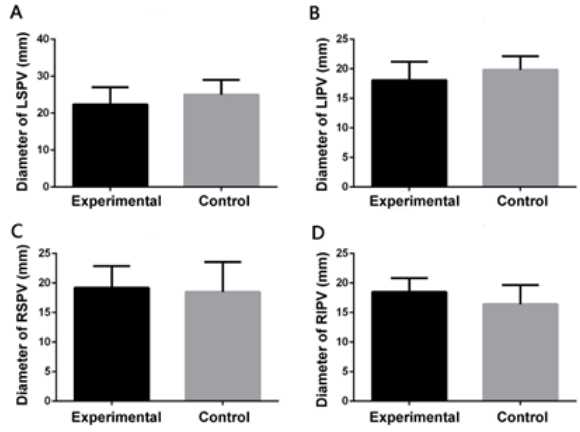

Figure 2. Comparative results of pulmonary vein morphology between the two groups of patients.

Comparison of Cryoablation Parameters

There were no significant differences in the lowest temperature and isolation time of the pulmonary veins during

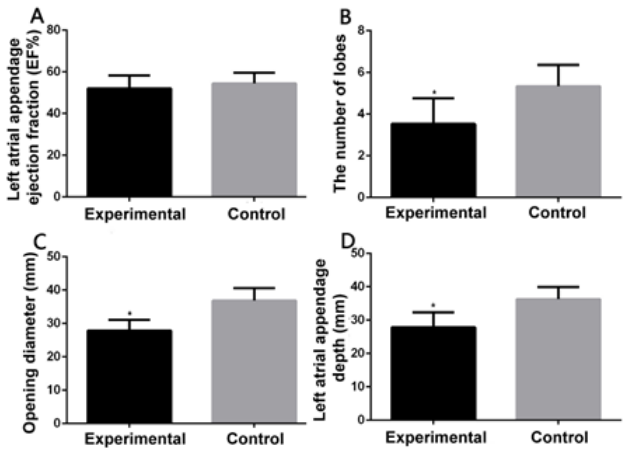

Figure 3. Comparison of left atrial appendiceal ejection fraction and anatomical parameters between control and recurrence groups.

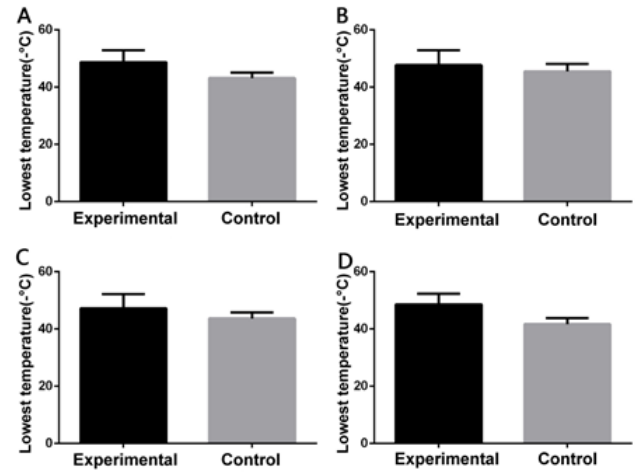

Figure 4. Comparison of lowest temperatures in the pulmonary veins during cryoablation.

cryoablation between the Control group and the Recurrence group (all $P > 0.05$) (Figures 4 and 5).

Comparison of mean left atrial pressure

The mean left atrial pressure in the Control group was significantly lower than that in the Recurrence group (9.23 ± 3.11 mmHg vs. 13.56 ± 3.27 mmHg, $P < 0.01$) (Figure 6).

Discussion

Atrial fibrillation (AF), also known as "AFib," is the most common arrhythmia in the elderly population, characterized by rapid and irregular heart rhythm. It is a major risk factor for stroke and heart failure, posing a serious threat to human health. There are many theories about the mechanism of AF, but currently, it is mainly believed to be related to the multi-wave reentry in the pulmonary vein and atrial substrate. However, clinical practice has repeatedly confirmed that pulmonary vein isolation (PVI), although the cornerstone of AF ablation, still has a 10-20% failure rate due to factors outside the pulmonary vein and the left atrial matrix plays an important role in the occurrence and maintenance of AF (10).

MiR-155 has been proven to participate in the inflammatory response of ventricular remodeling and plays an important role in the regulation of various tumors and inflammatory diseases (11). The expression level of miR-155 has also been regarded as an indicator of the degree of inflammatory response. This study found that miR-155 is directly related to the success rate of cryoablation in AF patients, providing a possible way to predict the success rate of cryoablation in patients with paroxysmal AF.

Through the study of the morphology of the pulmonary vein and left atrial appendage, it was found that miR-155 has no significant effect on the morphology of the pulmonary vein, but has a more significant effect on the morphology of the left atrial appendage. The number of lobes, opening diameter, and depth of the left atrial appendage in the control group were all higher than those in the experimental group, and they were correlated with the expression level of miR-155 (12). The left atrial appendage has strong compliance, and its shape will change progressively with age, changes in atrial pressure, atrial inflammatory response reconstruction, and the occurrence of arrhythmias. In contrast, the pulmonary vein is less affected by inflammatory regulation and left atrial pressure changes due to its rich elastic fibers. Previous reports have shown a correlation between the morphology of the left atrial appendage and the effectiveness of cryoablation for AF, which is similar to our study (13).

Studies have shown that miR-155 is involved in the chronic inflammatory response of myocardial cells, regulating genes involved in myocardial fibrosis, myocardial cell hypertrophy, fibroblast-to-fibroblast transformation,

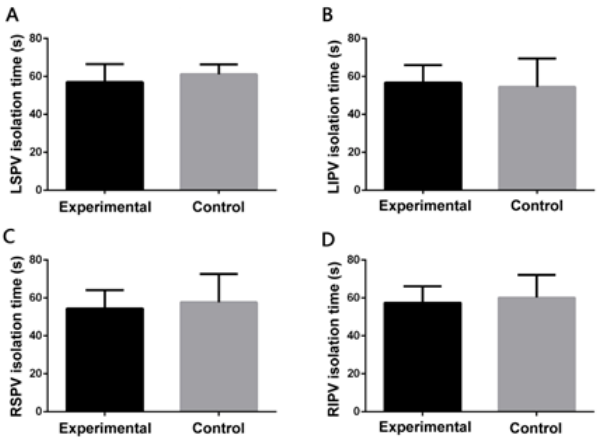

Figure 5. Comparison of isolation times of pulmonary veins during cryoablation procedures.

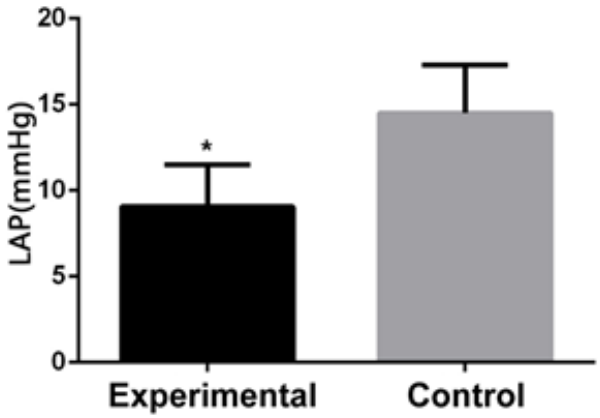

Figure 6. Comparative results of mean left atrial pressure in the two groups of patients.

microvascular regeneration, matrix metalloproteinase, and gap junction protein formation. These processes are all involved in the structural and electrophysiological remodeling of the heart, leading to increased atrial pressure and conduction block, which are important prerequisites for AF formation and also important factors leading to changes in the morphology of the left atrial appendage (14-18).

The left atrial appendage plays an important role in the occurrence and maintenance of AF. The abnormal electrical activity in the left atrial appendage can act as an AF trigger and can also form various forms of reentry with the left atrium and left upper pulmonary vein, leading to the maintenance of AF (19).

In non-valvular disease patients, left atrial pressure is mainly related to atrial compliance and left atrial volume load. The degree of left atrial fibrosis is also directly related to compliance (20-22). This study indirectly proves that miR-155 is related to left atrial pressure and fibrosis degree, but unfortunately, due to the lack of a three-dimensional navigation system during the experimental design, it was impossible to directly obtain the voltage map of the left atrium, which is a major regret of the study.

In terms of cryoablation, there were no differences between the experimental group and the control group in terms of the lowest freezing temperature and time to effectiveness (TTI). However, there was a significant difference in the one-year success rate between the two groups, with higher levels of miR-155 expression associated with higher recurrence rates. This further demonstrates that atrial fibrillation is not only related to the pulmonary veins and atria but also directly related to factors outside the pulmonary veins, such as the atrial substrate and left atrial appendage as other triggering foci (23-25). Left atrial substrate ablation, particularly in patients with persistent atrial fibrillation, has become a routine ablation procedure in some centers (26-29). In future studies, we will continue to investigate the relationship between miR-155 and the left atrial substrate in greater depth.

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Conflict of Interests
The authors declared no conflict of interest.

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