Radiofrequency Ablation Treating Atrial Fibrillation Can Reverse the Changes of Mirnas Regulating Ion Channel Proteins

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ABSTRACT

To investigate the possible molecular mechanisms of Radiofrequency Ablation (RFA) treating Atrial Fibrillation (AF), and the miRNA target for intervention in the future. We compared the changes of miRNAs regulating atrial ion channel proteins in the whole genome in 90 AF patients with 90 healthy subjects in pre-RFA and at 3 month post-RFA, found out that 21 miRNAs regulating ion channel proteins were differentially expressed >10-fold, and totally reversed in post-RFA compared with in pre-RFA. The temporal effect of miRNAs regulating outward K+ current such as Ikur, Ikr and Iks were more unanimous and bigger, while of which regulating IcaL and INa current channels was not so. miR-1266 level was increased in blood, but down-regulated in human rheumatic atrial tissue; dual luciferase test indicated that SCN5A was the direct target gene of miR-1266. Our results indicated that RFA treating AF may play a part via reversing the changes of miRNAs regulating ion channel proteins, especially outward K+ current like Ikur, Ikr and Iks channel which may play a major role in the electrical remodeling in AF. miR-1266 may be an antiarrhythmic miRNA and an AF intervention target in the future.

Keywords: Atrial Fibrillation (AF); Mirna; Genomics; Radiofrequency Ablation (RFA); Ion Channel Proteins

INTRODUCTION

As all well known that atrial fibrillation (AF) pathogenesis is very complex, and it involves multiple factors, genes, miRNA regulations and target changes [1-5], etc. Which resulted in the electrical and structural remodeling, and further make paroxysmal AF gradually evolving into persistent and permanent AF. Anyway, atrial fibrillation radiofrequency ablation (AF-RFA) is a main treating way for patients with AF at present [6,7]. But the postoperative recurrence rate is still higher [7], and the RFA treating molecular mechanism and recurrence machining is still unclear.

However, the miRNA regulating mechanism has been paid more attention in AF genesis and development now. David D, et al [8] has reported the relations between circulating microRNAs and atrial fibrillation. Based on our AF-RFA clinical practice and previous miRNA study [9-13], we has also shown that miRNAs involve in the regulation of AF, and the RFA can reverse the abnormal circulating microRNA expression in patients with atrial fibrillation. Recently, the miRhythm Study [14] has found out that plasma levels of miR-21 and 150 increased three fold after AF ablation. But all these miRNA changes almost mainly involved in the atrial structural remodeling, the alteration of miRNAs regulating ion channel proteins has not been reported. In this study, we really found out some miRNAs regulating ion channel proteins have been changed or reversed by the RFA, especially outward K+ current such as Ikur, Ikr and Iks channel, which may further rebalance the ion flow and reverse the electrical remodeling in AF. Furthermore, we wanted also to detect which channel may play important role in ion channel remodeling and its regulating miRNAs changes in post-RFA, and try to search the miRNA regulating mechanism and intervening targets.

MATERIAL AND METHODS

Study Population

Object of study: The 90 AF radiofrequency ablation patients were admitted to our hospital between January 2013 and June 2015, divided into paroxysmal AF (AF persisted ≤ 7 days), persistent AF (AF persisted > 7 days < 1year) and permanent AF (AF persisted ≥ 1 year) subgroup, 30 cases in each one respectively. The average age of the patients was 72.17 ± 4.76 years old, 44 cases were female, and 46 cases were male, alongside 90 healthy physical examination subjects as controls, the average age was 69.40 ±5.86 years old.

Each patient had more than five ECG in different time to support the diagnosis.

Exclusion criteria: Patients whose age > 80 years old, hyperthyrosis, diabetes mellitus, poor control of blood pressure (>140 and/or 90mmHg), left ventricular dysfunction (EF<40%), severe coronary artery disease, hepatic and renal dysfunction, acute and chronic infections disease, myocardial structural lesions were excepted. The patients who were accepted angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor inhibitors (ARB), statin drugs to control hypertension and hyperlipemia, beta-blockers (β-blocker) and other anti-arrhythmic medicine to control ventricular rate, had to stop to take at least >5 days pre-operation.

Informed consent: A statement to confirm that the all methods carried out in this study was in accordance with China National Ministry of Public Health “Biomedical research ethic review regulation”, China SFDA (State Food and Drug Administration) “The guiding principle of drug clinic trial ethic review”, and “Declaration of Helsinki”. The protocol of the study was approved by the Medical Ethics Committee of Shijitan Hospital. Every patient and his/her family member had to agree and sign the informed consent, and written informed consent was obtained from all subjects before RFA. Also, all authors promised to protect the patient’s privacy right. Any information which could identify individual participants during or after the research should not be open used.

Operation style: The operation style was surround pulmonary vein anotcut isolation (SPVAl) in paroxysmal AF patients, and “20+3L” in persistent and permanent AF patients, that is the SPVAl+3 line: left atrial roof line, mitral and tricuspid annulus narrow part ablation lines. The no recurrent patients of AF ablation operation later 3 months were entered into subgroup (3 times holter monitor shown no AF recurrence, 1 time every month detection post-operatan).

Experimental Instruments: The miRCURTYM LNA microRNA chips (v18.0 Exiqon) and the miRCURY Array Power Labeling kit (Cat #208032-3 A Exion) were used. We also used a wash buffer kit (Exion), TRizol Reagent (Invitrogen life technologies), miRNasey mini kit (Qiagen), NanodropR ND-1000 (Sigma) and Axon Gene Pix 4000B in the test.
miRNA differential expression in AF patients pre-RFA compared with at 3 months post-RFA

RESULTS

miRNA expression can be quantified using the following formula: 2−ΔΔCT.

miRNA Target Prediction

miRNA targets were predicted using the target-prediction programs in miRanda, TargetScan and miRBase. The target genes were presented according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, which is a database of biological systems, and it consists of the genetic building blocks of genes and proteins.

Luciferase combination experiment

After miR-1266 plasmid (pcDNA6.2-GW/miRNA-1266) and the negative control plasmid (pcDNA6.2-GW/miRNA NC) covalently transfected with SCNSA recombinant luciferase plasmid (pmirG-LO-SCNSA 3’UTR) respectively, compared with miR-1266 plasmid and blank group, the relative luciferase activity of each group was analyzed.

Statistical Analysis

The threshold value for significance that was used to define miRNA up regulation or down regulation was a fold change >1.5, with a value of P < 0.05 that was calculated using a t-test.

RESULTS

miRNA differential expression in AF patients pre-RFA compared with at 3 months post-RFA

Compared with the pre-RFA in AF patients, 503 miRNAs was up-regulated, but 81 miRNAs down-regulated more than 1.5-fold post-RFA (P<0.01). Meanwhile, the expression of 21 miRNAs that regulate main ion channel protein was changed more than 10-fold post-RFA compared with pre-RFA (Tab. 1); in which, miR-1266, miR-377-5p, miR-1284, miR-4796-5p and miR-296-3p were up-regulated more than 1.5-fold in pre-operation compared with control group, but down-regulated more than 10-fold in post- with pre-RFA compared; while miR-101-3p, miR-146b-5p, miR-186-5p, miR-151a-3p, miR-98-5p, miR-155-5p, miR-224-5p, miR-152, miR-199a-3p and miR-199b-3p were down-regulated more than 10-fold before RFA, up-regulated more than 30-fold after RFA (Fig.1,Tab.1).

Figure 1 The cluster analysis of miRNA expression in AF patients in pre- and post-RFA (Paro: Paroxysmal AF; Pers: Persistent AF; Perm: Permanent AF). It is indicated just only from the colour changes in fig.1 that miRNAs changes were difference entirely in 3 different AF groups in post-RFA; that means AF-RFA reversed or changed the miRNA abnormities in AF patients.

Expression of miRNAs regulating ion channel proteins

Expression of miRNAs correlated to SCNSA

Based on the cardiac action potential curve and target gene prediction, several miRNAs regulated the expression of SCNSA encoded α-subunit of sodium channel which determines the 0 phase of action potential duration (APD), including: miR-1266, miR-1284, miR-146b-5p, miR-98-5p (Figure.2).
Table 1: The main miRNAs regulating ion channel proteins expressed differently in pre- and post-RFA

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>pre-RFA/N.C. (N=90)</th>
<th>pre-/post RFA(N=90)</th>
<th>Difference Fold</th>
<th>P Value</th>
<th>Difference Fold</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-1266</td>
<td>1.96±0.025</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>249.86±0.218</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-377-5p</td>
<td>4.27±0.039</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>102.90±0.237</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsa-miR-366-5p</td>
<td>-8.88±0.009</td>
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<td></td>
<td>&lt;0.01</td>
<td>-46.06±0.156</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsa-miR-1284</td>
<td>1.94±0.056</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>-27.13±0.217</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsa-miR-4796-5p</td>
<td>2.68±0.106</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>-23.62±0.089</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-296-3p</td>
<td>4.30±0.049</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>-10.58±0.02</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-4666a-3p</td>
<td>-3.69±0.026</td>
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<td></td>
<td>&lt;0.01</td>
<td>13.83±0.093</td>
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<td>hsa-miR-30d-5p</td>
<td>-7.50±0.021</td>
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<td></td>
<td>&lt;0.01</td>
<td>14.15±0.088</td>
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<tr>
<td>hsa-miR-4306</td>
<td>-8.33±0.035</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>20.90±0.142</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-32B</td>
<td>-1.79±0.024</td>
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<td></td>
<td>&lt;0.01</td>
<td>28.49±0.172</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-101-3p</td>
<td>18.52±0.061</td>
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<td>&lt;0.01</td>
<td>34.94±0.181</td>
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<tr>
<td>hsa-miR-146b-5p</td>
<td>11.16±0.049</td>
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<td>&lt;0.01</td>
<td>39.16±0.219</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-186-5p</td>
<td>12.05±0.014</td>
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<td>&lt;0.01</td>
<td>101.88±0.235</td>
<td>&lt;0.001</td>
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<td>hsa-miR-151a-3p</td>
<td>11.83±0.016</td>
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<td></td>
<td>&lt;0.01</td>
<td>104.79±0.235</td>
<td>&lt;0.001</td>
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<td>hsa-miR-98-5p</td>
<td>-7.02±0.085</td>
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<td>&lt;0.01</td>
<td>130.54±0.173</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-155-5p</td>
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<td></td>
<td>&lt;0.01</td>
<td>132.57±0.218</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-224-5p</td>
<td>30.49±0.053</td>
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<td></td>
<td>&lt;0.01</td>
<td>148.29±0.328</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-361-5p</td>
<td>-5.12±0.026</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>150.68±0.254</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-152</td>
<td>16.34±0.071</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>184.27±0.356</td>
<td>&lt;0.001</td>
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<tr>
<td>hsa-miR-199-3p</td>
<td>11.30±0.062</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>221.90±0.562</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsa-miR-199-3p</td>
<td>11.30±0.051</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>221.98±0.751</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: "—" means going down; N.C.: Normal Control; RFA: Radiofrequency Ablation.

Table 2: MiRNA Targets Prediction Results

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target genes of ion channel proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-1266</td>
<td>SCN5A, KCNH2, KCNE1, KCNA6, KCNA7,</td>
</tr>
<tr>
<td></td>
<td>KCNC3, KCNB1, KCNG4, KCNH5, KCNH8,</td>
</tr>
<tr>
<td></td>
<td>KCNP4, KCN1, KCNJ1, KCNJ3, KCNJ5,</td>
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<td></td>
<td>KCN1, KCNK1, KCNMA1, KCNS1, KCNS2,</td>
</tr>
<tr>
<td></td>
<td>KCNU1</td>
</tr>
<tr>
<td>hsa-miR-377-5p</td>
<td>GJA5, GJA1, GJCNN1, GJCNN2, GJCNN3,</td>
</tr>
<tr>
<td>hsa-miR-366-5p</td>
<td>KCNC1, KCNA1B, CAMTA2</td>
</tr>
<tr>
<td>hsa-miR-1284</td>
<td>HCN4, SCNSA5, KCNC1</td>
</tr>
<tr>
<td>hsa-miR-4796-5p</td>
<td>HCN4</td>
</tr>
<tr>
<td>hsa-miR-296-3p</td>
<td>KCN1, HCN1, GJA5, KCNH2</td>
</tr>
<tr>
<td>hsa-miR-4666a-3p</td>
<td>KCNE4, KCNQ3, KCN1, KCNG3, KCNJ3,</td>
</tr>
<tr>
<td></td>
<td>HCN4, GACA1C, CAMSAP1, CAMTA1</td>
</tr>
<tr>
<td>hsa-miR-30d-5p</td>
<td>KCND2, GJA1, HCN1, HCN3</td>
</tr>
<tr>
<td>hsa-miR-4306</td>
<td>KCNC4, HCN4, GACNA1C, KCNN2, KCNN3,</td>
</tr>
<tr>
<td></td>
<td>KCNC1, KCNC4</td>
</tr>
<tr>
<td>hsa-miR-328</td>
<td>GACNA1C, GACNA1B</td>
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<tr>
<td>hsa-miR-101-3p</td>
<td>KCNH2, GJA1, KCNE1, KCNA5</td>
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<tr>
<td>hsa-miR-146b-5p</td>
<td>HCN1, SCNSA5</td>
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<td>hsa-miR-186-5p</td>
<td>GACNA1C, KCNJ2, KCNC4, KCNA5</td>
</tr>
<tr>
<td>hsa-miR-151a-3p</td>
<td>KCNA5, HCN1</td>
</tr>
<tr>
<td>hsa-miR-98-5p</td>
<td>KCNC4, KCNJ2, SCNSA5</td>
</tr>
<tr>
<td>hsa-miR-155-5p</td>
<td>KCNA5, KCNN3, HCN3, KCNH2, KCNN2, KC-</td>
</tr>
<tr>
<td></td>
<td>NC1, GJA5</td>
</tr>
<tr>
<td>hsa-miR-224-5p</td>
<td>KCND2, KCNN2, GJA3</td>
</tr>
<tr>
<td>hsa-miR-361-5p</td>
<td>KCNQ5, KCNJ2, RYIR2, KCNA5, CaMK2D</td>
</tr>
<tr>
<td>hsa-miR-152</td>
<td>KCNH2, KCNN3</td>
</tr>
<tr>
<td>hsa-miR-199a/b-3p</td>
<td>KCNH2, KCNN2, KCND2, GJA3</td>
</tr>
</tbody>
</table>

Expression of miRNAs regulating CACNA1C

There were 4 miRNAs that regulated the expression of CACNA1C encoding the main ion channel protein regulating the APD plateau, which were significantly altered pre-RFA/N.C. and pre-/post-RFA, including miR-377-5p, miR-328, miR-4306, miR-186-5p (Figure 3).

Expression of miRNAs regulating KCNA5

Expression of miRNAs regulating KCN2H2

KCN2H2 encodes one of the major cardiac voltage-gated potassium channels, the fast-rapid delayed rectifier potassium current (Ikur) ion channel protein, and plays an important role in the action potential repolarization related to several miRNAs, including miR-1266, miRNA-296-3p, miR-152, miR-199a-3p and miR-199b-3p, which were significantly changed pre-RFA/N.C. and pre-/post-RFA (Figure 5).

Expression of miRNAs regulating KCNE1

KCNE1 encodes the subunit of the slow-rapid delayed rectifier potassium current (Iks) ion channel protein, regulated miRNAs including miR-1266, miR-4480, miR-4766-5p, miR-101-3p, which were significantly changed pre-RFA/N.C. and pre-/post-RFA (Fig.6).
Expression of miRNAs regulating KCNE1

KCNE1 encodes a subunit of the inward rectifier potassium current (IKr), playing an important role during the repolarization and it is regulated by several miRNAs, including miR-186-5p, miR-98-5p, miR-101-3p, which were significantly changed pre-RFA/N.C. and pre-/post-RFA (Figure 7).

Expression of miRNAs regulating KCNJ2

KCNJ2 encodes a subunit of the inward rectifier potassium current (IK1), playing an important role during the repolarization and it is regulated by several miRNAs, including miR-186-5p, miR-98-5p, miR-101-3p, which were significantly changed pre-RFA/N.C. and pre-/post-RFA (Figure 7).

Expression of miRNAs regulating KCNC4

KCNC4 encodes a subunit of Acetylcholine-activated K+ current (IKAch) ion channel protein that plays an important role in the repolarization, regulated by several miRNAs, including miR-4306, miR-186-5p, miR-98-5p, which were significantly altered pre-RFA/N.C. and pre-/post-RFA (Figure 8).

Expression of miRNAs regulating KCND3

miR-133b regulating the transient outward K+ current (Ito) ion channel protein subunit encoding gene, KCND3, was shown to increase 2.89-fold in AF patients compared with controls, and decreased 1.98-fold pre- and post-RFA comparison.

Expression of miRNAs regulating KCNN3

KCNN3 encodes a small-conductance calcium-activated potassium channel3 (SK3), and it is regulated by several miRNAs, including miRNA-4732-3p, miR-30d-5p, miR-4306, miR-101-3p, miR-155-5p, miR-152, which were significantly altered pre-RFA/N.C. and pre-/post-RFA (Figure 9).

Expression of miRNAs regulating HCN1, HCN3 and HCN4

The f-channel isoforms of the hyperpolarization-activated current (If) that are expressed in the human atrium cardiomyocyte including HCN1, HCN2, HCN3 and HCN4.

miRNAs regulating HCN1: miRNA-296-3p expression increased 4.3-fold in AF patients pre-RFA compared with controls, and decreased 10.58-fold post-RFA compared with pre-RFA; miR-146-5p decreased 11.16-fold pre-RFA and increased 39.16-fold post-RFA; miR-4732, miR-30d-5p and miR-151a-3p expression are the same as described above.

miRNAs regulating HCN3: The miR-4732-3p, miR-30d-5p and miR-155-5p expression levels are shown in Figure 8.

miRNAs regulating HCN4: miRNA-1284 expression increased 1.94-fold in AF patients pre-RFA compared with controls, and decreased 27.13-fold post-RFA compared with pre-RFA; miR-4796-5p increased 2.68-fold pre-RFA and decreased 23.62-fold post-RFA; miR-4306 decreased 8.33-fold and increased 20.89-fold in patients pre- and post-RFA, respectively; miR-4732-3p expression was the same as described above.

Real-time PCR

The chip data of aforementioned main regulating ion channel microRNAs (Tab. 1) were validated by using real-time PCR. The expression levels of these 21 miRNAs was confirmed the previously observed significant upregulation or downregulation (Figure 10).

Figure 6: The fold changes of miRNAs regulated KCNE1.

Figure 7: The fold changes of miRNAs regulated KCNJ2.

Figure 8: The fold changes of miRNAs regulated KCNC4.

Figure 9: The fold changes of miRNAs regulated KCNN3.

Figure 10: RT-PCR verified the miRNA chip results of AF patients pre- with post-RFA compared. The expression levels of these 21 miRNAs was confirmed the previously observed significant upregulation or downregulation.
miR-1266 expressing levels in human left atrial tissue

The miR-1266 expressing levels in left atrial tissues of rheumatic AF patients were validated by using real-time PCR. The discarded valves and atrial tissues from 20 rheumatic AF patients (control group was 20 non-AF patients) who were undergoing mitral valve replacement surgery were tested, and we found out that the miR-1266 markedly decreased in atrial tissues of the AF patients compared with non-AF patients of mitral valves replacement (p=0.002, Figure 11).

The results of dual luciferase combination experiment

The luciferase relative activity was significantly reduced in miR-1266 groups compared with negative control group (P=0.002); compared with blank control group, which in miR-1266 and negative groups was higher significantly (P=0.01, Fig. 12). So, the SCN5A gene is a target of miR-1266.

Figure 11: miR-1266 expressing levels in human rheumatic left atrial tissue, which was confirmed with RT-PCR, that the miR-1266 markedly decreased in atrial tissues of the AF patients compared with non-AF patients of mitral valves replacement.

Figure 12: The miR-1266 with SCN5A gene dual luciferase combined experimental result, which indicated that the SCN5A gene is a target of miR-1266.

DISCUSSION

miRNAs are the key molecules that regulate cardiac ion channel proteins. miRNAs regulate and maintain cardiac electrophysiological function through combination with the gene 3’-untranslated region (UTR). Abnormal regulation and expression of ion channel proteins and the changes in ion flow lead to atrial electrical remodeling. Arrhythmia is caused by an imbalance of ion flow which depended on the ion channels balance regulated by antiarrhythmic and arrhythmic miRNAs [13,15]. This article mainly investigated the effect of RFA, its possible regulating mechanisms and the influence of RFA on the expression of miRNAs that may play an important regulatory role in ion channel protein expression. We determined that changes in miRNA level in AF patients may be one of the causes of the atrial electrical remodeling of AF; furthermore, these changes were significantly reversed by RFA.

Sodium Current

SCN5A encodes α-subunit of the cardiac Na+ channel and regulates sodium ion flow. The sodium channel of voltage dependence decides the action potential effective refractory period (AERP) [16]. miRNAs related to SCN5A are shown in Figure 2. miR-1266 increased 1.96-fold pre-operatively, which consistent with the RT-PCR result in human rheumatic atrial tissue (released into blood, Fig.11), and decreased 249.86-fold post-operatively in patients. The miR-1266 and SCN5A dual luciferase experimental result indicated that the SCN5A was the direct target gene of miR-1266 (Fig. 12). SCN5A expression was down-regulated before RFA, which resulted in the reduction of sodium current density, but it was reversed dramatically after RFA. These consequences are consistent with the results of animal experiments in dogs, the atrial sodium current decreased 28% and 52% after pacing for 7 or 42 days, respectively, conduction velocity was also significantly decreased [17]. It has been recently shown that the Ina peak current density decreased with a decline in the pore protein Nav1.5 [18-20]. But miR-146-5p and miR-98-5p was significantly decreased 11.16-fold and 7.02-fold respectively in pre-RFA, which may be the arrhythmic miRNAs, but miR-1266 and miR-1284 the antiarrhythmic miRNA; so, the colonial regulating effect might be a little increase of Ina channel protein expression in our study that may be a compensatory change of Ina current decline. So, we suspected that Ina channel and current remodeling might be an adaptation changes after other ion flow remodeling, such as Ca++ and K+ current etc., rather than a dominant alteration.

Calcium Current

The L-type Ca2+ channel α-subunit gene CACNA1C was regulated by several kinds of miRNAs (Fig. 3). miR-377-5p expression increased 4.27-fold before RFA and decreased 102.89-fold after RFA in AF patients. This plays a main role in the regulation of CACNA1C expression that results in a reduction of the ICaL shortening of action potential duration (APD) and AERP, increases cardiac vulnerability to atrial fibrillation. L-type Ca2+ current reduced in about 60% to 70% of patients with AF. ICaL was decreased in dogs underwent rapid atrial pacing [17], which was consistent with our results. Lu et al. reported that the miR-328 level was elevated by 3.5-fold in AF patients with rheumatic heart valvular disease [21,22], but in our study, miR-328 expression decreased 1.79-fold before RFA and increased 20.52-fold after RFA. The different reason may be that our research subjects and sample are different from those in their study. We used the blood of non-valve disease AF patients, but they took atrial tissues from mitral valve replaced AF patients; so, the miR-328 retention in atrial tissue may be easily induced AF susceptibility, when released into blood may inhibit AF coming up; also, our study revealed that miR-328 and the miRNAs regulating Ca++ channel were entirely reversed by the RFA intervention. Further, it indicated that miR-328 may be a potentially useful target site in the regulation of ICaL. The level of miR-328, miR-4306 and miR-186-5p expression decreased before RFA may be so same like Ina current that was the compensatory changes resulted from the ICaL decline, and the increases of outward K+ ion current.

Potassium Current

Kv1.5

The ultrarapid delayed rectifier potassium current (Ikur) is thought to play a major role in the repolarization process of human atrial myocytes. Ikur is carried by functional channels that are assembled using KCNA5-encoded pore-forming α-subunits [23]. The main miRNAs by which the KCNA5 was regulated are shown in Figure 4. The regulating tend and amplitude of all miRNAs was more unanimous and bigger, namely, the all regulating miRNA expression was deeply decreased in pre-RFA, but augmented dramatically in post-RFA; this resulted in the KCNA5 expression increased markedly before RFA, and the outward K+ current increased, which led...
to the shortening of APD and AERP. AF susceptibility enhanced in pre-RFA, but these were reversed in post-RFA. Our result was consistent with the theory of atrial remodeling in AF [24]. Therefore, we think of Ikur current change may play a leading role in the electrical remodeling in atrial fibrillation, as the initiator of evil in the ion flow changes. This view also and mainly was consistent with the result of the effectiveness of the Ikur current inhibitor Vernakalant treatment of atrial fibrillation [25-27]. Furthermore, our result was consistent with Christophersen’s study, six novel non- synonymous mutations was found in KCNA5 and KCNAB2 in 307 patients diagnosed with AF alone, three mutant proteins decreased and weakened KV1.5 current, whereas other 3 proteins preserved and strengthened the K+ current [28], all these enhanced AF susceptibility.

But other previous research [29] found that the density of Ikur was reduced in atrial myocytes from patients with AF. In the left and right auricle of patients with chronic AF, the Ikur density was reduced by 57% and 51%, respectively, which accompanied a decrease in KCNAP expression. One possible explanation is that electrical remodeling makes KV1.5 increase in early AF, but then decrease gradually in persistent or chronic AF because of the reduction of other ion current, such as L-type Ca2+ current (ICaL), etc.

IKr and IKs

KCNH2 encodes α subunit of the voltage-dependent delayed rectifier potassium channel (IKr), which regulated by several kinds of miRNAs (Fig. 5). miR-1266 expression increased 1.96-fold before RFA and decreased 2.49.86-fold after RFA, but the other miRNAs decreased before and increased after RFA. The synthetic levels of KCNH2 regulation and expression was increased, Ik was increased, APD and ERP were shortened and AF susceptibility was enhanced in pre-RFA, but these were reversed in post-RFA, which was consistent with the theory of electrical remodeling [30], and furtherly confirmed that the RFA intervening was effective.

KCNN3 with AF alone [46]. It has been reported that the mRNA and protein of HCN2 and HCN4 in atrial fibrillation electrical remodeling play a pilot and basic role. Therefore, we think of Ikur current change may play a leading role in the electrical remodeling in AF; the IKAch, If, SK, Ik1 and Ito current may also play a certain role, but ICaL and INa current may be a constitutive or adaptability change. This view was mainly consistent with the theory of atrial remodeling in AF [24]. Therefore, we think of Ikur current inhibitor Vernakalant treatment of atrial fibrillation [25-27]. Furthermore, our result was consistent with Christophersen’s study, six novel non- synonymous mutations was found in KCNA5 and KCNAB2 in 307 patients diagnosed with AF alone, three mutant proteins decreased and weakened KV1.5 current, whereas other 3 proteins preserved and strengthened the K+ current [28], all these enhanced AF susceptibility.

IK1

The inward rectifier potassium current (IK1) was increased in patients with AF and plays an important role at the end stage of repolarization [32]. KCNJ1 encodes α subunit of the IK1 channel, regulated main miRNAs in figure 7. Among these miRNAs, mirR186-5p, mirR-98-5p and mirR-361-5p were down-regulated in pre-RFA and up-regulated in post-RFA. The increased in an increase in KCNJ2 levels, an enhancement of IK1 and higher AF susceptibility. However, these changes were reversed by RFA. Previous research has shown that the IK1 current amplitudes and densities were increased in patients or experimental models of AF [33,34], especially, in chronic AF, the IK1 current density was 2-fold higher than controls, which contributed to the decrease in resting membrane potential and involved in atrial APD shortening, and easily developed fibrillation waves and triggered AF.

Ito

Previous studies showed that the transient outward K+ current (Ito) was reduced in human and rabbit atrial cells from patients or animals with AF [35]. KCND3 encodes the pore-forming subunit Kv4.3 and plays an important role in the Ito current. Our results showed that miR-133, which regulates KCND3, increased before and decreased after RFA. Ito reduction slowed the AP phase 1 and elevated the plateau, prolonging ERP. The decrease in Ito was more pronounced in the left atrium than in the right atrium. This may be the basis of AF development [36-39].

IKAch

Acetylcholine-activated K+ current (IKAch) is a small inward rectifying K+ current that mainly exists in the atrial muscle cell membrane [40]. KCNC4 production, which is regulated by several miRNAs (Fig. 8), plays an important role in the IKAch current. miR-4306, miR-186-5p and miR-98-5p expression were decreased before RFA and increased after RFA in our study. So, the KCNC4 expression was up-regulated, IKAch enhanced and APD shortened. But, it has been reported [41] that in patients with persistent AF, the IKAch current density decreased by about 50%, along with the decrease of regulating miRNA level. The decreased IKAch density may result in the increase in atrial muscle excited heterogeneity which might promote reentrance and trigger occurrence [38,42]. This may be related with the difference of the AF phase, such as paroxysmal or persistent.

SK

Recent studies have shown that SK channels are the small-conductance calcium-activated potassium channels in human atrial tissue and that they play an important role in the action potential duration, contributing to the cardiac repolarization current [43]. KCN3 encoding SK3 was regulated by several miRNAs (Fig. 9). All of these miRNAs levels was decreased before RFA and increased after RFA, which leads to an increase in KCN3 before RFA, an increase in outward potassium currents and elevated AF susceptibility. However, studies have shown that miR-499 significantly increased in patients with AF, leading to SK3 down-regulation and possibly contributing to the electrical remodeling in AF [44,45]. This conclusion is different from our result in which the miR-499 expression there was no significant change, but SK current was increased that was also consistented with the theory of AF electrical remodeling [24]. Also, a genome-wide association study has recently associated an intronic single-nucleotide polymorphism (SNP) in KCN3 with AF alone [46].

If

Pacemaker current (If) plays an important role in formatting and maintaining the heart rhythm. Abnormal activities of the If channel were theionic basis of myocardial cell ectopic rhythm [47]. It has been reported when rapid pacing the pulmonary vein muscle sleeve cells, the If current abnormally increased, and at the sympathetic nervous excitement, more obvious the current was added, which could lead to the venous muscle sleeve cells increasing automaticity, forming ectopic rhythm [48], in accordance with our study. We found that the comprehensive regulating results of miRNAs modified the If ion channel protein HCN1, HCN3 and HCN4 was decreased, which led to the ion channel proteins, ion flows, and the excitability of ectopic rhythm increases in pre-RFA, but all reversed in post-RFA. So, the If channel regulating miRNA may also in atrial fibrillation electrical remodeling play a pilot and basic role. It has been reported that the mRNA and protein of HCN2 and HCN4 constituted the If current in atrial fibrillation were increased, especially HCN4 [49]. But this article found that HCN1, the main regulator of normal heart rhythm, its regulatory miRNAs also significantly decreased in atrial fibrillation, especially HCN3 which always thought has little to do with AF, but also had significant changes in AF patient pre-RFA. So, we suspected that HCN1 and HCN3, especially the latter in the incidence of atrial fibrillation may play a very important role, should receive more attention in the future. However, we did not find a difference in HCN2-related miRNA levels. This might be related with the difference phase of AF development.

The significance of miRNA changes

Above all, the regulatory trend and amplitude of the miRNAs regulating outward potassium channels (such as KCNAP5) were unanymous and bigger, so, the colonial regulating effect in outflow K+ ions in atrial fibrillation was increased dramatically, especially the outward Iikur, Ik or Ik1, and Ik1 current, which may play a major role in the electrical remodeling in AF; the IKAch, If, SK, IK1 and Ito current may also play a certain role, but ICaL and INa current may be a compensatory or adaptability change. This view was mainly
consistent with the result of the Ilur current inhibitor Vernakalant treating atrial fibrillation effectiveness.

In conclusion, this study shows that atrial fibrillation radiofrequency ablation operation is not only to have the effect of pulmonary vein electrical isolation, but also can really change or restore the main miRNAs abnormalities regulating ion channel proteins, which may be important for further rebalancing the ion flow, reversing the electrical remodeling in AF, and maintaining the sinus rhythm. miR-1266 regulated multiple ion channels, its regulating trend was consistent with the theory of AF electrotonal remodeling and its amplitude was bigger, so, it may become the future target for AF intervention. However, a larger samples and further research is still needed.

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Conflict of interest
There were no potential conflicts of interest in this study, including related consultancies, shareholdings and grant funding.

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The authors confirm that all ongoing and related trials for this intervention are registered

Supplementary Information
AF clinic research ethics approved document.jpeg
AF ethics voting paper.jpeg
AF miRNA study for apply foundation ethics approved document.jpeg
Informed consent1.jpeg
Informed consent2.jpeg
Informed consent3.jpeg
Informed consent4.jpeg
Informed consent5.jpeg

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