Identification of atrial fibrillation-associated microRNAs in left and right atria of rheumatic mitral valve disease patients

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MicroRNA (miRNA) is associated with the development and pathology of atrial fibrillation (AF). In this study, we performed miRNA profiling of left and right atrium samples from individuals with AF-associated rheumatic mitral valve disease (RMVD) to identify miRNAs that are differentially expressed between RMVD patients with AF and RMVD with sinus rhythm (SR) as controls, as well as between left and right atrium samples from RMVD with AF patients. We performed hematoxylin and eosin staining as well as scanning and transmission electron microscopy to examine in detail any morphological and physiological changes in cardiomyocytes from RMVD patients with AF or SR. Raman spectroscopy was performed to identify molecular and structural information of left and right atrium samples from RMVD with AF and SR. We also performed miRNA array profiling to separately profile miRNA expression patterns of right and left atrium samples from three independent RMVD patients with AF and in a mixed pool of 10 RMVD patients with SR. Morphological and physiological analysis showed distinct shapes and structures of cardiomyocytes from the left and right atria of RMVD patients with AF or SR. The intensity of Raman spectroscopy of atrial tissues from RMVD patients with AF and with SR was different. miRNA profiling showed differential miRNA expression between RMVD patients with AF or SR, and between the left and right atria of RMVD patients with AF. Importantly, miRNAs showed consistent expression changes among all three patients, suggesting that these miRNAs have potential as markers for AF pathology. Our results revealed potential biomarker miRNAs for atrial fibrillation pathology in patients with RMVD. Meanwhile, our data suggested that miR-10b and miR-138-2, which were both significantly increased in the left atrium, are responsible for morphological and physiological phenotype differences between the left and right atria.

Key words: microRNA, atrial fibrillation, rheumatic mitral valve disease, biomarker

INTRODUCTION

Rheumatic mitral valve disease (RMVD), a chronic acquired heart disease, is a significant global health threat and is estimated to affect over 15 million people (Carapetis et al., 2005). Many RMVD patients are children living in developing countries (Marijon et al., 2012). With the advancement of molecular medicine, efforts have been made to identify molecular markers of disease for clinical applications (Ozer et al., 2009; Banerjee et al., 2014; Leão et al., 2014). However, few RMVD biomarkers are known. Meanwhile, atrial fibrillation (AF) is the most common type of heart arrhythmia and this condition is
present in many RMVD patients (Burstein and Nattel, 2008). Although accumulating evidence suggests a connection between RMVD and AF (Zhang et al., 2014; Kim et al., 2015; Garcia-Villarreal, 2016), the detailed mechanisms of AF pathology are largely unclear. Moreover, differences between RMVD patients with AF and RMVD with sinus rhythm (SR) are poorly understood, as is how AF contributes to RMVD pathology and vice versa. Thus, understanding differentially expressed genes in RMVD patients with and without AF should provide insights into the relationship between RMVD and AF, and provide information for clinical diagnosis and treatment.

As a subtype of non-coding RNA, microRNA (miRNA) is associated with the development and pathology of cardiovascular disease, and miRNAs are therefore promising therapeutic candidates (Hata, 2013; Dangwal and Thum, 2014). Circulating miRNAs have recently been identified and may serve as passive markers for many diseases, particularly cardiovascular diseases (Chen et al., 2008; Wang et al., 2010; Melman et al., 2015; Thomou et al., 2017; Zhang et al., 2017), further indicating their potential role as clinically relevant biomarkers. miRNAs have been associated with both RMVD and AF pathologies (Lu et al., 2010; Dong et al., 2015; Li et al., 2015). For example, in AF, miRNA-328 contributes to adverse electrical remodeling, whereas miRNA-26 governs potassium currents (Lu et al., 2010; Luo et al., 2013). A recent comprehensive comparison of differentially expressed miRNAs in RMVD patients with SR or AF by Liu et al. provided information for understanding the roles miRNAs play in regulating RMVD and AF (Liu et al., 2014). The Liu et al. study, together with previous reports, showed that miRNA expression profiles in the left and right atrium differ, suggesting that left and right atrial functions are regulated by distinct mechanisms during the pathology of AF in RMVD. Based on these findings, miRNA profiling of single patients could validate these results and provide additional information for identifying biomarkers to distinguish RMVD with AF or SR and, more importantly, decipher the mechanisms underlying RMVD and AF pathology.

In this study, we collected samples from three RMVD patients with AF and 10 RMVD patients with SR. We first observed morphological and physiological changes in cardiomyocytes by hematoxylin & eosin (H&E) staining, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). We also separately profiled the miRNA expression patterns in samples from left and right atrial appendages from the three RMVD patients with AF as well as the miRNA expression patterns in a pooled sample of left and right atrial tissue from RMVD patients with SR.

**MATERIALS AND METHODS**

**Patients and samples** Study patients were recruited from the Department of Cardiology, First Hospital of the First Affiliated Hospital, Xi’an Jiaotong University inpatients. This study was approved by the Committee for the Conduct of Human Ethics of the First Affiliated Hospital of Xi’an Jiaotong University, and all patients signed informed consent forms. Samples from right and left atrial appendages were obtained from three patients who exhibited clinical characteristics of RMVD with AF; two were males. Samples of right and left atrial appendages were also collected from 10 patients exhibiting clinical characteristics of RMVD with SR; seven were males. All patients in this study were > 45 years old. The appendages were immediately frozen in liquid nitrogen after surgical excision and stored at −80 °C before experiments. The diagnosis of AF was made based on medical records and electrocardiograms.

**H&E staining** Samples were first prepared for paraffin sectioning. Briefly, tissue was fixed in 4% formaldehyde for 4 h and dehydrated with ethanol, followed by replacement of ethanol with dimethylbenzene. The dehydrated tissues were paraffin-embedded and cut into 5-μm-thick sections. For H&E staining, sections were immersed in hemalum for 5 min, and rinsed with tap water. Nuclear staining was achieved by incubation in eosin aqueous solution for ~2 min. Slides were washed in water and dehydrated in solutions with increasing amounts of alcohol and xylene. The slides were then sealed with neutral resin.

**SEM and TEM observations** For SEM observation, appendages were fixed in 4% paraformaldehyde overnight and then coated with plasma for 2 min. Specimens were observed using an XL-30 ESEM (Philips). For TEM observation, appendages were fixed overnight in 2.5% glutaraldehyde and then incubated in 1% osmium tetroxide for 2 h. The samples were rinsed in distilled water and incubated in 2% uranyl acetate for 2 h at room temperature, and then dehydrated in graded ethanol concentrations. Finally, samples were embedded with fresh resin. Ultrathin sections (70 nm) were prepared and observed with a TEM (JEM, JEOL).

**Laser confocal micro-Raman spectroscopy** Micro-Raman spectra were recorded on a dispersive confocal Raman microscope (Renishaw InVia Reflex, 1200 l/mm). Data were collected using a 50× lens and a 785-nm diode laser line for excitation.

**Array analysis of miRNAs** miRCURY LNA Arrays (Exiqon) were used to detect expression of all annotated miRNAs in the miRBase database (http://www.mirbase.org/). Total RNA was isolated from atrial appendages using Trizol (Invitrogen) according to the manufacturer’s protocol. RNA samples from the right and left atrial...
appendages from three patients exhibiting clinical characteristics of RMVD with AF were examined. Scanning of the miRNA array slides was performed with an Axon GenePix 4000B microarray scanner (Axon Instruments). GenePix Pro v6.0 software (Axon) was used to read the raw intensity of the image. The actual intensity of the signal was calculated by subtracting background signals from the overall intensity. miRNAs that showed

Fig. 1. Morphological and physiological alteration of cardiomyocytes from RMVD patients with SR or AF. H&E staining of cardiomyocytes from RMVD patients with SR (A) or AF (B). TEM results of cardiomyocytes from RMVD patients with SR (C) or AF (D) under different magnifications. SEM results of cardiomyocytes from RMVD patients with SR (E) or AF (F) under different magnifications.

Fig. 2. The intensity of Raman spectroscopy of atrial tissues from RMVD patients with AF or SR. The peak intensities of left (A) and right (B) atrial specimens in AF, and left (C) and right (D) atrial specimens in SR, differ.
a change in expression of > 2-fold and a t-test P value < 0.05 were selected as differentially expressed miRNAs.

**Real-time RT-PCR** Expression level of miRNAs were further determined by RT-PCR in 11 RMVD patients with SR and 11 RMVD patients with AF. RT-PCR experiments were performed as previously described (Chan et al., 2014). Briefly, real-time quantitative PCR was performed according to the manufacturer’s protocol (Bioteke). A twenty-microliter reaction contained 2.5 μl reverse transcription products diluted five-fold, 600 nM of each primer, 10 μl 2×9 SYBR Green PCR master mix, 0.4 μl ROX, and 4.1 μl water. The reactions were prepared in a 96-well plate, and the thermal cycler program was as follows: 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s, and then 62 °C for 32 s, on an ABI 7500 Real-Time PCR System. The thermal denaturation protocol was run at the end of the PCR to determine the number of products that were present in the reaction. All reactions were run in triplicate.

**RESULTS**

**Morphological and physiological changes in cardiomyocytes from RMVD with SR or AF** To understand differences in characteristics of RMVD patients with SR or AF, we first inspected the morphology of cardiomyocytes in tissue samples. H&E staining showed that cardiomyocytes were normally arranged in atrial tissues from RMVD patients with SR (Fig. 1A), whereas typical myocardial hypotrophy and hyperplasia of interstitial collagen fibers were observed in atrial tissues from RMVD patients with AF (Fig. 1A).

![Fig. 3. miRNA profiling comparison of atrial tissues from RMVD patients with AF or SR. Number of up-regulated miRNAs from left atrium (A) and right atrium (B) from RMVD patients with AF compared with RMVD patients with SR. Number of down-regulated miRNAs from left atrium (C) and right atrium (D) from RMVD patients with AF compared with RMVD patients with SR.](image-url)
Table 1. Differentially expressed miRNAs in left and right atria between patients with SR or AF

| Up-regulated miRNAs in LA | Up-regulated miRNAs in RA | Down-regulated miRNAs in LA | Down-regulated miRNAs in RA |
|---------------------------|---------------------------|----------------------------|----------------------------|
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-3p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
| hsa-miR-1307              | hsa-miR-508-5p            | hsa-miR-23a*               | hsa-miR-5204-5p            |
RMVD patients with AF, as were necrotic cardiomyocytes and compensatory cardiomyocyte hypertrophy (Fig. 1B). To further examine morphological changes in atrial tissues among RMVD patients with and without AF, we used TEM and SEM. TEM results showed that cardiomyocytes in the left atrium of RMVD patients with AF had irregular shapes and enlarged nuclei compared with atria from RMVD patients with SR. Varying amounts of glycogen were also observed in the left atrium of RMVD patients with AF (Fig. 1C and 1D). Meanwhile, in RMVD patients with either AF or SR, myofibrillar disorder and increased interstitial chondriosomes were observed in the left atrium, whereas cardiomyocytes in the right atrium had a normal shape but a disordered arrangement. Besides increased numbers of interstitial chondriosomes, few other abnormalities were observed in the right atrium of RMVD patients with either AF or SR (Fig. 1C, 1D). TEM results suggested that major morphological and physiological changes in myocardial tissues occurred in the left atrium. SEM results showed an irregular endocardium surface of the right atrium of RMVD patients with SR, whereas a shuttle shape predominated in RMVD patients with AF (Fig. 1E, 1F).

Raman spectroscopy of atrial tissues from RMVD patients with AF or SR

Raman spectroscopy is a spectroscopic technique used to observe vibrational, rotational and other low-frequency modes in a system, and is increasingly used in the biological sciences to obtain rich molecular and structural information to identify biological samples (Driskell et al., 2008; Cissell and Deo, 2009; Fisk et al., 2017; Desroches et al., 2018; Devitt et al., 2018; Jafarzadeh et al., 2018). Here we used Raman spectroscopy to analyze molecular and structural information in the left and right atria in RMVD patients with AF or SR. Our results showed shifted peaks and, most importantly, changes in peak heights among the four groups (Fig. 2), which suggests the variability of molecular structure of atrial tissues from RMVD patients with AF or SR. The peak intensities of left (Fig. 2A) and right (Fig. 2B) atrial specimens in AF, and of left (Fig. 2C) and right (Fig. 2D) atrial specimens in SR, are different from each other. This indicates that molecular and structural information in the four groups varies. Changes in miRNA species and abundance may contribute to the variation, and this hypothesis was tested by the follow-
Differentially expressed miRNAs in RMVD with AF

miRNA expression profiling in RMVD patients with AF or SR  To identify specific miRNAs associated with RMVD patients with AF, we profiled miRNA expression patterns of the right and left atria separately in three RMVD patients with AF. For the control miRNA expression pattern, we analyzed a pooled sample of right and left atria from 10 RMVD patients with SR. Compared to the control group, 94, 102 and 102 miRNAs were up-regulated by more than 2-fold ($P < 0.05$) in the left atrium of the three patients (Fig. 3A), whereas 163, 167 and 175 miRNAs were up-regulated by more than

Table 2. Differentially expressed miRNAs in both LA and RA between patients with SR or AF

| Up-regulated miRNAs in both LA and RA | Down-regulated miRNAs in both LA and RA |
|---------------------------------------|----------------------------------------|
| hsa-miR-664*                          | 71.45802                                |
| hsa-miR-138                           | 67.31552                                |
| hsa-miR-532-5p                        | 61.7922                                 |
| hsa-miR-181a                          | 28.72962                                |
| hsa-miR-25                            | 23.47413                                |
| hsa-miR-378c                          | 20.09109                                |
| hsa-miR-194                           | 13.16721                                |
| hsa-miR-518d-5p                       | 12.84173                                |
| hsa-miR-612                           | 0.022097                                |
| ebv-miR-BART14*                       | 0.023272                                |
| hsa-miRPlus-C1076                     | 0.031703                                |
| hsa-miR-1915*                         | 0.032517                                |
| hsa-miR-1207-3p                       | 0.038556                                |
| hsa-miR-139-3p                        | 0.040052                                |
| hsa-miR-130b*                         | 0.046186                                |
| hsa-miR-3614-3p                       | 0.048278                                |
| hsa-miR-129-5p                        | 0.049082                                |
| hsa-miR-636                           | 0.050779                                |
| hsa-miR-892a                          | 0.051943                                |
| hsa-miR-3158-3p                       | 0.052771                                |
| hsa-miR-760                           | 0.053109                                |
| hsa-miR-551a                          | 0.05548                                 |
| hsa-miR-3183                          | 0.056411                                |
| hsa-miR-3614-5p                       | 0.056449                                |
| hsa-miR-1204                          | 0.05959                                 |
| hsa-miR-1226                          | 0.062765                                |
| hcmv-miR-UL148D                       | 0.06539                                 |
| hsa-miR-3622b-3p                      | 0.070451                                |
| hsa-miR-550b                          | 0.072384                                |
| hsv1-miR-H2*                          | 0.072675                                |
| hsa-miR-639                           | 0.074675                                |
| hsa-miR-885-5p                        | 0.074785                                |
| ebv-miR-BART10*                       | 0.076009                                |
| hsa-miR-2113                          | 0.076309                                |
| hsa-miR-4312                          | 0.076874                                |
| hsa-miR-585                           | 0.079907                                |
| hsa-miR-485-3p                        | 0.082754                                |
| hsa-miR-1267                          | 0.087765                                |
| hsa-miRPlus-A1031                     | 0.088138                                |
| ebv-miR-BART9                         | 0.088768                                |
| hsa-miR-654-5p                        | 0.093376                                |
2-fold ($P < 0.05$) in the right atrium of the three patients (Fig. 3B). Meanwhile, 153, 133 and 129 miRNAs were down-regulated by more than 2-fold ($P < 0.01$) in the left atrium of the three patients (Fig. 3C) and 237, 235 and 243 miRNAs were down-regulated by more than 2-fold in the right atrium of the three patients (Fig. 3D). In the left atrium, 47 miRNAs were up-regulated in all three patients and in the right atrium, 131 were up-regulated ($P < 0.05$) in all three patients (Fig. 3A and 3B, Table 1, Supplementary Table S1). Interestingly, 32 miRNAs were up-regulated in both the left and right atria (Table 2), suggesting that miRNAs expressed on both sides of the heart may participate in AF in RMVD patients in addition to those miRNAs that were differentially expressed between the two sides ($P < 0.01$). Meanwhile, 129 miRNAs were down-regulated ($P < 0.05$) in the left atrium of all three patients and 178 were down-regulated ($P < 0.05$) in the right atrium of all three patients (Fig. 3C and 3D, Table 1). Expression of 50 overlapping miRNAs was downregulated by more than 2-fold in both the left and right atria (Table 2) ($P < 0.05$). These results indicated that the differentially expressed miRNAs in three RMVD patients with AF constitute a firm basis for further discovery of miRNA biomarkers for diagnostic and clinical applications for RMVD patients with AF.

Characterization of differentially expressed miRNAs between the left and right atria of RMVD patients with SR showed that 117 and 237 miRNAs were enriched by more than 2-fold ($P < 0.05$) in the left and right atria, respectively (miRNAs with more than 10-fold change ($P < 0.05$) in expression are also listed in Table 3). These results suggested that miRNAs were differentially regulated between the left and right atria in RMVD. In addition, right atrial samples from the three RMVD patients with AF were mixed as the right atrial group, and left atrial samples from the three RMVD patients with AF were mixed as the left atrial group. A comparison of miRNA expression levels between the left and atria from the RMVD patients with AF showed that 42, 44 and 38 miRNAs and 36, 17, and 83 miRNAs were up- and down-regulated, respectively, in the left atrium of the three patients (Fig. 4A and 4B). Importantly, we found two miRNAs, miR-10b and miR-138-2, that were up-regulated ($P < 0.05$) in the left atrium of all three RMVD patients with AF. Since SEM and TEM indicated that the left atrium had the most severe morphological and physiological changes, these two miRNAs were considered candidates for additional study.

We further validated the expression changes with qPCR (Fig. 5). As expected, our results showed that in RMVD patients with AF, the expression of miR-181a-2 was significantly increased ($P < 0.05$) while miR-25 was significantly decreased ($P < 0.05$). Meanwhile, our data for patients with both RMVD and AF confirmed that miR-10 and miR-138-2 were up-regulated in the left atrium compared to the right atrium, while let-7f and miR-195-5p were down-regulated ($P < 0.05$).

**DISCUSSION**

Since its discovery, microRNA-mediated gene regulation has been shown to influence nearly every biological process in human beings (Fabian et al., 2010; Sun et al., 2010). With the rapid development of the big data era, gene profiling at the single-patient level can provide important information. Here, we profiled miRNA expression in individuals suffering from RMVD with AF and revealed critical miRNAs that displayed identical expression alterations in different patients, suggesting that RMVD patients with AF shared common gene
expression variation that may play important roles during AF pathology in RMVD patients. These miRNA varieties could direct future clinical applications.

Our results revealed that 47 miRNAs were up-regulated in the left atrium of all three RMVD patients with AF compared with RMVD patients with SR. Among these miRNAs, has-miR-3613-3p, has-miR-3178, has-miR-3196, has-miR-1307-3p, has-miR-181a and has-miR-30a* were also shown in a previous report to be up-regulated (Liu et al., 2014). Interestingly, miR-181a was found to play critical roles in cardiac hypertrophy, heart mitochondria and hypertension, and its expression correlates with cardiothoracic surgical outcomes (Jackson et al., 2013; Bloch et al., 2015; Raut et al., 2016; Das et al., 2017). In addition, miR-30 is involved in the prevention of cardiac ischemia/reperfusion mitochondrial impairment (Forini et al., 2014). These previous findings support our results and demonstrate the role of miRNAs in cardiac disease. Our results further suggest a functional role in AF for several miRNAs. Thus, additional functional validation of these overlapping miRNAs is warranted. Our results also revealed that 129 miRNAs were down-regulated in the left atrium of all three RMVD patients with AF. Among them, has-miR-195-5p and has-let-7f were also previ-
ously found to be down-regulated in RMVD patients with AF (Liu et al., 2014). Interestingly, miR-195 regulates cardiac apoptosis induced by ischemia/reperfusion and is closely associated with aortic aneurysmal disease (Zampetaki et al., 2014; Gao et al., 2016; Hang et al., 2016). Taken together, our results were consistent with most previous findings and provide new insights into the pathology of AF.

In the right atrium of all three RMVD patients with AF, we found that 131 and 178 miRNAs were up- and down-regulated, respectively, compared with RMVD patients with SR. Among these miRNAs, hsa-miR-25*, hsa-miR-221*, hsa-miR-4324, hsa-miR-125b-2 and hsa-miR-181a-2 were previously shown to be down-regulated in RMVD patients with AF (Liu et al., 2014). Unexpectedly, we found that hsa-miR-181a was up-regulated in the left atrium of all three RMVD patients, suggesting that miR-181a-2 and miR-181a may play opposite roles in the right and left atria. Thus, this specific miRNA cluster should be further examined to define its function in AF and in cardiac development. Meanwhile, miR-25 is critical during heart failure (Dirkx et al., 2013; Wahliquist et al., 2014). Since AF is closely related to heart failure (Xiao et al., 2011), our finding may provide new evidence for miRNAs that regulate AF associated with heart failure. Among the down-regulated miRNAs in the right atrium, miR-221 was reported to promote cardiac hypertrophy, whereas circulating miR-221 could serve as a biomarker for early prediction of acute myocardial infarction (Wang et al., 2012; Coskunpinar et al., 2016). Our findings further validate these previous reports, and suggest that these differentially expressed miRNAs exist in circulation and thus could be used as biomarkers for disease prediction. In addition, miR-12b, another miRNA that was down-regulated in the right atrium of all three RMVD patients with AF, is closely related to cardiac fibrosis and calcific aortic valve disease (Ohukainen et al., 2015; Nagpal et al., 2016). These results are suggestive of a relationship among cardiac fibrosis, calcific aortic valve disease and AF.

Interestingly, levels of both miR-10b and miR-138-2 were significantly increased in the left atrium of all three RMVD patients with AF compared to their respective expression levels in the right atrium. A recent report showed that miR-10b is associated with cardiomyocyte apoptosis in aortic stenosis patients (Gallego et al., 2016), suggesting that the different phenotypes we observed for the left and right atrium by TEM and SEM are due to cardiomyocyte apoptosis. Importantly, in addition to its role in heart development (Morton et al., 2008; He et al., 2013; Xiong et al., 2016), miR-138 also participates in cardiomyocyte apoptosis, which further suggests that there are differences in apoptosis between the left and right atria of RMVD patients with AF.

In this study, many virus-derived miRNAs (e.g., hsv2-miR-H6* from herpes simplex virus in Table 1) are detected as differentially expressed miRNAs between RMVD patients with AF and SR. Viral myocarditis is common in the general population and patients, most of whom are virus carriers (Andréoletti, 2011). That is why virus-derived miRNAs are detected in our study. These viruses, in turn, can trigger rapid progression of primary heart disease (Bowles et al., 2002; Saguner et al., 2017). However, understanding the potential roles and mechanisms of these viruses in the development of rheumatic mitral valve disease needs further work.

In summary, our results revealed potential biomarker miRNAs for RMVD patients with AF. By comparative expression profiling of the left and right atria, our data indicated that several miRNAs are differentially expressed between both RMVD patients with AF and SR and the left and right atria in RMVD patients with AF. These results provide preliminary insights into the mechanism of AF pathology in RMVD.

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