Expression of miR-133 and miR-30 in chronic atrial fibrillation in canines

HONGLI LI[1], SHUFENG LI[2], BO YU[2*] and SHAOWEN LIU[1*]

[1]Department of Cardiology, Shanghai First People's Hospital, College of Medicine, Shanghai Jiaotong University, Shanghai 200080; [2]Department of Cardiology, Second Affiliated Hospital of Harbin Medical University, Harbin 150086, P.R. China

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Abstract. Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. The most significant histological property of AF is atrial fibrosis, but the underlying mechanism is not clear. In this study we investigated the expression of miR-133 and miR-30, anti-fibrotic microRNAs (miRNAs), in chronic AF in canines. A total amount of 42 mongrel canines of either gender, weighing between 20 and 28 kg, were randomly assigned to the sham-operated and AF groups. All canines were subjected to weekly physical examinations and electrocardiogram. Alterations in tissue structure were assessed in atrial tissue samples by using hematoxylin and eosin and Masson's trichrome. The expression of miR-133 and miR-30 was determined by TaqMan real-time polymerase chain reaction (RT-PCR) and northern blot analyses of atrial tissue. The data were analyzed using the program SPSS 11.5 for Windows. At follow-up, rapid pacing from the left superior pulmonary vein induced sustained AF in the AF group. In the left atrium, increased interstitial fibrosis and chronic inflammation were observed. RT-PCR and northern blot analyses showed that miR-133 and miR-30 expression was downregulated in the AF group. Our results show that both miR-133 and miR-30 play an important role in controling structural changes in chronic AF.

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, occurring in 1-2% of the general population (1). The prevalence of AF increases with age, from <0.5% at 40-50 years, to 5-15% at 80 years (2). The most important characteristic of AF is the changes in atrial structure and electrophysiology, which promotes persistent AF even in the absence of progressive underlying heart disease (3,4). Two principal interrelated forms of atrial remodeling have been identified: atrial structural remodeling (ASR) and atrial electrical remodeling (3,4). ASR is characterized by prominent fibrosis between and within atrial muscle bundles (5). The mechanism of ASR remains unclear.

Over the years, with the rapid evolution of microRNA (miRNA) study, researchers have begun to appreciate the roles of these small non-protein-coding miRNAs in the cardiovascular system (6,7). miRNAs primarily silence gene expression at the post-transcriptional level by acting on the 3'-untranslated region of genes to help define the proteome of cells. Growing evidence has indicated that miRNAs are important in the growth, development and stress responses of the heart (7). The implications of miRNAs in the pathological process of the cardiovascular system have also been appreciated over the years (7,8). Of particular relevance to this study, miRNAs have been documented to play critical roles in regulating cardiac excitability and the associated arrhythmogenicity in a number of pathological settings (8). It has been reported that miR-133 and miR-30 are abundant in cardiac muscles and regulate fibrogenesis in cardiac tissues (9). However, it remains unclear whether the expressions of miR-133 and miR-30 are affected in chronic AF. In this present study, we investigate the expression pattern of miR-133 and miR-30 in chronic AF of canines.

Materials and methods

Animal models. The canine model has been fundamental in enhancing our knowledge of ASR and atrial electrical remodeling. In this study, we use canines experimentally with induced chronic AF. The electrophysiology of the canines was evaluated by serial electrocardiograms (ECGs) at 1, 3 and 5 weeks after rapid pacing, and the animals were euthanized at 1, 3 and 5 weeks after rapid pacing. Another group of canines were monitored and euthanized at 1 to 5 weeks after sham-operation, and their hearts were used as the controls. All animal experiments were approved by the Animal Care and Ethics Committee of the Shanghai Jiaotong University, Shanghai, China.

Correspondence to: Professor Shaowen Liu, Department of Cardiology, Shanghai First People's Hospital, College of Medicine, Shanghai Jiaotong University, 100 Haining Road, Shanghai 200080, P.R. China
E-mail: shaowenliu@yahoo.cn

*Contributed equally

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Surgical procedure for the AF model. Forty-two mongrel canines of either gender, weighing between 20 and 28 kg, were randomly assigned to the sham-operated group (n=21) and the AF group (n=21). All canines were anesthetized [pentobarbital sodium 25 mg/kg injected intravenously (iv)], intubated, placed on a fluoroscopy table and mechanically ventilated with room air and supplemental oxygen. Anesthesia was maintained by additional doses of pentobarbital sodium (5 mg/kg iv), if needed. In order to induce sustained AF in the AF group, a left lateral thoracotomy was then performed in the 4th intercostal space for optimal visualization of the left superior pulmonary vein (LSPV). A bipolar screw-in lead was affixed to the LSPV, and the other end of the electrode cable was tunneled subcutaneously and exposed at the back of the canines where it was used for the pacing in the chronic phase. A pacemaker (Medtronic Inc., Minneapolis, MN USA) was implanted in a subcutaneous pocket and connected to a screw-in lead in the LSPV. After a 1-week recovery, the stimulator was programmed to burst pace at 400 beats/min for 5 weeks. Rhythm monitoring was recorded intermittently via electrodes placed on the skin. The canines were examined on a weekly basis for AF induction.

Histology. Transmural tissue samples from the left atrium (LA) free wall of the 2 groups were immediately fixed in 10% formalin for 24 h and embedded in paraffin, and cut into 3-mm sections for histological assessment. Paraffin sections were stained with hematoxylin and eosin (H&E), and Masson's trichrome.

Real-time polymerase chain reaction (RT-PCR). To detect miRNAs from tissues, total RNA was isolated using the mirVana miRNA isolation kit (Ambion) according to the protocol of the manufacturers. Total and miRNA-specific cDNA was generated with iScript cDNA Synthesis kit (Bio-Rad), and mirVana quantitative RT-PCR primer sets for miR-133 and miR-30 (Ambion).

Northern blotting. Three micrograms of total RNA were fractionated on a denaturing 12% polyacrylamide gel containing 8 mol/l urea, transferred to Nytran membrane by the capillary method, and fixed by UV cross-linking according to the manufacturer's instructions. Membranes were hybridized with specific digoxigenin-labeled LNA probes for miR-133 or miR-30. Detection was performed with an antibody to digoxigenin.

Statistical analysis. Quantitative data are presented as the means ± SD. For comparison between multiple groups, data was analyzed by ANOVA, and with the Student-Newman-Keuls post-hoc analysis. Values of $P<0.05$ were considered to indicated statistically significant differences. SPSS 11.5 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

Results

Rapid pacing induced sustained AF. Rapid pacing from the LSPV induced sustained AF in the AF group. Sustained AF was documented by the elimination of electrical activity in electrodes placed on the skin in the AF group (Fig. 1). There was no clinical evidence of heart failure in any canine.

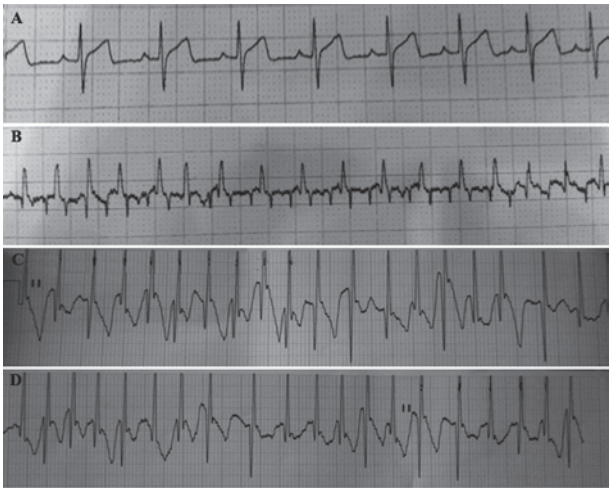

Figure 1. Continuous recordings show electrocardiogram leads II. (A) Before rapid pacing. (B) One week after rapid pacing. (C) Sustained atrial fibrillation (AF) induced by rapid pacing for 3 weeks. (D) Sustained AF induced by rapid pacing for 5 weeks.

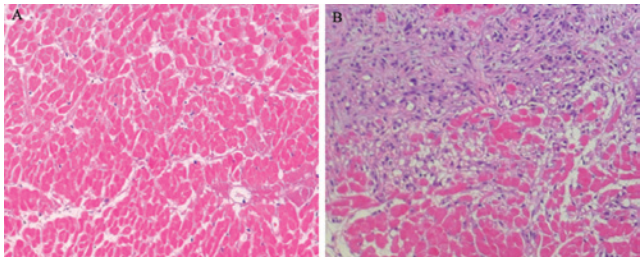

Figure 2. Hematoxylin and eosin staining of the left atrial tissue in (A) the control and (B) atrial fibrillation groups.

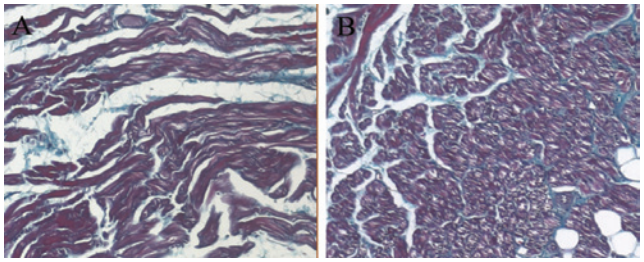

Figure 3. Masson's trichrome staining of the left atrial tissue in (A) the control and (B) atrial fibrillation groups.

Histology studies. Transmural tissue sections from LA were stained using H&E (Fig. 2), or trichrome stain (Fig. 3) to compare tissue structure and the distribution of fibrous tissue in the control and AF groups. The atria of the control group showed a normal histological structure. In the AF group, LA sections had a significant increase in fiber separation with some inflammatory infiltration in the late phases of chronic AF (Fig. 2). These changes were not observed in the early phases of AF (data not shown). However, myocyte morphology was similar to that in the control canines, without signs of cellular necrosis (Fig. 2). At higher magnification, infiltrates of inflammatory cells indicative of chronic inflammation were identified in the AF group. In Masson's trichrome-stained sections, fiber separation and inflammatory infiltrates were

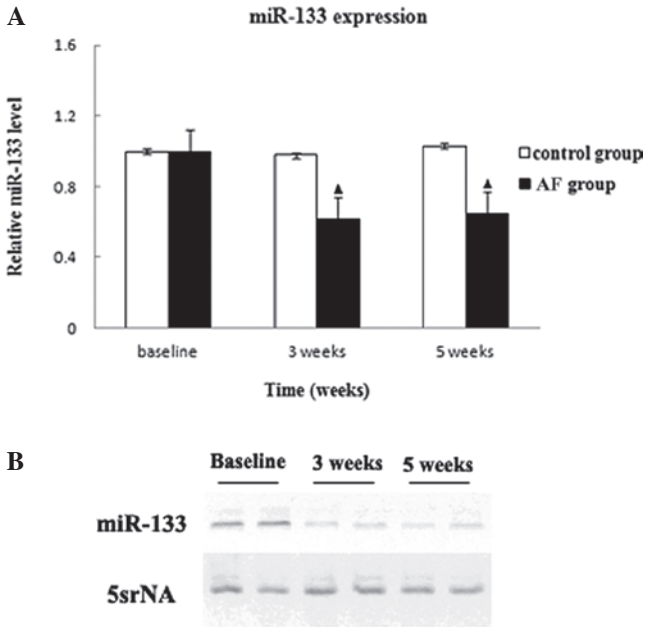

Figure 4. Expression of miR-133 in left atrial tissue. (A) Mature miR-133 is downregulated in the heart of 3- and 5-week canines after rapid pacing (n=7, respectively), as detected by real-time PCR (▲, compared with that of the-control group and baseline, P<0.05). (B) Northern blot analysis for miR-133 in cardiac tissue. The upshift reflects miR-133. 5srNA is the loading control.

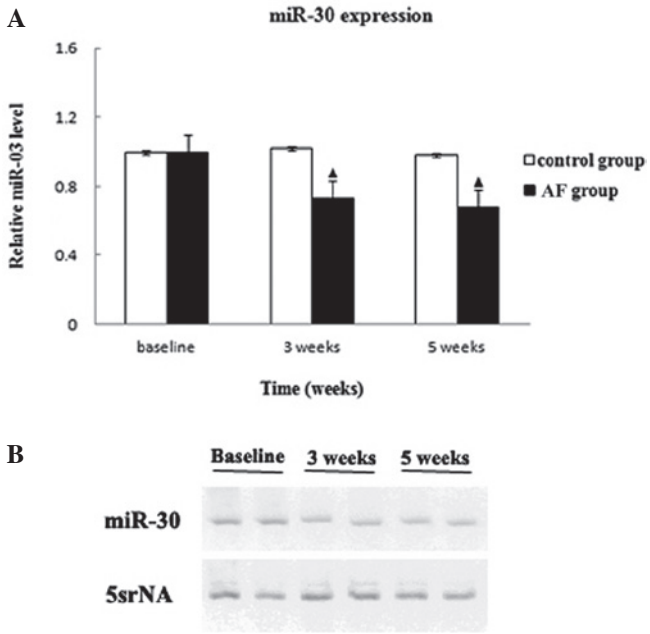

Figure 5. Expression of miR-30 in left atrial tissue. (A) miR-30 is down-regulated in the heart of 3- and 5-week canines after rapid pacing (n=7, respectively), as detected by real-time PCR (▲, compared with that of the control group and baseline, P<0.05). (B) Northern blot analysis for miR-30 in cardiac tissue. The upshift reflects miR-30. 5srNA is the loading control.

visible in the AF group (Fig. 3). These results indicate that fibrosis is a major aspect of the remodeling process after the creation of AF.

miR-133 expression in pathological LA. We investigated the expression of mature miR-133 in LA. RT-PCR analysis verified a consistent decrease in miR-133 expression in the AF group. Compared with that of the baseline, the level of miR-133 was significantly downregulated in the LA 3 weeks after rapid pacing. At later stages, mature miR-133 levels remained decreased in comparison with those of the control group (Fig. 4A). Northern blot analysis further confirmed the decrease in the expression of miR-133 at weeks 3 and 5 after rapid pacing (Fig. 4B).

miR-30 expression in pathological LA. We investigated the expression of mature miR-30 in LA. RT-PCR analysis verified a consistent decrease in miR-30 expression in the LA tissue of the AF group. Compared with the baseline data, the level of miR-30 was significantly downregulated in LA 3 weeks after rapid pacing. At later stages, mature miR-30 levels remained decreased in comparison with those of the control group (Fig. 5). Northern blot analysis of miR-30 expression further confirmed the decrease in the expression of miR-30 at weeks 3 and 5 after rapid pacing (Fig. 4).

Taken together, these results indicate that the reduced miR-133 and miR-30 expression may cause an accumulation of collagens and thereby contribute to tissue fibrosis in the diseased heart.

Discussion

AF is the most commonly encountered clinical arrhythmia. The most important characteristic of AF is the alterations in atrial structure and electrophysiology, which promote persistent AF even in the absence of underlying heart disease (3,4). Atrial structure remodeling is characterized by prominent fibrosis between and within atrial muscle bundles (5). The mechanism of ASR remains unclear. In this study, we describe the expression patterns of miR-133 and miR-30 in chronic AF in canines.

First we observed the electrical activity at the baseline levels and weekly post-operation. A wealth of data suggest that re-entry is the primary mechanism of AF (10). An alternative theory indicates that there is a focal mechanism for AF (11). In this study, we show that rapid electrical stimulation from the LSPV can induce sustained AF. This suggests that there are focal discharges from the pulmonary veins (PVs) during chronic sustained AF in canines. These focal discharges are most likely due to automaticity and triggered activity, which are known to develop in the PV myocytes as a result of pacing-induced remodeling. However, the contribution of rapid pacing to the remodeling process may be unclear.

Secondly, the histological properties of AF in the LA were investigated using H&E and trichrome staining. In the AF group, LA sections were significantly increased in fiber separation with some inflammatory infiltration in the late phases of chronic atrial dilatation. Signs of cellular hypertrophy, myolysis, necrosis, or other degenerative changes were not identified. Therefore, this suggests that fibrosis is related to chronic AF in the LA. In other studies, extensive interstitial fibrosis in the LA has also been reported, accompanied with cellular hypertrophy, loss of myofibrils, and signs of necrosis in a canine chronic heart failure model (12). However, LA cannot be completely contributed to fibrosis in this model.

Finally, the expression of miR-133 and miR-30 during AF was evaluated. miR-133 and miR-30 families are among

the most highly expressed miRNAs in cardiac myocytes (6). Earlier reports have shown that miR-133 and miR-30 control ventricular fibrosis in a chronic heart failure model (9). Unfortunately, these studies did not examine tissue fibrosis in the atrium; therefore, it is unknown whether miR-133 and miR-30 are also involved in regulating atrial fibrosis. In our study, we show that miR-133 and miR-30 levels decrease substantially in the course of AF. Importantly, the downregulation of miR-133 and miR-30 occurred very early in AF, before the observed atrial fibrosis. It has already been reported that miR-30 and miR-133 are evidenced as anti-fibrotic miRNAs (9,13,14). The early loss of these miRNAs suggests that this downregulation does not represent a disease consequence caused by, for instance, cell death, inflammation, or fibrosis, but may represent an evolutionarily conserved mechanism that contributes to adverse cardiac remodeling and tissue fibrosis. However, their involvement in regulating fibrosis needs to be researched further.

In conclusion, our results show that both miR-133 and miR-30, as anti-fibrotic miRNAs, may play an important role in the control of structural changes in chronic AF.

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