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MicroRNA 208 in Atrial Fibrillation

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Abstract

MicroRNAs (miRNAs) are critical regulators of most major cellular processes and seem to play a vital role in the pathogenesis of numerous diseases including atrial fibrillation, the most commonly encountered cardiac rhythm disorder. Among the several miRNAs that appear to be involved in pathogenesis of atrial fibrillation, miRNA 208a is linked to fibrosis and proper cardiac conduction.

We quantified the expression levels of miRNA 208a in left atrial appendage tissue of patients with paroxysmal (n=2), persistent (n=10), and long-standing persistent (n=7) arrhythmia using quantitative PCR. In paroxysmal atrial fibrillation, miRNA 208a was expressed moderately, whereas the expression was enhanced in persistent atrial fibrillation and significantly reduced in long-standing persistent atrial fibrillation. The difference between persistent and long-standing persistent atrial fibrillation was significant at p=0.02.

The findings from our study suggest a decline in miRNA 208a expression with ongoing arrhythmia, possibly preceded by a rise in expression from paroxysmal to persistent atrial fibrillation or even long-standing persistent. The significant changes in miRNA 208a expression over the course of the disease may be used as an additional diagnostic tool to monitor the progression of atrial fibrillation.

Keywords: MicroRNAs; Atrial fibrillation; Arrhythmia; Expression

Introduction

Atrial fibrillation is the most common form of cardiac atrial arrhythmia, with a prevalence of 0.4%–1% in the general population and 5.5% in patients over 55 years [1-3]. According to Go et al., the estimated prevalence will become more than double by the end of 2050. Atrial fibrillation increases the risk of stroke and is responsible for more than half of the deaths resulting from cardiovascular diseases [2]. The arrhythmia also accounts for stroke and stroke related death and generally doubles the mortality from cardiovascular disease [4,5]. Different pathological mechanisms are believed to be associated with the development and maintenance of atrial fibrillation. Several factors have been implicated in the pathogenesis of atrial fibrillation such as atrial electrical remodeling via changes in gene expression associated with gap junction proteins (connexins), structural remodeling caused by fibrosis of the myocardium, and inflammation [6,7]. Recent studies have highlighted the role of microRNAs (miRNAs) in the pathogenesis of atrial fibrillation [8]. Several miRNAs have contributed to the pathogenesis of atrial fibrillation by regulating the availability of their target mRNAs and the corresponding proteins. For example, the overexpression of muscle-specific miRNA 1 slows down cardiac conduction by inhibiting the translation of the calcium channel subunit Kir2.1 and connexin 43 [9].

Members of the miRNA 208 family (miRNA 208a and miRNA 208b) are considered to be heart specific. miRNA 208a, which is located intronic to the Myh6 gene, is associated with cardiac conduction in mice as well as cardiac hypertrophy and fibrosis in both humans and mice [10,11]. The overexpression of miRNA 208a in transgenic mice was sufficient to induce cardiac arrhythmia, whereas mice lacking miRNA 208a exhibited abnormal cardiac conduction [11]. There is also evidence that miRNA 208 plays a role in cardioprotection by post-conditioning after ischemia/reperfusion injury as well as in differentiation of cardiac embryonic stem cells, demonstrating it various roles in cardiac biology [12,13].

We therefore hypothesized that dysregulation of this miRNA plays a crucial role in inducing atrial fibrillation. Therefore, in this study we quantified the expression of miRNA 208a in the left atrial appendage tissue of patients suffering from atrial fibrillation as well as commercial heart tissue RNA obtained from healthy donors.

Material and Methods

Sample collection

All experiments performed with human tissue strictly adhered to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from each patient. Specimens obtained during surgery were frozen immediately. A total of 19 samples of left atrial appendage tissue were obtained from patients suffering from atrial fibrillation. In addition, 3 samples of aortic tissue showing no signs of atrial fibrillation were collected. We compared

normal left atrial adult total RNA (purchased from US Biological, Massachusetts, USA) with 3 of our samples by employing miRXplore Microarrays (Miltenyi Biotech, Bergisch Gladbach, Germany). According the manufacture's recommendations and as described previously [14]. To validate the microarrays data set in all 19 samples we picked miRNA208, the most differentially expressed one.

RNA isolation

Isolation of total RNA was achieved using the Trizol reagent (Life Technologies) according to the manufacturer's instructions. In brief, 100 mg of tissue was homogenized with 1 mL of Trizol solution using a tissue lyser (Qiagen, Hilden, Germany). Then 0.2 mL of chloroform was added to the homogenized solution and the lysate was centrifuged to accomplish phase separation. The aqueous phase containing the total RNA was carefully removed and subsequently precipitated by adding isopropyl alcohol, washed, and re-suspended in nuclease-free water.

cDNA synthesis

For synthesis of cDNA, the TaqMan MicroRNA Reverse Transcription Kit was utilized together with the reverse transcription primers from the human miRNA 208 assay (Assay ID 000511; Applied Biosystems, Weiterstadt, Germany). Reverse transcription was performed following the manufacturer's protocol with 10 ng of RNA as the input amount.

Real-Time Quantitative Reverse Transcription PCR (qRT-PCR)

Relative quantification of miRNA 208a expression was performed on an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems) with nuclear RNA RNU48 as the internal control (Assay ID 001006; Applied Biosystems). The samples were run in triplicates with 1.3 µL of the corresponding cDNA in a total volume of 20 µL. Control without reverse transcriptase was run for each sample and control without template was run for every cDNA synthesis and PCR reaction. The thermal cycle profile consisted of one initial 2 min step at 50°C, one 10 min denaturation step at 95°C, and 40 cycles of 15-s denaturation at 95°C and 60s elongation at 60°C.

Statistical analysis

Statistical analysis was performed using excel/WIN STAT and SPSS. The Data obtained from each experiment are presented as mean ± standard deviation. Differences between miRNA expression levels and other continuous variables were tested with two-sided Mann–Whitney U-test and differences in gender distribution were analysed using Fisher's exact-test. For analysing the association among different parameters, Pearson's correlation coefficient was utilized. Significant differences were considered at $p<0.05$.

Results

Patients characteristics

Patients diagnosed with atrial fibrillation were divided into sub-categories according to the latest guidelines established by the European Society for Cardiology in 2012. Atrial fibrillation episodes shorter than 48 h were considered paroxysmal, whereas arrhythmias lasting for more than 7 days or being treated pharmacologically or electrically within these 7 days were considered persistent. Atrial fibrillation persisting for more than 1 year was classified as long-standing persistent. Patient characteristics are shown in (Table 1).

Patient Characteristics	**Atrial fibrillation (AF)-Classifications**		
AF	paroxysmal (n=2)	persistent (n=10)	long persistent (n=7)
Age, ys	7.350 ± 0.707	65.2 ± 4.2	66.3 ± 3.6
Gender, male: female	1:01	8:02	4:03
BMI	27.17 ± 0.00	31.27 ± 1.96	29.87 ± 2.28
LA-D, mm	45	47.43 ± 3.43	51.86 ± 2.09
LV-EF, %	60	52.20 ± 4.63	53.29 ± 5.88
NYHA	3.0 ± 0	3.10 ± 0.18	3.00 ± 0.218
CAD	0	5 (50%)	1 (14.3)
AoV-Vitium	1 (50%)	2 (20%)	1 (14.3)
TV-Vitium	1 (50%)	3 (30%)	4 (57.1)
MV-Vitium	2 (100%)	10 (100%)	7 (100%)
VD	1 (50%)	4 (40%)	6 (85.7%)#
CHF	2 (100%)	10 (100%)	6 (100%)
aHTN	1 (50%)	10 (100%)	5 (71.4%)
DM	1 (50%)	2 (20%)	1 (14.3%)
Stroke	1 (50%)	2 (20%)	5 (71.4%)*
CHA2DS2-VASc	6	4.1 ± 0.53	5.00 ± 0.72
CHADS2	2	2.7 ± 0.26	2.29 ± 0.42

Table 1: Patients' characteristics, *$p<0.05$, #$p\approx 0.05$

AF: Atrial Fibrillation; BMI: Body Mass Index; LA-D mm: Left Atrium Diameter in Millimetre; LV-EF%: Left Ventricle Ejection Fraction in Percentage; NYHA: New York Heart Association; CAD: Coronary Arterial Disease; Aov-Vitium: Aortic Valve Vitium; TV-Vitium: Tricuspid Valve Vitium; MV-Vitium: Mitral Valve Vitium; VD: Vascular Disease; CHF: Chronic Heart Failure; Ahtn: Arterial Hypertension; DM: Diabetes Mellitus; CHA2DS2-Vasc: Congestic Heart Failure/ Insufficiency

Micro RNA 208a expression

The relative expression level of miRNA 208a was 2.33 ± 1.99-fold of calibrator in atrial fibrillation tissue, whereas it was 1.4 E−4 ± 2.4 E−4 in RNA obtained from healthy donors control aortic tissue RNA (data not shown), thus confirming the cardiac specific expression of this miRNA.

The comparison among duration-based atrial fibrillation sub-categories revealed miRNA 208a expression levels of 2.32 ± 2.12 in paroxysmal patients, 3.26 ± 2.30 in persistent atrial fibrillation, and 1.14 ± 0.67 in long-standing persistent arrhythmias (Figure 1). The difference in miRNA 208a expression levels between persistent and

long-standing persistent atrial fibrillation proved to be significant with p=0.02. All other differences were not significant.

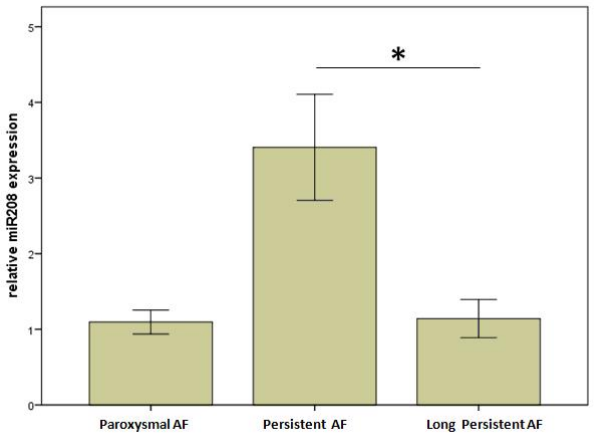

Figure 1: Relative miRNA 208a expression in atrial fibrillation categories. The average relative expression of miRNA 208a in paroxysmal, persistent, long-standing persistent atrial fibrillation is shown. The error bars represent standard deviation, and the asterisk indicates a significant difference between the average miRNA 208a expression in persistent and long-standing persistent atrial fibrillation. Differences in age, sex, or BMI between the groups were not significant; comparison between one group and all other groups treated as a whole revealed no significant differences as well. There was also no significant correlation between age or BMI and miRNA 208a expression.

Discussion

The results of our study demonstrate a significantly higher miRNA 208a expression in patients with a history of persistent atrial fibrillation tissue than in patients with long-standing persistent atrial fibrillation tissue. Additionally our data suggest a medium expression level of miRNA 208a in paroxysmal atrial fibrillation and a very low expression in aortic tissue (data not shown). These results indicate a decline in miRNA 208a expression over time from persistent to long-standing persistent atrial fibrillation.

There is growing evidence indicating changes in gene expression associated with sustained atrial fibrillation. Chung et al. found higher levels of C-reactive protein (CRP), an inflammatory marker, in persistent arrhythmia than in paroxysmal arrhythmia, indicating a stepwise higher inflammatory response in more active atrial fibrillation [15].

miRNA 208a is part of the miRNA network and the miRNAs from this network are encoded intronic and coexpressed with myosin heavy chain genes. The Myh6 gene encodes the fast α-myosin heavy chain (αMHC) and also co-expresses miRNA 208a, whereas the slow myosin genes Myh7 and Myh7b encode the microRNAs 208b and 499, respectively [10,11]. In mice, where αMHC is the predominant isoform, miRNA 208a is the dominant miRNA and is utilized for the expression of the slow myosin isoforms. In humans, αMHC is predominantly expressed in the cardiac atrium, whereas the slow isoform β-myosin heavy chain (encoded by the Myh7 gene) is the main form in the ventricle [10,16]. It remains unclear whether miRNA 208a fulfills a similar role related to the expression of slow myosin isoforms in humans as in mice. In a study related to contractile dysfunction in permanent atrial fibrillation, Mihm et al. reported decreased expression of αMHC in human atria, whereas βMHC expression was enhanced [17]. This switch in the αMHC/βMHC ratio is observed in rodent models of cardiac dysfunction and also in human heart failure leading to reduced contractile velocity [18-20]. The decline in miRNA 208a expression that we observed in case of enduring atrial fibrillation may be partly attributed to the switch in the ratio between fast and slow myosin heavy chain isoforms. However, if miRNA 208a performs analogous role in humans as in mice, there should be an upregulation of miRNA 208a expression prior to the increase in slow myosin heavy chain expression. There may be an upregulation of miRNA 208a at the beginning of cardiac arrhythmia, as indicated by the rise in miRNA 208a expression levels from paroxysmal to persistent atrial fibrillation in our study, although this difference was not significant. A comparison between atrial miRNA 208a expression in patients with sinus rhythm and different types of atrial fibrillation may provide useful information regarding miRNA expression during the course of atrial fibrillation.

Callis and colleagues reported that the transgenic overexpression of miRNA 208a as well as the lack of miRNA 208a expression in mice is sufficient to induce arrhythmias [11]. Therefore the balance of miRNA 208a expression seems to play a pivotal role for proper cardiac conduction in mice. Our results suggest the possible rise and decline of miRNA 208a expression levels with ongoing atrial fibrillation and hence show that imbalances in miRNA 208a expression are associated with arrhythmias in humans as well.

Recently, circulating miRNA 208a has gained importance as a biomarker for detecting cardiovascular diseases [21]. Wang et al. demonstrated that circulating miRNA 208a can be used to detect acute myocardial infarction with high sensitivity and specificity [22]. In addition, overexpression of miRNA 208a predicts poor clinical outcome in patients with dilated cardiomyopathy [23]. Therefore, our results suggest a biomarker role for circulating miRNA 208a to support the diagnosis of the severity of atrial fibrillation status, keeping in mind, that a significant proportion of patients gets misclassified by clinical categorization [24].

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