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Plasma MicroRNAs Relate to Atrial Fibrillation Recurrence after Catheter Ablation: Longitudinal Findings from the MiRhythm Study

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Abstract

Introduction: Genetic and transcriptomic factors play important roles as mediators of new-onset and recurrent atrial fibrillation (AF). MicroRNAs (miRNAs) regulate expression of gene networks involved in key aspects of atrial remodeling. Associations between circulating miRNAs and AF recurrence are unknown. We tested the hypothesis that cardiac miRNAs associated with electrical and structural remodeling predict recurrent AF rhythm in post-ablation patients.

Methods: We quantified plasma expression of 86 cardiac miRNAs using RT-qPCR in 83 consenting participants undergoing ablation for AF. MiRNA expression was re-measured 1-month post-ablation in a subset of 43 of 83 study participants. Then all 83 patients were followed over a 12-month period for AF recurrence and plasma miRNA expression was compared between baseline and 1-month post-ablation and between those with and without an AF recurrence.

Results: The mean age of study participants was 59 years, 34% were female, and 63% had paroxysmal AF. Plasma levels of miRNAs 125a-5p and 10b were 3-fold lower after ablation compared to pre-ablation ($p<0.01$). Pre-ablation plasma expression of miRNAs 125a and 10b, as well as miRNAs 60, 30a-3p and 199b, were higher among patients with an AF recurrence compared to those without recurrence after ablation ($p<0.05$) even after adjustment for clinical risk factors.

Conclusion: The plasma miRnome is dynamic after AF ablation and associated with AF recurrence. Higher pre-ablation levels of circulating gene regulators implicated in atrial remodeling and AF, including miRNAs 125a-5p and 10b, were associated with AF recurrence and that these same miRNAs decreased post-ablation. Our investigation highlights dynamic gene regulatory networks in patients undergoing ablation and identifies potentially new AF treatment targets.

Keywords: Atrial fibrillation; Ablation; Circulation; MicroRNA; Recurrence

Introduction

AF is associated with stroke, dementia, myocardial infarction, heart failure, and diminished quality of life [1]. Catheter ablation (CA) of AF is commonly employed to reduce arrhythmia burden, manage symptoms, and improve AF-specific quality of life [2]. Although CA is highly effective in some patients, AF recurrence after ablation occurs in up to 65% of patients undergoing this procedure within 1 year [2]. Unfortunately, little is known about what drives AF recurrence after CA. Patient selection plays an important role in procedural success and risk-stratification for recurrence is based on pre-existing clinical patient characteristics, such as age or pattern of AF, as well as degree of atrial enlargement. However, clinical prediction tools for AF recurrence show modest discriminative ability [3]. New AF biomarkers are needed, not only to predict treatment response after CA, but also to better understand the mechanisms driving arrhythmia occurrence and recurrence.

Susceptibility to AF is heritable [4]. Commonly occurring genetic polymorphisms correlate with AF recurrence after ablation. Single nucleotide polymorphisms in or near the *4q25/PITX2* gene [5], *16q22/ZFHX3* gene [6], epoxide hydrolase-2 (*EPHX2*) gene [7], interleukin-6 receptor (*IL6R*) gene [8] and the heme oxygenase-1 (HO1) gene promoter region [9], are associated with AF and AF recurrence after

CA. In addition, altered atrial gene expression has been shown in human and animal models of AF, highlighting the importance of dynamic gene regulatory networks as mediators of atrial remodeling and AF. While some genes (i.e., *PITX2*) associated with AF, are involved in electrical remodeling, others influence atrial fibrosis or inflammation (*ZFHX, EPHX, IL6R, HO-1*) [5-9].

MicroRNAs (miRNAs) comprise a class of RNA species that play an important role as dynamic master regulators of gene expression in many diseases. MiRNAs are involved in the pathogenesis of numerous cardiovascular diseases, such as coronary artery disease, heart failure, and arrhythmia [10]. Circulating miRNAs are stable and provide insights into dynamic cardiac gene regulatory processes. Since atrial expression of transforming growth factor-β (TGF-β), matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and connexin-40 (Cx40) are associated with AF recurrence and are regulated by cardiac-enriched or specific miRNAs, atrial miRNAs likely play important roles in atrial remodeling and AF susceptibility [11,12]. We hypothesize that circulating levels of cardiac miRNAs correlate strongly in patients with AF and relate to AF prevalence in several distinct cohorts [13-16].

In light of our prior results implicating circulating miRNAs in the pathophysiology of AF, we hypothesized that plasma miRNA levels change after ablation and differ between individuals with AF recurrence as compared to those who remained free from AF after ablation. We further hypothesized that the identified miRNAs would have mechanistic associations with electrostructural cardiac remodeling. To test these hypotheses, we quantified pre-ablation plasma expression of 86 miRNAs in 83 participants and repeated profiling 1-month post-ablation in a subset of 43 participants in a prospectively recruited, contemporary cohort with AF referred for CA and then examined associations between miRNA expressions with AF recurrence over 12 months [17-25].

Methods

Study population

The miRhythm study is an ongoing, prospectively recruiting cohort study of AF and gene regulation based at the University of Massachusetts Medical Center (UMMC). Briefly, miRhythm study started recruitment in April 2011 and as of November 2016, 584 participants have been enrolled in this study at the UMMC. These participants consist of 227 patients with AF who underwent CA, 114 *patients with AF who underwent a cardiac surgery, and 243 patients who underwent cardiovascular evaluation in cardiology clinics.

94 of 227 patients who underwent CA for symptomatic AF (from April 2011 to January 2014) consented for the pre-ablation measurement of miRNA expression. Our cohort for this study was composed of 83 of these participants who completed 12-month follow-up at UMMC. A subset of 43 participants also consented for repeat profiling 1-month post-ablation.

Participants consented to gene and RNA profiling prior to their ablation. Baseline venous blood samples were drawn from femoral venous sheaths prior to CA and stored using methods previously documented to preserve miRNA [16]. Quantification of plasma miRNA levels were performed with this blood. Demographic, clinical, and baseline laboratory data were abstracted from the UMMC AF Treatment Registry and hospital electronic medical records by trained study staff. Follow-up venous blood samples were obtained at a 1-month follow-up visit and were processed and stored using identical methods. All study protocols were approved by the UMMC Review Board (IRB# 14875).

Catheter ablation for atrial fibrillation

Patients underwent either cryoballoon (n=33) or radiofrequency (n=50) CA, performed by one of 5 clinical electrophysiologists at the UMMC (a tertiary care medical center). A standardized CA approach as stated below was followed in nearly all cases. In cryoballoon CA procedures, an Arctic Front cryoballoon catheter (28 or 23 mm, Medtronic Inc.) was used to perform PVI. For each vein, cryoablation was performed twice for 150 to 240 seconds. In radiofrequency CA procedures, wide area circumferential ablation was conducted with an open-irrigated or 8-mm radiofrequency ablation catheter. In every patient, a circular mapping catheter was employed to assess entrance and exit blockage. Also, at the discretion of the performing electrophysiologist, additional linear lesions were made in the LA roof, the basal posterior wall, and the LA isthmus in participants with persistent AF. After CA, patients were administered proton pump inhibitors for 4 weeks, and antiarrhythmic medications were usually discontinued at a routine 3-month follow-up appointment if there were no symptomatic AF recurrences or evidence of AF on routine per-protocol 1 or 3-month electrocardiograms reported. Oral anticoagulation was prescribed for 3 to 6 months after CA, and long-term use was decided by stroke risk as predicted by $CHADS_2$ or CHA_2DS_2-VASc scores, at the discretion of the treating physician.

Atrial fibrillation monitoring

All study participants included in this analysis had follow-up for at least 12 months after ablation. We screened participants at, 6 and 12 months with ECG and 7-day cardiac event monitoring at 3 or 6 months to assess for recurrence of AF. Additional monitoring (ECG or cardiac event monitoring) was ordered at the discretion of the treating electrophysiologist if the participant reported a symptomatic recurrence (i.e., palpitations, shortness of breath).

Atrial fibrillation recurrence adjudication and study definitions

To confirm the presence of AF, an electrophysiologist blinded to miRNA results reviewed all ECG and event monitor recordings. We defined AF recurrence as the presence of AF on a 12-lead ECG or any AF episode of 20 seconds duration or longer on an event monitor. Clinically significant AF recurrence was defined as any AF recurrence occurring after a traditional blanking period of 3 months after CA, up to 12 months [2]. If a study participant had a cardioversion or repeat AF ablation, they were deemed to have had a clinically significant AF recurrence. For the purposes of this analysis, we decided a priori to focus on relations between miRNA expression and clinically significant AF recurrences (AF recurrences after the usual 3-month blanking period).

Variable	Total Sample (n=83)	No clinically significant AF Recurrence (n=48)	Clinically significant AF recurrence (n=35)
Age (in years)	59 ± 10	58 ± 10	60 ± 9

Female (%)	28 (34)	11 (26)	17 (49)
Caucasian Race	79 (95)	46 (96)	33 (94)
Physiological Characteristics			
Body mass index (kg/m^2)	34 ± 6	32 ± 5	34 ± 14
Systolic blood pressure (mm Hg)	124 ± 18	126 ± 21	123 ± 16
Diastolic blood pressure (mm Hg)	75 ± 12	76 ± 11	74 ± 13
CHA_2DS_2-VASc Score	2 ± 1	2 ± 1	3 ± 1
Medical History			
Current smoking	10 (12)	6 (12)	4 (11)
Diabetes mellitus	16 (19)	6 (12)	10 (29)
Hypertension	61 (73)	36 (76)	25 (69)
Heart failure	10 (10)	4 (10)	6 (17)
Stroke/TIA	5 (6)	2 (4)	3 (8)
Laboratory Characteristics			
Left atrium volume index† mL/m^2	39 ± 10	40 ± 9	38 ± 12
Creatinine (mg/dL)	9 ± 0.2	1 ± 0.2	0.9 ± 0.2
C-Reactive Protein (mg/dL)	5 ± 8	4 ± 6	5 ± 8
Treatment Characteristics			
Any antiarrhythmic drug	59 (72)	32 (66)	27 (75)
Beta Blocker	54 (48)	23 (48)	22 (61)
Calcium channel Blocker	23 (28)	13 (28)	10 (29)
Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use	40 (48)	24 (50)	16 (44)
Warfarin	44 (53)	20 (44)	24 (67)
Aspirin	44 (53)	25 (50)	19 (53)
Digoxin	3 (4)	1 (4)	2 (6)
Statin Drug	39 (47)	21 (42)	18 (50)

Data are given as mean ± SD or no. (%).
AF: Atrial Fibrillation.
† Left atrial volume index available in 72 participants (28 with late AF recurrence).

Table 1: Characteristics of miRhythm study cohort stratified by presence of clinically significant AF recurrence after catheter ablation.

Selection of miRNAs

Greater than 90% of all cardiac miRNA expression originates from only 19 miRNAs [17]. Additional studies have identified other cardiac-enriched circulating miRNAs to be associated with atrial structural and electrical remodeling and AF [13,16,25] For the purposes of this investigation, we selected 86 miRNA expressed in the heart or associated with pathways involved in pathogenesis of AF or atrial remodeling for high-throughput miRNA expression profiling [25]. We identified validated target genes for these miRNAs through miRDB and mirTarBase (under Strong Evidence) and predicted target genes through Target Scan.

MiRNA expression profiling

We isolated RNA and quantified plasma expression levels of 86 candidate miRNAs, 3 negative controls (SNORD61; SNORD95, RNU6-2 RNA), a Reverse Transcription Control, and a PCR Positive Control prior to ablation in 83 study participants as well as 1-month post-ablation in 43 participants with QIAGEN chemistry-based assays for venous blood samples. We utilized the BioMark System, a high-throughput system that has been validated extensively and is able to detect single miRNA copies at 26–27 cycle thresholds [14] to quantify miRNA levels. We report MiRNA levels in cycle quantification units (ΔCq). We used standard methods for cDNA conversion, pre-amplification, and quantitative reverse transcription polymerase chain reaction (RT-qPCR) with QIAGEN miScript Assays per manufacturer's protocols as we have previously described in detail [25].

Statistical Analyses

We reported descriptive statistics as number (%) and continuous variables were reported as mean (± SD) for the 83 miRhythm study participants included in our analyses. We analyzed differences in patient characteristics with a 2-sided Student's T-test or a χ^2-square test as appropriate with a p-value of 0.05 as a threshold for statistical significance. Due to our limited sample size, our study was underpowered to account for multiple testing. To account for expression bias, we used global mean normalization for the analysis, as we have done previously and is recommended [15]. We compared baseline miRNA expression levels to levels 1-month after CA after each set of miRNAs underwent global mean normalization. We used a 2-sided Student's T-test with a p-value of 0.05 and an absolute fold-change of >3 chosen a priori as a threshold for statistical significance. We then used logistic regression analyses to examine relations between baseline plasma miRNA expression levels and clinically significant recurrence of AF. In light of prior studies associating stroke risk factors with AF recurrence and to avoid over-fitting our regression model, we performed multivariable adjustment using the CHA_2DS_2-VASc score. In this analysis, AF recurrence was considered as our dependent variable and miRNA expression levels were considered as our exposure variable. Statistical significance in this regression model was defined based on a 2-sided p-value cutoff of 0.05 and an absolute fold-difference of >1. For the purpose of better understanding mechanisms underlying associations between miRNAs, AF recurrence, and atrial remodeling, we examined univariate associations between clinical factors (age, heart failure, type of AF) as well as echocardiographic markers of atrial structural remodeling (left atrial volume index) and inflammation (C-reactive protein levels) with plasma miRNA expression at baseline.

Results

We display the characteristics of our study cohort stratified by AF recurrence in Table 1. The average age of study participants was 69.4 ± 9.6 years, about one-third of participants were women, the mean body mass index of participants was 3, and most participants had at least one associated cardiovascular comorbidity. The mean left atrial volume index fell at the upper limits of normal, 63% of participants had

paroxysmal AF, and a similar proportion had tried and failed at least one antiarrhythmic drug, indicating that most study participants had a consensus class 1A indication for CA [4]. Over a 12-month follow-up period, 38 participants (40.4%) had clinically significant AF recurrence. As has been previously reported, participants with AF recurrence had higher mean CHA_2DS_2-VASC risk score (3 *vs.* 2) and were more likely to be women than those who remained free from AF recurrence. Participants with AF recurrence were otherwise similar to those without recurrence.

Levels of miRNAs 125a-5p and 10b decrease after catheter ablation for AF

As shown in Figure 1, expression levels of miRNAs 125a-5p (-3.30 *vs* 1.83) and 10b (-0.01 *vs* 3.23) were significantly lower 1-month post-ablation as compared to baseline among 43 study participants with available miRNA data from these two important time-points ($p<0.01$ and fold change >3). Participant clinical and laboratory characteristics did not differ between those participants who had miRNA levels quantified (n=43) and those who did not (n=40, Supplemental Table 1).

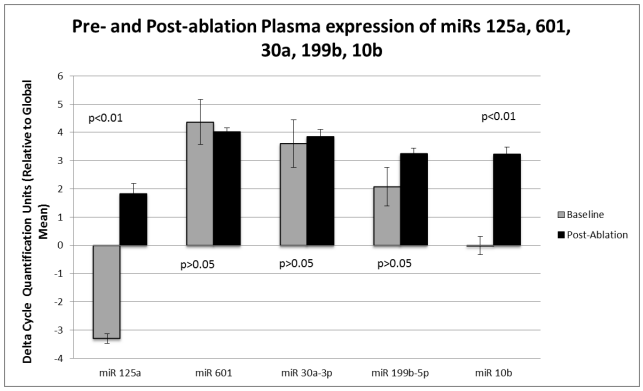

Figure 1: Plasma expression of miRNAs pre- and post-catheter ablation in patients with Atrial Fibrillation.

Plasma expression of 5 cardiac miRNAs, including miRNAs 125a-5p and 10b, are associated with AF recurrence

In this prospective analysis of 83 patients undergoing CA for symptomatic AF, we observed that 5 miRNAs were associated with clinically significant AF recurrence over 12 months of follow-up (Table 2).

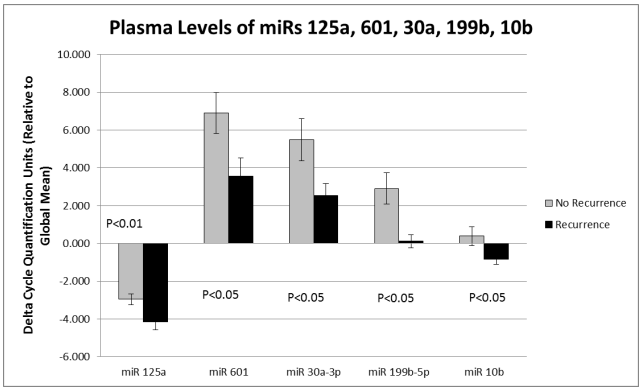

Figure 2: Plasma expression of miRNAs in patients with and without evidence of Atrial Fibrillation Recurrence at 12-months post-ablation. (Note: expression levels measured in delta cycle quantification units (ΔCq). Expression levels are inversely related to ΔCq. Lower ΔCq indicates a higher expression).

Plasma expression of miRNAs 125a-5p (-4.16 *vs.* -2.95, $p<0.01$), 601 (3.55 *vs* 6.89, $p<0.05$), 30a-3p (2.53 *vs* 5.48, $p<0.05$), 199b (0.11 *vs.* 2.9, $p<0.05$) and 10b (-0.83 *vs* 0.38, $p<0.05$) were all higher among patients with AF recurrence compared to those without recurrence (Figure 2), even after adjustment for CHA_2DS_2-VASc score. Furthermore, as shown in Supplemental Table 2 additional miRNAs (100-5p, 146a, 148b, 30c) were associated with AF recurrence but did not meet our pre-specified criteria of a 1-fold or greater difference in expression fold change.

	N		Average Expression (ΔCq)				Multivariable Adjusted		
MiRNA	**Total**	**Late AF Recurrence Cases**	**No clinically significant AF Recurrence**	**Clinically significant AF Recurrence**	**Fold Change**	**Odds Ratio**	**95% CI**		**P-value**
miRNA 125a-5p	80	32	-2.95	-4.16	1.28	0.45	0.26	0.7	0.001
miRNA 601	50	20	6.89	3.55	3.15	0.85	0.73	0.97	0.02
miRNA 10b	76	33	0.38	-0.83	1.24	0.69	0.47	0.94	0.04
miRNA 30a-3p	58	28	5.48	2.53	2.97	0.87	0.75	0.98	0.04
miRNA 199b-5p	65	28	2.91	0.11	2.79	0.7	0.46	0.9	0.04

Legend AF: Atrial Fibrillation;
*Fold-change is the difference in miRNA expression between individuals with late AF recurrence compared with no late AF recurrence

Table 2: Significant Plasma miRNAs associated with clinically significant atrial fibrillation recurrence after catheter ablation.

MiRNA associations with factors known to promote AF recurrence

Supplemental Table 3 shows the strength of univariate associations among baseline factors related to atrial remodeling, reverse remodeling after ablation, and/or AF recurrence with baseline plasma miRNA levels, including baseline left atrial volume index, C-reactive protein level, age, and heart failure. None of the 5 candidate miRNAs were significantly associated with left atrial volume index, serum C-reactive protein level, age or heart failure.

miRNA	Function (Target Genes)	Target Status	Associated Phenotype
miRNA-125a-5p	Anti-proliferative activity (ERBB2/3 MMP11)	Validated	Cardiac fibrosis, AF, and HFrEF
	Anti-apoptotic activity (P53, BAK1, TNFAIP3)	Validated	
miRNA-10b	Cardiac development (HOX genes, TBX5)	Validated	Congenital heart disease, diffuse myocardial fibrosis in HCM, isoproterenol-induced myocardial injury and fibrosis
	Angiogenesis (mib 1, FLT 1)	Predicted	
miRNA-199b	Regulation of NFAT/calcineurin signalling (Dyrk1a) and proteasome activity/ ubiquitin ligases	Validated	Expression of fetal cardiac genes, cardiac hypertrophy, cardiac fibrosis, end-stage DCM, and doxorubicin-induced cardiotoxicity,
miRNA-30a-3p	Excessive autophagy through beclin1	Predicted	AF induced myocardial fibrosis, cardiac hypertrophy
	Myocardial fibrosis (snail1), periostin	Predicted	
miRNA-601	NF-kappa B-signalling, PTP4A1	Predicted	No associated cardiac phenotypes prior to our findings. Known associations with colon cancer.
	Actin cytoskeleton formation		
	Fas-induced apoptosis		

AF: Atrial Fibrillation; HFrEF: Heart Failure with reduced Ejection Fraction; HF: Heart Failure; MR: Mitral Regurgitation; DCM: Dilated Cardiomyopathy; CM: Cardiomyopathy; AS: Aortic Stenosis; LVH: Left Ventricular Hypertrophy; HCM: Hypertrophic Cardiomyopathy; ERBB 2/3: Erb-B2 Receptor Tyrosine Kinase 2/3; MMP11: Matrix Metallopeptidase 11; P53: Phosphoprotein p53; BAK1: Apoptosis Regulator BAK; TNFAIP3: TNF Alpha Induced Protein 3; Dyrk1a: Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A; Beclin1: Moesin-Like BCL2 Interacting Protein; Snail1: Protein snail homolog 1; Mib1: Mindbomb E3 Ubiquitin Protein Ligase 1; FLT1: Fms-Related Tyrosine Kinase 1; HOX genes: Homeotic genes; TBX5: T-box transcription factor 5; PTP4A1: Protein Tyrosine Phosphatase Type IVA, Member 1. *Grey shading indicates associations with AF recurrence AND dynamic down-regulation 1-month post-CA
The validated targets for each miRNA were obtained from miRDB and mirTarBase (under Strong Evidence), and the predicted targets were obtained from Targetscan.

Table 3: Known functions and Phenotypes of 5 plasma miRNAs associated with clinically significant AF recurrence after catheter ablation.

Discussion

MiRNAs are key regulators of cardiac gene expression and can be reliably detected in the circulation [16,25]. MiRNAs are secreted and/or released by damaged cardiomyocytes and fibroblasts, may be reliably detected and are stable in the circulation, and play an important role in both intracellular and intercellular signaling processes. Our group and others have related circulating miRNAs to distinct forms of cardiovascular diseases, including coronary artery disease, myocardial infarction, heart failure, and AF [10,25]. Recently, animal studies have shown that cardiac gene expression, atrial structure, and vulnerability to AF is enhanced when atrial miRNA expression is altered [18].

The major finding of the present study is that plasma levels of 2 miRNAs (125a-5p and 10b) were lower 1-month after CA for AF and that levels of these same miRNAs were higher at baseline among participants who experienced a clinically significant AF recurrence. Based on our new findings and prior data showing that miRNAs 125a-5p and 10b are master regulators of genes implicated in atrial fibrosis and cardiac remodeling, we hypothesize that these miRNA-dependent pathways are of pathophysiological significance as mediators of AF progression and recurrence.

MiRNAs 125a-5p and 10b are dynamic and associated with AF recurrence

In a prior case-control study, plasma levels of miRNA 125a-5p were higher among 121 participants with AF compared to 99 hospital referents participants who were free from AF [25]. In our study, plasma miRNA 125a-5p levels were lower 1-month post-ablation as compared with pre-CA levels, perhaps from reduced atrial miR-125a-5p expression or release into the circulation. In addition, higher expression of miR-125a-5p at baseline was also associated with recurrence of AF, suggesting that this dynamically regulated miRNA plays a role in atrial remodeling and AF.

A comprehensive search of miRNA target databases identified several miRNA-125a gene targets involved in atrial structural and electrophysiological remodeling. These targets include ERBB2/3 (receptor tyrosine kinase of EGF receptor family), HuR (RNA-binding protein), and MMP11/12 (matrix metalloproteinases), as well as p53 (key tumor suppressor gene), Bak1 (pro-apoptotic protein), and TNFAIP3 (negative regulator of NF-kB signaling pathway; Table 3) [20-23]. miR-125a inhibits MMP11/12 activity and thus higher cardiac miR-125a expression is likely associated with greater fibrosis and extracellular collagen deposition. Conversely, lower levels of miR-125a would be expected to correlate with lower burden of atrial remodeling and lesser vulnerability to AF [23].

Plasma levels of miRNA-10b were also noted to be lower 1 month after CA as compared to baseline and levels were >3-fold higher among study participants with an AF recurrence. The miRNA-10 family is highly conserved across species and plays a crucial role in development and cellular differentiation through regulation of Hox genes [24]. MiRNA-10b is a key regulator of genes that control cellular processes, including proliferation and apoptosis [26]. MiRNA 10b also suppresses angiogenesis by up-regulating mib1 and FLT, thereby altering VEGF signaling and resulting in vascular branching defects [27,28]. Furthermore, tissue and circulating levels of miRNA 10b are associated with diffuse myocardial and ventricular structural abnormalities in the setting of ischemic cardiomyopathy [29]. Our findings further implicate miRNA-10b as a contributor to atrial remodeling and AF susceptibility.

Plasma miRNAs 199B, 30A, and 601 are related to AF recurrence

MiR-199b, another plasma miRNA related to AF recurrence in our study, has previously been implicated in the pathogenesis of heart disease, especially heart failure [30]. MiRNA 199b targets Dyrk1a, which in turn is an activator of the NFAT/calcineurin-signaling pathway.30 Up-regulation of this pathway induces pathologic expression of "fetal" cardiac genes, such as β-myosin heavy chain, which is known to be up regulated in both murine and human models of heart failure [30]. MiRNA 199b also targets several ubiquitin ligases involved in proteasome activity, such that an increase in the ubiquitin-proteasome system as mediated by miRNA 199b leads to pathologic structural remodeling and ventricular dilatation [31]. Furthermore, in mouse models, over-expression of miRNA 199b results in accelerated ventricular hypertrophy and fibrosis, whereas antagonism of miRNA 199b in these same models results in leads to reversal of hypertrophy and fibrosis [30]. Although miRNA 199b has not previously been linked to atrial electrophysiological remodeling, in light of the strong relations between left ventricular hypertrophy, markers of ventricular pressure overload (i.e., B-type natriuretic peptide), impairments in atrial structure and function, and vulnerability to AF, we speculate that levels of miRNA 199b may identify individuals with ongoing ventricular hypertrophic remodeling whose atria are subjected to pressure or volume overload.

Higher plasma levels of miR-30a-3p were observed in patients with AF recurrence after CA. MiRNA 30a-3p may be related to AF-induced myocardial fibrosis through its down-regulatory effects of snail1 and periostin, genes that promote fibrosis [32]. Indeed, there is significant sequence homology across miR-30 family members, and miR-30c was statistically significantly ($p<0.05$) associated with AF recurrence after adjustment in our cohort, but did not meet our pre-specified criteria for >1 fold difference between those with and without recurrence. MiRNA 30c upregulates levels of connective tissue growth factor (CTGF), a key gene involved in cardiac fibrosis, and it is enriched in cardiac fibroblasts [33]. Experiments by Duisters et al. showed that levels of miR-30c in ventricular tissue were lower than among participants with ventricular hypertrophy than in tissue from study participants free from pathological remodeling [34]. When viewed in light of prior data associating members of the miR-30 family with ventricular remodeling, we speculate that miR-30 is up-regulated in patients with greater degrees of pathological atrial structural remodeling and vulnerability to AF recurrence.

Plasma levels of miR-601 were related to AF recurrence after CA. Prior associations between miR-601 with cardiovascular disease have not been identified. However, miRNA 601 is cardiac-enriched and regulates NF-kappaB signaling, actin cytoskeleton formation and Fas-induced apoptosis [34]. Although miR-601 does not have known associations with cardiac structural modeling or relations with AF, our findings implicate this miRNA as a mediator of atrial fibrosis, remodeling and AF recurrence.

Reverse cardiac remodeling drives susceptibility to AF recurrence

Prior data shows that AF is heritable and that atrial gene expression drives pathological atrial structural remodeling and AF susceptibility [4]. Cardiac miRNAs are central regulators of gene processes implicated in AF and cardiac miRNAs are present in the circulation and are dynamically regulated in cardiovascular disease [17-19]. Our findings implicate important gene regulatory pathways as mediators of reverse electrical cardiac remodeling after ablation and potentially identify novel, mechanism-based circulating biomarkers of AF recurrence.

Strength and Limitations

Using proven methods of RT-qPCR, we quantified cardiac specific or enriched miRNAs in the plasma of a moderately sized prospective cohort of participants undergoing CA for symptomatic AF. Participants had detailed AF characterization as well as a standardized and intensive follow-up for AF recurrences over the 12 months after CA. Despite these strengths, our study did have notable limitations. Our small sample size was underpowered to control for multiple testing and introduced the potential for false positive and negative associations. Furthermore, we were underpowered to control for variance in ablation technique, as is common in procedure based studies. For example, miRNA concentration may be related to tissue damage, presumably with cryoablation resulting in less trauma to the atrium versus RF heating, or related to the specific cellular features at the target lesion. The observational nature of the data and the limited number of covariates in the multivariable models introduced the risk of residual confounding. While we need to validate our study in an independent cohort to replicate these findings, our study is primarily hypothesis generating. It is the first study to show associations between microRNAs and longitudinal outcomes 1-year post-ablation and investigate the mechanistic linkages of these mircroRNAs to cardiac remodeling.

Conclusions

In our contemporary and prospective study including patients with AF, we observed significant down-regulation of miRNAs 125a and 10b after CA, showing that the circulating miRnome is dynamic after controlled atrial injury and perhaps reflecting reverse atrial remodeling due to lower AF burden. We also showed that these two miRNAs as well as miRNAs 60, 30a-3p, and 199b were higher among patients with clinically significant AF recurrence compared to those who remained free from AF. Our results suggest a novel role for circulating miRNAs as mediators of cardiac reverse remodeling and potentially as mechanism-based biomarkers of AF recurrence after CA. Further studies need to validate our findings and investigate mechanisms through which these microRNAs promote vulnerability to AF recurrence.

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