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Influence of atrial fibrillation on microRNA expression profiles in left and right atria from patients with valvular heart disease

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Cooley N, Cowley MJ, Lin RCY, Marasco S, Wong C, Kaye DM, Dart AM, Woodcock EA. Influence of atrial fibrillation on microRNA expression profiles in left and right atria from patients with valvular heart disease. *Physiol Genomics* 44: 211–219, 2012. First published December 6, 2011; doi:10.1152/physiolgenomics.00111.2011.—Chronic atrial fibrillation (AF) is a complication associated with the dilated atria of patients with valvular heart disease and contributes to worsened pathology. We examined microRNA (miRNA) expression profiles in right and left atrial appendage tissue from valvular heart disease (VHD) patients. Right atrial (RA) appendage from patients undergoing coronary artery bypass grafting and left atrial (LA) appendage from healthy hearts, not used for transplant, were used as controls. There was no detectable effect of chronic AF on miRNA expression in LA tissue, but miRNA expression in RA was strongly influenced by AF, with 47 miRNAs (15 higher, 32 lower) showing differential expression between the AF and control sinus rhythm groups. VHD induced different changes in miRNA expression in LA compared with RA. Fifty-three (12 higher, 41 lower) miRNAs were altered by VHD in LA, compared with 5 (4 higher, 1 lower) in RA tissue. miRNA profiles also differed between VHD-LA and VHD-RA (13 higher, 26 lower). We conclude that VHD and AF influence miRNA expression patterns in LA and RA, but these are affected differently by disease progression and by the development of AF. These findings provide new insights into the progression of VHD.

mitral valve disease; atrial dilatation; noncoding RNA; coronary artery bypass grafting

THE PROGRESSION FROM HEALTHY to diseased myocardium involves complex changes in gene expression and subsequent changes in protein expression and activity. Some of these are protective, but others contribute to a worsening of the pathology. In recent years it has been shown that some of these pathological events involve posttranscriptional regulation by microRNAs (miRNA)(4, 33). miRNAs are short noncoding RNA species of 20–22 nucleotides in length that bind target sequences in the 3′-untranslated region of target genes to induce mRNA instability or to inhibit translation (10). Each miRNA can influence multiple gene products, and conversely, each gene product is the target of multiple miRNAs (17). Thus, changes in miRNA expression pattern have the potential to influence key cellular responses involved in cardiac pathology.

Atrial fibrillation (AF) is currently the most prevalent arrhythmia worldwide. The incidence of AF is increasing along with the aging of the population, increasing the prevalence of stroke and thromboembolic complications. AF contributes significantly to death and disability and is a major burden on the cost of health care. Chronic AF is a complication associated with the dilated atria of patients with valvular heart disease (VHD) or heart failure, and, in both of these diseases, AF worsens outcomes.

To gain insight into factors contributing to the development and perpetuation of AF, we performed genome-wide miRNA expression profiling using atrial appendage tissue from patients undergoing surgery for mitral valve disease (VHD) and those undergoing coronary artery bypass grafting (CABG). In addition, healthy left atrial (LA) appendage tissue was studied. The VHD group included patients with a history of chronic AF, defined as being present for >1 yr. We found that miRNA expression profiles showed differences between the different patient groups. We identified a number of miRNAs associated with VHD and miRNAs that differed in expression between the VHD patients with and without chronic AF. In addition, we demonstrated that LA and right atrium (RA) respond differently to the progression of VHD and the development of AF.

METHODS

Atrial tissue samples. All of the studies were approved by the Human Ethics Committee of the Alfred Hospital, Melbourne, Australia, and all patients gave informed consent. Atrial appendage tissue was obtained from patients undergoing heart surgery at the Alfred Hospital, Melbourne. RA appendage was removed from patients undergoing mitral valve surgery (VHD patients) as well as those undergoing coronary artery bypass grafting (CABG patients). All CABG RA tissues used as control were from patients with normal left ventricular (LV) function. Left atrial appendage was removed from hearts of VHD patients with severely dilated LA. Healthy LA tissue was obtained from donor hearts not used for transplantation. VHD patients were classified as AF based on clinical assessment of chronic AF over at least 1 yr. Patients not showing AF over this period are classified as sinus rhythm (SR). In all cases, tissue removed at surgery was placed immediately in ice-cold saline. Our previous studies demonstrate that such tissues retain the ability to undergo responses to stimulation (35). Surgically removed tissues were treated with RNAl-

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Table 1. *Characteristics of the patient groups used in the study*

	VHD-LA-SR	VHD-LA-AF	VHD-RA-SR	VHD-RA-AF	CABG-RA
n	4	7	6	4	8
Age	73 ± 6	74 ± 2.5	54 ± 8	74 ± 1	67 ± 3
Sex (males)	1	2	4	1	7
LA volume, ml	144 ± 16	147 ± 34	128 ± 14	194 ± 46	99 ± 8
Diabetes	1	0	0	1	5
β-Blockers	1	3	2	0	0
ACE inhibitors/AT1-blockers	3	5	3	2	3
Statins	2	2	1	3	8

VHD, valvular heart disease; LA and RA, left and right atria; CABG, coronary artery bypass grafting; SR, sinus rhythm; AF, atrial fibrillation. *n*, the number of samples used in the comparative analysis. Some of the LA and RA samples are from the same patient. LA volume was measured by echocardiography. Diabetes refers to a chronic history of diabetes for > 1 yr. There were no significant differences between the subgroups of VHD patients in terms of drug treatment. But the CABG groups different from the VHD group in that all CABG patients were using statins, whereas <40% of VHD patients were using statin therapy. Age and LA volume data are expressed as means ± SE.

ater (Invitrogen) within 10 min of excision. RNAlater was removed after overnight treatment, and the samples were stored at −80°C.

Microarrays. Total RNA was extracted using TRIzol reagent (Ambion). miRNA hybridization was performed by the Ramaciotti Centre for Gene Function Analysis (Sydney, Australia) in three batches, onto Agilent G4470B (miRBase 10.1) and G4470C (miRBase 12.0) microarrays, which are glass slides containing eight arrays of ~15K hairpin probes, capable of recognizing a total of 966 mature miRNA sequences. Data have been deposited at http://www.ncbi.nlm.nih.gov/geo/, accession number GSE28954.

Microarray normalization, filtering, and batch removal. Because miRNA microarray data were generated in three batches, across six slides, and on two different array designs, we took care in the normalization and batch removal analysis of these data. Raw data from each array batch were normalized independently using robust multiarray average (15), which utilizes the multiple probes per miRNA to obtain a robust estimate of the true expression level of each miRNAs, on the $\log_2$ scale. Data from the three batches were combined and quantile normalized. Control probes ($n = 22$) and miRNAs detected on none of the arrays ($n = 508$) using the *IsGeneDetected* flag were filtered out, leaving 436 miRNAs. To remove any remaining batch effect, we fitted a linear model using *limma* (26) in the R/Bioconductor project (v2.11.1) (11), (8):

$$y_i = \mu_i + \beta_{1i} + \beta_{2i} + \beta_{3i} + \beta_{4i} + \beta_{5i} + \beta_{6i} + \varepsilon_i, \qquad (1)$$

where y_i are the quantile normalized expression levels for miRNA i, μ is the intercept, and β_1–β_6 represent the batch effect common to all arrays on each of the six array slides, and ε is the residual variation, which we subsequently used as the normalized, filtered, batch-batch removed expression data for each miRNA.

Differential expression. The correlation between technical replicates was estimated as 0.51 using the duplicate correlation method (27) and was used to more accurately assess the standard errors when fitting (*Eq. 2*) below.

To detect differentially expressed miRNAs, we fitted a treatment means parameterization linear model describing the seven different experimental groups:

$$y'_i = LA\,.\,Healthy_i + LA\,.\,VHD\,.\,AF_i + LA\,.\,VHD\,.\,SR_i + RA\,.\,CABG\,.\,AF_i + RA\,.\,CABG\,.\,SR_i + RA\,.\,VHD\,.\,AF_i + RA\,.\,VHD\,.\,SR_i + \varepsilon'_I \qquad (2)$$

where y'_i are the normalized residuals from (*Eq. 1*), ε'_i are the residual errors, and each term represents the average expression of miRNA i in each of the seven biological groups.

Detecting differential expression between groups is achieved by fitting contrasts to this linear model, for example:

$$VHD\text{-}RA_i\ vs.CABG - RA_i = (RA\,.\,VHD.AF_i + RA.VHD.SR_i)/2 - (RA.CABG\,.\,AF_i + RA.CABG.SR_i)/2$$

Each contrast was compared with the null hypothesis of no differential expression via an empirical Bayes, moderated *t*-statistic, implemented in *limma*, in the R/Bioconductor project. The moderated *t*-statistic has been shown to be more appropriate than the Student's *t*-statistic for genomic data and is widely used in the analysis of whole genome microarrays (26). Unadjusted *P* values produced for each miRNA

Table 2. *Validation of the miR array data*

miRNA	qRT-PCR			Array
	VHD-RA-AF	*VHD-RA-SR*	*AF/SR fold*	*AF/SR fold*
hsa-miR-21	0.48 ± 0.15	0.11 ± 0.01.	4.2	3.5
hsa-miR-146b-5p	0.05 ± 0.02	0.012 ± 0.002	4.2	6.5
hsa-miR-133a	0.8 ± 0.16	1.4 ± 0.012	0.56	0.45
hsa-miR-133b	0.42 ± 0.16	0.97 ± 0.20	0.43	0.51
hsa-miR-30c	0.09 ± 0.01	0.13 ± 0.01	0.69	0.54
hsa-miR-490-3p	0.007 ± 0.001	0.012 ± 0.001	0.58	0.33
	VHD-LA	*VHD-RA*	*LA/RA fold*	*LA/RA fold*
hsa-miR-10b	0.61 ± 0.28	0.12 ± 0.01	5.1	6.2
hsa-miR-886-5p	0.012 ± 0.002	0.011 ± 0.004	1.01	1
hsa-miR-17	0.057 ± 0.006	0.046 ± 0.007	1.25	0.5
hsa-miR-100	0.13 ± 0.02	0.17 ± 0.01	0.76	0.39
	VHD-RA	*CABG-RA*	*VHD/CABG fold*	*VHD/CABG fold*
hsa-miR-490-3p	0.010 ± 0.001	0.015 ± 0.001	0.66	0.59

Selected microRNAs (miRNA or miR) identified in the microarray analyses were measured by qRT-PCR. miRNA expression levels were compared between the VHD-RA-SR and VHD-RA-AF groups, between VHD-LA and VHD-RA, or between VHD-RA and CABG-RA, as indicated. Values from qRT-PCR shown are miRNA expression relative to U6, means ± SE, $n = 4$–6. Values obtained by qRT-PCR and array expressed as $\log_2$ fold change (FC) showed a linear correlation, $r = 0.94$, $P < .001$, power = 0.99 at 0.05 (Sigma Stat).

under each contrast were independently adjusted for multiple testing by the false discovery rate (FDR) (1). Differentially expressed miRNAs were those defined with an FDR <0.05 and fold change (FC) >1.5, up or down.

Heirarchical clustering. Heirarchical clustering images were generated upon the residuals from (*Eq. 1*), using GenePattern (v3.2.3, http://pwbc.garvan.unsw.edu.au/gp, Ref. 24), using Pearson correlation distance, and pairwise average-linkage clustering, with a row-normalized, relative color scheme.

Array validation by qRT-PCR. Total RNA was extracted from human atrial appendages by homogenizing 10–20 mg of tissue using the TissueRuptor (QIAGEN) in 1 ml TRIzol reagent (Invitrogen) according to the manufacturer's instructions. miRNAs were measured using the TaqMan system (Applied Biosystems): miR-17 (ID 002308), miR-886-5p (ID 002193), miR-100 (ID 000437), miR-10b (ID 002218), miR-490-3p (ID 001037), miR-30c (ID 000419), miR-133b (ID 002247), miR-21 (ID 000397), miR-133a (ID 002246), and miR-146b-5p (ID 001097). Expression levels were calculated relative to the endogenous U6 control (ID 001973). Data from qRT-PCR showed a linear correlation with array data [correlation coefficient of 0.94, $P < 0.001$ and the power was 0.99 at 0.05 (Sigma Stat)]. Differences between groups were calculated by one-way ANOVA followed by Fisher's exact test (Sigma Stat). Where tissues were paired (same patient), a paired *t*-test was used. $P < 0.05$ was taken as significant.

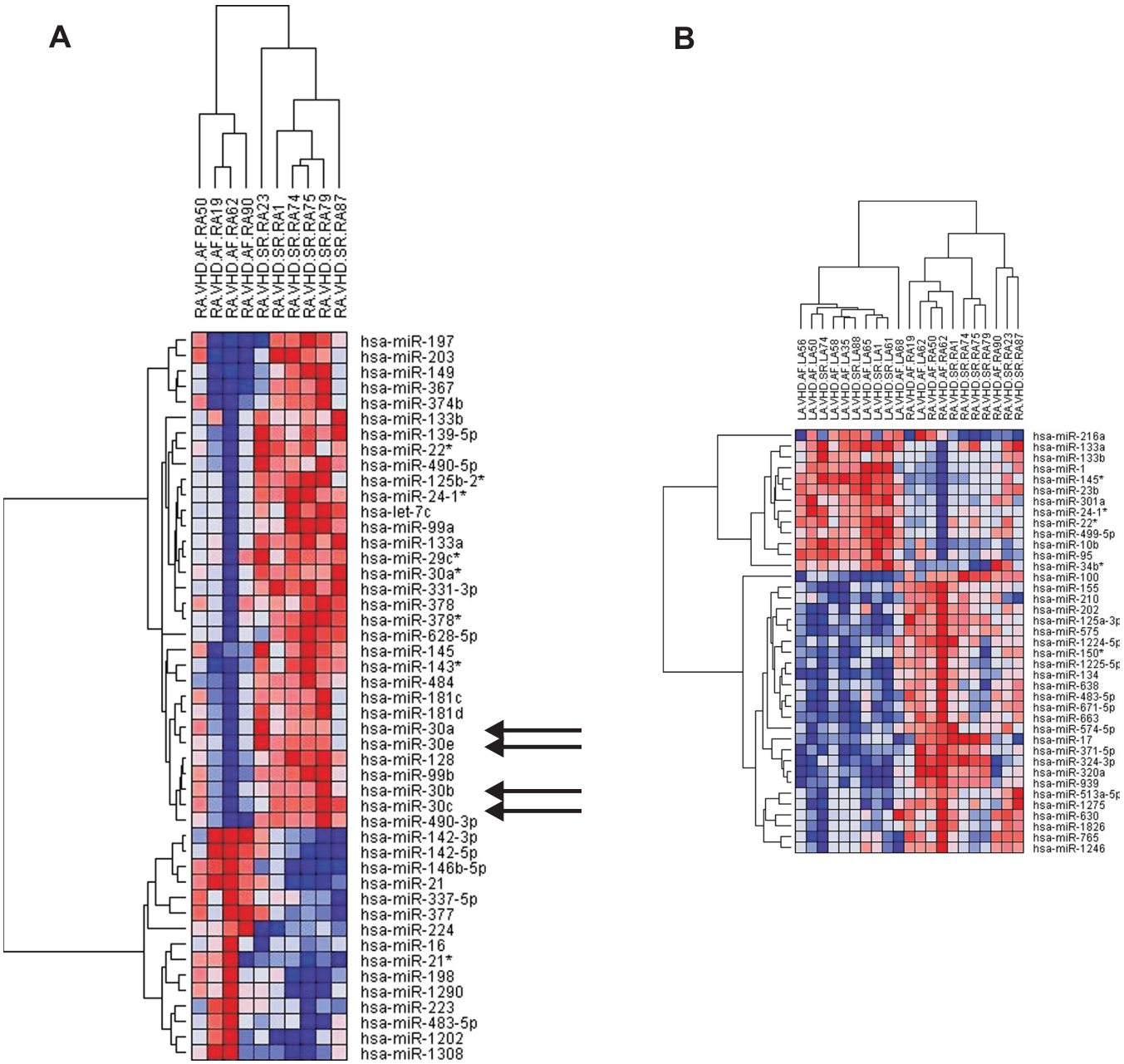

Fig. 1. Valvular heart disease (VHD) and atrial fibrillation (AF) alter miRNA expression in left (LA) and right atria (RA). Heat maps and hierarchical clustering of microRNA (miRNA) expression and sample subgroup. miRNAs that showed significant difference in expression [fold change (FC) > 1.5, false discovery rate (FDR) < 0.05] are shown on the *y*-axes and patient number, atrial subgroup, and disease status are shown on the *x*-axis. SR, sinus rhythm. *A*: groups compared were VHD-RA-AF (4 samples) and VHD-RA-SR (6 samples). *B*: groups compared were VHD-LA (11 samples) and VHD-RA (10 samples). Red indicates higher relative expression, blue lower expression. miR-30 family members are indicated by the arrows.

RESULTS

Patient groups studied. Chronic AF associated with mitral valve disease (VHD) was studied using RA and LA appendage tissues from VHD patients. CABG atrial samples, used in the study as control RA tissue, were all from patients with normal LV function. Healthy LA appendage tissue from unused transplant donor hearts was used as control for comparison with LA tissue from VHD patients. The tissue groups and the numbers of samples and the characteristics of the groups are shown in Table 1. AF in VHD patients was defined based on a history of chronic AF for at least 1 yr prior to surgery. This subgroup was compared with samples from patients who remained in SR.

Validation of the miRNA array data. We sought to identify miRNAs potentially associated with AF in VHD patients by comparing miRNA expression profiles in LA and RA tissue from patients suffering chronic AF with those who remained in SR. The studies used a microarray system encompassing a total of 966 individual miRNA sequences, described under METHODS. Array data were validated using qRT-PCR measurements of 11 miRNAs with different signal strengths and different responses to disease status or different LA/RA expression profiles (2 were higher in RA tissue from patients with AF than in SR, and 4 were lower; whereas 1 was higher in VHD-LA than in VHD-RA, 2 were lower and 1 was unchanged). We found excellent correspondence between changes detected on the arrays and those found by direct qRT-PCR measurements (Table 2). The exception was miR-17, which was 50% lower in expression VHD-LA compared with RA from the array data, but was unchanged when assayed by q-RT-PCR (Table 2).

Table 3. *miRNA expression differences between VHD-RA-AF compared with VHD-RA-SR*

miRNA	FC	Direction	*P* Value	False Discovery Rate
hsa-miR-146b-5p	6.5546	up	9.375E-06	0.001588
hsa-miR-142-3p	3.7638	up	0.0018188	0.023099
hsa-miR-21	3.5135	up	4.194E-05	0.00343
hsa-miR-1202	3.4125	up	0.0045582	0.037691
hsa-miR-142-5p	2.5911	up	0.000645	0.013653
hsa-miR-21*	2.554	up	0.0010058	0.016877
hsa-miR-483-5p	2.3163	up	0.0039893	0.03494
hsa-miR-223	2.2202	up	0.0010793	0.017133
hsa-miR-1308	2.193	up	0.005793	0.045274
hsa-miR-224	1.8652	up	0.0003876	0.010826
hsa-miR-16	1.7736	up	0.0003339	0.0106
hsa-miR-377	1.5647	up	0.0022217	0.025967
hsa-miR-337-5p	1.547	up	0.0044363	0.037691
hsa-miR-1290	1.5468	up	0.0031016	0.031217
hsa-miR-198	1.5362	up	0.0007135	0.014498
hsa-miR-490-3p	3.0632	down	8.251E-06	0.001588
hsa-miR-378*	2.2356	down	0.0001676	0.007738
hsa-miR-133a	2.1906	down	0.000259	0.009398
hsa-miR-143*	2.1808	down	8.515E-05	0.004325
hsa-miR-22*	2.1315	down	0.0016117	0.020993
hsa-miR-378	1.9828	down	0.003299	0.032229
hsa-miR-133b	1.9674	down	0.0027509	0.029114
hsa-miR-30a*	1.9245	down	0.0002356	0.009382
hsa-miR-29c*	1.9139	down	0.0038026	0.03404
hsa-miR-24-1*	1.9079	down	0.0033752	0.032351
hsa-miR-197	1.8873	down	0.0013192	0.018112
hsa-miR-30c	1.8584	down	0.0016087	0.020993
hsa-miR-139-5p	1.858	down	4.726E-05	0.00343
hsa-miR-125b-2*	1.8441	down	7.338E-05	0.004324
hsa-miR-30e	1.8362	down	0.0004928	0.011379
hsa-miR-99b	1.8246	down	7.661E-05	0.004324
hsa-miR-99a	1.7935	down	0.0020262	0.025105
hsa-miR-181c	1.771	down	0.0002401	0.009382
hsa-miR-331-3p	1.7535	down	5.939E-06	0.001588
hsa-miR-374b	1.7212	down	0.0004149	0.010826
hsa-miR-628-5p	1.7153	down	0.0045447	0.037691
hsa-miR-128	1.7018	down	0.0012997	0.018112
hsa-miR-181d	1.6812	down	0.0010299	0.016877
hsa-miR-145	1.6682	down	0.0031339	0.031217
hsa-miR-203	1.633	down	0.0050888	0.041033
hsa-miR-367	1.5954	down	0.005777	0.045274
hsa-let-7c	1.5817	down	3.237E-05	0.003289
hsa-miR-484	1.5752	down	0.0022945	0.025967
hsa-miR-490-5p	1.5532	down	0.0008714	0.015325
hsa-miR-30b	1.5447	down	0.0012841	0.018112
hsa-miR-149	1.5282	down	0.0004262	0.010826
hsa-miR-30a	1.5061	down	0.0037241	0.03404

miRNAs that were differentially expressed between VHD-RA-SR and VHD-RA-AF were defined by FC >1.5, false discovery rate <0.05, $P < 0.01$. miRNAs described as 'up' are higher in VHD-RA-AF than in VHD-RA-SR, whereas 'down' means expression is higher in the SR group.

Table 4. *miRNA expression profiles in atria from VHD patients compared with controls*

miRNA	FC	Direction	*P* Value	False Discovery Rate
		VHD-LA vs. Healthy-LA		
hsa-miR-34b*	3.1768	up	3.629E-06	0.0006145
hsa-miR-10b	2.035	up	0.0023091	0.0165213
hsa-miR-454	1.8914	up	0.0003075	0.0045944
hsa-miR-146a	1.8735	up	0.0037623	0.0224854
hsa-miR-101	1.8253	up	0.0070396	0.0357869
hsa-miR-20a*	1.8141	up	0.0030578	0.0194172
hsa-miR-199b-5p	1.7073	up	0.001883	0.0145271
hsa-miR-301a	1.6556	up	0.0054207	0.0302608
hsa-miR-221	1.6357	up	0.0014551	0.0131995
hsa-miR-148b	1.624	up	0.0008227	0.0085296
hsa-miR-23a	1.6226	up	0.0018076	0.0145271
hsa-miR-26b	1.5006	up	0.0028219	0.0181461
hsa-miR-886-3p	5.8884	down	0.00001057	0.0007275
hsa-miR-21*	4.764	down	2.446E-08	0.00001243
hsa-miR-17	3.714	down	0.00004172	0.0011809
hsa-miR-939	3.1014	down	0.00001897	0.0008238
hsa-miR-663	2.8861	down	0.0017262	0.0144522
hsa-miR-193a-5p	2.6307	down	0.00013	0.0026417
hsa-miR-212	2.5486	down	0.00003815	0.0011809
hsa-miR-197	2.3636	down	2.124E-06	0.0005396
hsa-miR-202	2.2536	down	0.0002346	0.0037244
hsa-miR-324-3p	2.0791	down	0.0002033	0.0033931
hsa-miR-191*	1.9862	down	0.00001653	0.0008238
hsa-miR-1238	1.9622	down	0.00001146	0.0007275
hsa-miR-371-5p	1.9069	down	0.0005797	0.0072918
hsa-miR-134	1.8815	down	0.0060748	0.0321456
hsa-miR-490-3p	1.8668	down	0.0012912	0.0119263
hsa-miR-940	1.8519	down	0.0001096	0.0023192
hiv1-miR-H1	1.85	down	0.002188	0.0160444
hsa-miR-33b*	1.8077	down	0.00001442	0.0008142
hsa-miR-125a-3p	1.8057	down	0.0021261	0.0158834
hsa-miR-320a	1.793	down	0.0009066	0.0092114
hsa-miR-150	1.7914	down	0.0001453	0.002733
hsa-miR-1234	1.7766	down	0.00002085	0.0008238
hsa-miR-1224-5p	1.7563	down	0.0024315	0.0170738
hsa-miR-425*	1.7516	down	0.00004861	0.0012996
hsa-miR-1225-3p	1.7185	down	0.00002996	0.0010148
hsa-miR-605	1.714	down	0.0010199	0.0099641
hsa-miR-563	1.7139	down	0.00005671	0.0014404
hsa-miR-20a	1.7044	down	0.0016844	0.0144522
hsa-miR-770-5p	1.6918	down	0.0053837	0.0302608
hsa-let-7f-1*	1.6853	down	0.00006345	0.0015349
hsa-miR-125a-5p	1.6583	down	0.00002844	0.0010148
hsa-miR-132	1.6485	down	0.0020148	0.0152762
hsa-let-7b*	1.6281	down	0.0007696	0.0083179
hsa-miR-149	1.6048	down	8.627E-06	0.0007275
hsa-miR-487b	1.5819	down	0.0025441	0.0170738
hsa-miR-550	1.5771	down	0.0001675	0.0029333
hsa-miR-1274a	1.5632	down	0.006957	0.0357869
hsa-miR-423-5p	1.5483	down	0.0007562	0.0083179
hsa-miR-1228	1.5435	down	0.0005494	0.0072918
hsa-miR-1237	1.5174	down	0.00009094	0.0020085
hsa-miR-602	1.5031	down	0.0001396	0.0027276
		VHD-RA vs. CABG-RA		
hsa-miR-146b-5p	2.625	up	0.0010108	0.04132
hsa-miR-21	2.0131	up	0.0012316	0.04171
hsa-miR-376c	1.6797	up	0.0005774	0.04104
hsa-miR-376a	1.5402	up	0.0009218	0.04132
hsa-miR-490-3p	1.7982	down	0.000751	0.04104

miRNAs that were differentially expressed between VHD-LA and healthy-LA or CABG- RA and VHD-RA were defined by FC >1.5, false discovery rate <0.05, $P < 0.01$. miRNAs described as 'up' are higher in VHD-LA than in healthy-LA or in VHD-RA than in CABG-RA, whereas 'down' means expression is higher in the control group.

miRNA expression patterns are influenced by AF status in RA tissue from VHD patients. Using array methodology, we examined miRNA expression patterns in RA tissue from VHD patients, with and without chronic AF [VHD-RA-AF (4 samples) and VHD-RA-SR (6 samples)]. We identified 47 differentially expressed miRNAs (FDR < 0.05, FC > 1.5). Of these, 15 were higher in the group with chronic AF and 32 showed lower expression compared with those who remained in SR (Fig. 1*A*, Table 3). As noted above, findings were confirmed using qRT-PCR for three miRNAs whose expression was higher in the AF group and five miRNAs that showed lower expression in AF (Table 2). The biggest difference was in miR-146b-5p, which was expressed 6.5-fold higher in VHD-RA-AF than in VHD-RA-SR based on array analysis and 4.2-fold when measured by qRT-PCR (Table 2). The well-studied profibrotic miR-21 was also more highly expressed in AF tissues [by 3.4-fold based on array data and 4.2-fold from qRT-PCR (Table 2)] along with its antisense miRNAs, miR-21* (2.3-fold higher in AF). Members of the miR-133 and miR-30 families were prominent among the miRNAs that showed lower expression in VHD-RA-AF than VHD-RA-SR (Tables 2 and 3). Expression of miR-133a and miR-133b were reduced by ~50% in the AF group compared with the SR group, from array data or qRT-PCR (Tables 2 and 3).

Despite the clear differences in miRNA expression profile in RA tissue, there were no differences in miRNA expressions in LA tissue from VHD patients with chronic AF compared with samples from patients who remained in sinus rhythm (VHD-LA-AF cf. VHD-LA-SR) that satisfied the statistical criteria (FDR < 0.05, FC > 1.5). Thus, we were unable to detect any AF-associated miRNA expression changes in LA tissue.

miRNA expression changes associated with VHD in LA and RA tissue. Our studies had demonstrated AF-related changes in miRNA expression profile only in RA, with no detectable differences being observed between profiles in VHD-LA-SR and VHD-LA-AF. We next examined the influence of VHD on miRNA expression profiles in LA samples from VHD patients by comparing these with LA tissue from healthy controls (VHD-LA; 11 samples, including SR and AF subgroups vs. healthy-LA, 5 samples). A total of 53 miRNAs showed an expression profile that was significantly different between VHD-LA and healthy-LA (FDR <0.05, FC > 1.5). Of these, 12 miRNAs were more highly expressed in VHD-LA compared with healthy-LA and 41 were expressed at lower level

Table 5. *miR expression profiles in VHD-LA compared with VHD-RA*

miRNA	FC	Direction	*P* Value	False Discovery Rate
hsa-miR-10b	6.1906	up	4.441E-12	2.256E-09
hsa-miR-1	2.8681	up	0.0009073	0.0132362
hsa-miR-145*	2.0034	up	0.0000552	0.0030655
hsa-miR-24-1*	1.9751	up	2.453E-05	0.0019947
hsa-miR-133b	1.9209	up	6.035E-05	0.0030655
hsa-miR-22*	1.8082	up	0.0003849	0.0098344
hsa-miR-95	1.7015	up	8.285E-06	0.0008418
hsa-miR-499-5p	1.6832	up	0.0033171	0.0274255
hsa-miR-34b*	1.6435	up	0.0065317	0.0414765
hsa-miR-133a	1.6373	up	0.0006335	0.0123775
hsa-miR-216a	1.6372	up	0.00223	0.0226566
hsa-miR-23b	1.5802	up	0.0003145	0.008877
hsa-miR-301a	1.5493	up	0.0033472	0.0274255
hsa-miR-575	5.2054	down	3.252E-08	0.00000826
hsa-miR-663	2.5906	down	0.0006822	0.0124625
hsa-miR-100	2.5318	down	3.056E-07	0.00005176
hsa-miR-125a-3p	2.4611	down	4.64E-07	0.00005893
hsa-miR-483-5p	2.2749	down	9.011E-05	0.0035211
hsa-miR-1225-5p	2.1285	down	0.005056	0.0347091
hsa-miR-17	2.0856	down	0.0028269	0.0251943
hsa-miR-513a-5p	2.0771	down	8.944E-05	0.0035211
hsa-miR-939	2.0489	down	0.0005005	0.0109321
hsa-miR-630	2.0017	down	0.0039083	0.0287742
hsa-miR-1246	1.9948	down	0.007759	0.0453057
hsa-miR-134	1.8914	down	0.0009755	0.0135635
hsa-miR-1826	1.8619	down	0.0002358	0.0074854
hsa-miR-671-5p	1.7948	down	0.0007758	0.0132362
hsa-miR-638	1.7491	down	0.0062224	0.0410514
hsa-miR-765	1.6701	down	0.0013397	0.0165996
hsa-miR-202	1.6586	down	0.0035749	0.0279392
hsa-miR-1275	1.6322	down	0.0027979	0.0251943
hsa-miR-155	1.6283	down	0.001171	0.0148711
hsa-miR-1224-5p	1.5744	down	0.0027583	0.0251943
hsa-miR-320a	1.571	down	0.0015575	0.0183998
hsa-miR-324-3p	1.5507	down	0.0044228	0.0313197
hsa-miR-210	1.531	down	0.0037252	0.0282447
hsa-miR-371-5p	1.528	down	0.004439	0.0313197
hsa-miR-574-5p	1.5208	down	0.0018913	0.0208865
hsa-miR-150*	1.5157	down	0.0042637	0.030942

miRNAs that were differentially expressed between VHD-LA and VHD-RA were defined by fold change >1.5, false discovery rate <0.05, $P < 0.01$. miRNAs described as 'up' are higher in LA than in RA, whereas 'down' means expression is higher in the RA group.

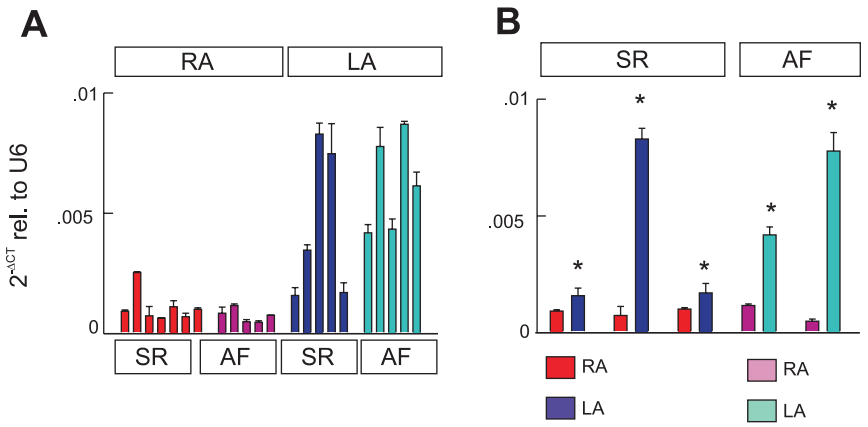

Fig. 2. miR-10b expression in LA and RA of individual VHD patients. Expression profiles of miR-10b in individual samples from the VHD patient group, VHD-RA-SR, VHD-RA,AF, VHD-LA-SR, VHD-RA-AF. Each bar represents miR-10b expression measured by qRT-PCR (means ± SE, $n = 3$ determinations). *A*: for the entire patient cohort, expression of miR-10b in LA samples was higher than in RA (0.005 ± 0.0008 vs. 0.0009 ± 0.0001, means ± SE, $P < 0.001$). *B*: values shown are miR-10b expression in LA and RA, where both were obtained from an individual patient. *$P < 0.01$ relative to expression in RA.

(Table 4). We also compared miRNA profiles in VHD-RA with CABG-RA. Based on array data, only five miRNAs were differentially expressed between these two groups, with four being more highly expressed in VHD-RA than in CABG-RA and one miRNA being expressed at a lower level (FDR <0.05, FC > 1.5; Table 4). The miRNAs that were more highly expressed in VHD-RA than in CABG-RA included miR-21, which, as shown above, was further heightened in the AF subgroup relative to the SR subgroup of RA tissue (Fig. 1, Tables 2 and 3). Only miR-490-3p showed similar expression changes with VHD in both LA and RA, where VHD was associated with reduced expression in both. miR-490-3p also showed lower expression with AF in the VHD-RA group (Tables 2–5).

miRNA expression profiles in VHD RA and LA. As shown above, VHD caused different changes in miRNA expression in LA and RA. To identify miRNA expression differences between LA and RA from VHD patients, we next compared miRNA expression patterns in VHD-LA and VHD-RA. Thirteen miRNAs were more highly expressed in VHD-LA than in VHD-RA, and 26 were more highly expressed in VHD-RA (FDR <0.05, FC >1.5; Fig. 1*B*, Table 5). The chamber specificity of miR-10b expression is depicted in Fig. 2, where expression levels in individual patients are shown. In Fig. 2*A*, expression levels in individual patients in relation to LA or RA origin, as well as AF/SR status. In Fig. 2*B*, expression of miR-10b is shown in LA and RA of the same patient. For each patient, miR-10b expression was higher in LA than in RA.

DISCUSSION

Using array technology, we investigated miRNA expression profiles in atrial appendage tissue from a number of patient groups, with a view to identifying changes in miRNA expression associated with VHD, and in particular with the development of AF on a background of VHD. The availability of both LA and RA appendage tissue provided an opportunity to examine the effects of disease on both of these tissues separately. Our data identified a number of miRNAs whose expressions were different in LA and RA. Furthermore, we showed that miRNA expression patterns in LA and RA are influenced differently by VHD and by chronic AF. Previously confirmed cardiac targets and possible functional significance of some of the miRNAs identified in our studies are described in Table 6.

We found that the development of AF in VHD patients was associated with measurable changes in miRNA expression in RA but not in LA (Fig. 1*A*, Table 3). A recent study investigating miRNA expression in VHD patients in relation to AF also demonstrated changes in RA, many of which were also identified in the current study. However, this study did not investigate changes in LA tissue, and so a comparison with RA was not possible (36). The lack of detectable difference between VHD-LA-AF and VHD-LA-SR in terms of miRNA expression profile may be partially due to tissue availability. LA appendage tissue is removed only from the most dilated atria, and therefore the VHD-LA group derives from the most severely affected patients. In contrast, RA appendage is re-

Table 6. *Selected targets and potential functions of miRNAs shown to be regulated by VHD and AF*

miR	Target	Functions	Reference
miR-21	sprouty	promotes cell growth	(30)
	PTEN	promotes Akt activation and cell growth	(25)
miR-146b-5p	IKKα	NF-κb activation	(23)
miR-133a & b	HCN2&4	arrhythmia	(6)
	HERG	arrhythmia	(14)
	CTGF	fibroblast growth	(19)
miR-30 family	CTGF	fibroblast growth	(6)
miR-10b	TIAM1	promotes cell growth and cell migration	(31)
miR-100	mTOR	regulates growth	(21)

IKKα, inhibitor of nuclear factor κB kinase (α-subunit); PTEN, phosphate and tensin homolog (a PIP_3 phosphatase); HCN2&4, hyperpolarization and cyclic nucleotide activated K^+ channels involved in pacemaker function; HERG, human ether-a-go-go, a repolarising K^+ channel (I_{KR}); CTGF, connective tissue growth factor; TIAM1, a RAC GEF (guanylnucleotide exchange factor); mTOR, mammalian target of rapamycin, a growth-promoting protein.

moved from all patients undergoing valve surgery. This suggests the possibility that miRNA expression differences in the AF vs. SR groups are partly a reflection of the severity of the disease. On this basis, it is possible that miRNA expression changes in LA are already maximal at the time of surgery, regardless of AF status. We also found differences in miRNA expression between VHD-LA and VHD-RA. miR-10b was expressed preferentially in LA, whereas miR-100 was higher in RA (Figs. 1*B* and 2, Tables 2 and 5). miR-100 has previously been studied in relation to heart disease (29), whereas miR-10b has been primarily studied in relation to cancer progression (31), but our data suggest a role in pathology in the LA.

The miRNAs that were differentially expressed in VHD-RA-AF vs. VHD-RA-SR included profibrotic miRNAs [miR-21 (12) and miR-377 (34)] that might be expected to contribute to AF by promoting fibrosis, an established AF substrate (3, 9) (Tables 2, 3, 5). miR-133 and miR-30 family members showed lower expression in the AF group. Both the miR-133 and miR-30 families regulate connective tissue growth factor expression and thereby contribute to ventricular remodeling in heart failure (6). In addition, miR-133 family members might directly influence arrhythmia outcomes by direct targeting of critical ion channels, including hyperpolarization and cyclic nucleotide regulated channel (HCN2&4) pacemaker K^+ channels and the human ether-a-go-go (HERG, I_{kr}) repolarizing K^+ channel (2, 5, 6, 20), both of which have been implicated in the pathogenesis of AF (7, 13, 16, 28, 37) (Table 3). miR-133a and miR-133b are muscle-specific miRNAs, and altered expression levels are associated with hypertrophy, fibrosis, and arrhythmia (5, 14, 20). miR-133a and miR-133b are localized on chromosome 18, along with miR-1 family members, and the expression of these miRNAs is regulated by muscle-specific transcription factors including myocytes enhancer factor 2, serum response factor, and myocardin (32). Thus, our data suggest transcriptional repression of this locus in RA of VHD patients with chronic AF. In contrast, miR-30 family members are expressed from different locations on chromosomes 1, 6, and 8, some being intronic and some intragenic. The finding that several of these miRNAs are downregulated in tissue from patients with AF suggests a co-coordinated repression of miR-30 members under these disease conditions (Fig. 1*A*, Table 3). Changes in miRNA expression in RA observed in relation to the VHD or AF share some similarities with changes reported previously in LV tissue of heart failure patients (6, 18, 22).

VHD was associated with more pronounced changes in miRNA expression in LA than in RA (Table 4). This most likely reflects the primary role of LA in the genesis of the disease. Most of the miRNAs showing altered expression in VHD-LA have not previously been studied in heart, and a number of miRNAs currently recognized as promoting cardiac fibrosis were not included in this group (12, 33). In RA samples, the established profibrotic miRNAs, miR-21, showed increased expression in VHD compared with CABG (Table 4). The contribution of miR-21 to cardiac pathology has been well studied, especially in relation to ventricular remodeling in heart failure. miR-21 contributes to fibrosis by targeting Sprouty family members and phosphate and tensin homolog, which, in turn, repress growth signaling (12, 30). The increased expression of miR-21 in VHD-RA therefore likely contributes to fibrosis associated with the progression of VHD. Interestingly, miR-146b-5p showed the largest increases in VHD-RA compared with CABG-RA. While this miRNA has not previously been investigated in relation to heart disease, potential targets of miR-146b-5p predicted by Targetscan include Sprouty family members that are confirmed targets of miR-21. These remain to be confirmed as real targets of miR-146b-5p in heart. miR-21 and miR-146b-5p have been reported to be regulated coordinately in inflammatory diseases (23), and it is possible that they are also regulated in parallel in atrial disease. This possibility remains to be investigated. The only miRNA to be regulated similarly with VHD in LA and RA was miR-490-3p. This miRNA showed reduced expression in both LA and RA and was further decreased in the VHD-RA-AF group compared with VHD-RA-SR (Tables 2–5). This miRNA has not been extensively studied, but the data suggest a possible role in the pathology of VHD yet to be identified.

In summary, we have described miRNA expression changes associated with VHD and with AF in VHD patients. Some of the miRNAs identified as differentially expressed have been identified previously as promoting hypertrophy (miR-23a, miR-1, miR-133a), fibrosis (miR-133a, miR-30, miR-1, miR-21), or arrhythmia (miR-1, miR-133a) within the myocardium (6, 12). In addition to these well-studied miRNAs, we identified changes in miRNAs not previously associated with cardiac pathology and some of these were altered specifically in LA. miRNA profiles in LA and RA respond differently to the challenge of VHD and the AF associated with VHD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.C., R.C.L., and S.M. performed experiments; N.C., M.J.C., and C.W. analyzed data; N.C. and D.M.K. interpreted results of experiments; N.C. prepared figures; N.C. and E.A.W. edited and revised manuscript; N.C., M.J.C., R.C.L., S.M., C.W., D.M.K., A.M.D., and E.A.W. approved final version of manuscript; A.M.D. and E.A.W. conception and design of research; E.A.W. drafted manuscript.

REFERENCES

1. **Benjamini Y, Hochberg Y.** Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc Series B* 57: 289–300, 1995.
2. **Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu YS, Dalton ND, Elia L, Latronico MVG, Hoydal M, Autore C, Russo MA, Dorn GW, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G.** MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13: 613–618, 2007.
3. **Corradi D, Callegari S, Maestri R, Benussi S, Alfieri O.** Structural remodeling in atrial fibrillation. *Nat Clin Pract Cardiovasc Med* 5: 782–796, 2008.

4. **Divakaran V, Mann DL.** The emerging role of microRNAs in cardiac remodeling and heart failure. *Circ Res* 103: 1072–1083, 2008.
5. **Diwan A, Matkovich SJ, Yuan Q, Zhao W, Yatani A, Brown JH, Molkentin JD, Kranias EG, Dorn GW 2nd.** Endoplasmic reticulum-mitochondria crosstalk in NIX-mediated murine cell death. *J Clin Invest* 119: 203–212, 2009.
6. **Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE.** miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circ Res* 104: 170–178, 2009.
7. **Ehrlich JR, Zicha S, Coutu P, Hebert TE, Nattel S.** Atrial fibrillation-associated minK38G/S polymorphism modulates delayed rectifier current and membrane localization. *Cardiovasc Res* 67: 520–528, 2005.
8. **Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J.** Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 5: R80, 2004.
9. **Gramley F, Lorenzen J, Koellensperger E, Kettering K, Weiss C, Munzel T.** Atrial fibrosis and atrial fibrillation: the role of the TGF-beta1 signaling pathway. *Int J Cardiol* 143: 405–413, 2009.
10. **Humphreys DT, Westman BJ, Martin DI, Preiss T.** MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc Natl Acad Sci USA* 102: 16961–16966, 2005.
11. **Ihaka R, Gentleman R.** A language for data analysis and graphics. *J Comput Graphic Stats* 5: 299–314, 1996.
12. **Jiang X, Tsitsiou E, Herrick SE, Lindsay MA.** MicroRNAs and the regulation of fibrosis. *FEBS J* 277: 2015–2021, 2010.
13. **Lai LP, Su MJ, Lin JL, Lin FY, Tsai CH, Chen YS, Tseng YZ, Lien WP, Huang SK.** Changes in the mRNA levels of delayed rectifier potassium channels in human atrial fibrillation. *Cardiology* 92: 248–255, 1999.
14. **Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, Olson EN.** microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev* 22: 3242–3254, 2008.
15. **Lopez-Romero P, Gonzalez MA, Callejas S, Dopazo A, Irizarry RA.** Processing of Agilent microRNA array data. *BMC Res Notes* 3: 18, 2010.
16. **Luo XB, Lin HX, Pan ZW, Xiao JN, Zhang Y, Lu YJ, Yang BF, Wang ZG.** Down-regulation of miR-1/miR-133 contributes to re-expression of pacemaker channel genes HCN2 and HCN4 in hypertrophic heart. *J Biol Chem* 283: 20045–20052, 2008.
17. **Massirer KB, Pasquinelli AE.** The evolving role of microRNAs in animal gene expression. *Bioessays* 28: 449–452, 2006.
18. **Matkovich SJ, Van Booven DJ, Youker KA, Torre-Amione G, Diwan A, Eschenbacher WH, Dorn LE, Watson MA, Margulies KB, Dorn GW 2nd.** Reciprocal regulation of myocardial microRNAs and messenger RNA in human cardiomyopathy and reversal of the microRNA signature by biomechanical support. *Circulation* 119: 1263–1271, 2009.
19. **Matkovich SJ, Wang W, Tu Y, Eschenbacher WH, Dorn LE, Condorelli G, Diwan A, Nerbonne JM, Dorn GW 2nd.** MicroRNA-133a protects against myocardial fibrosis and modulates electrical repolarization without affecting hypertrophy in pressure-overloaded adult hearts. *Circ Res* 106: 166–175, 2009.
20. **Meder B, Katus HA, Rottbauer W.** Right into the heart of microRNA-133a. *Gene Dev* 22: 3227–3231, 2008.
21. **Nagaraja AK, Creighton CJ, Yu Z, Zhu H, Gunaratne PH, Reid JG, Olokpa E, Itamochi H, Ueno NT, Hawkins SM, Anderson ML, Matzuk MM.** A link between miR-100 and FRAP1/mTOR in clear cell ovarian cancer. *Mol Endocrinol* 24: 447–463, 2010.
22. **Prasad SVN, Duan ZH, Gupta MK, Surampudi VSK, Volinia S, Calin GA, Liu CG, Kotwal A, Moravec CS, Starling RC, Perez DM, Sen S, Wu QY, Plow EF, Croce CM, Karnik S.** Unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks. *J Biol Chem* 284: 27487–27499, 2009.
23. **Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN.** MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. *FASEB J* 25: 544–560, 2011.
24. **Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP.** GenePattern 20. *Nat Genet* 38: 500–501, 2006.
25. **Sayed D, Abdellatif M.** AKT-ing via microRNA. *Cell Cycle* 9: 3213–3217, 2010.
26. **Smyth GK.** Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3: Article3, 2004.
27. **Smyth GK, Michaud J, Scott HS.** Use of within-array replicate spots for assessing differential expression in microarray experiments. *Bioinformatics* 21: 2067–2075, 2005.
28. **Stillitano F, Lonardo G, Zicha S, Varro A, Cerbai E, Mugelli A, Nattel S.** Molecular basis of funny current (If) in normal and failing human heart. *J Mol Cell Cardiol* 45: 289–299, 2008.
29. **Sucharov CC, Dockstader K, McKinsey TA.** YY1 protects cardiac myocytes from pathologic hypertrophy by interacting with HDAC5. *Mol Biol Cell* 19: 4141–4153, 2008.
30. **Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S.** MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 456: 980–984, 2008.
31. **Tian Y, Luo A, Cai Y, Su Q, Ding F, Chen H, Liu Z.** MicroRNA-10b promotes migration and invasion through KLF4 in human esophageal cancer cell lines. *J Biol Chem* 285: 7986–7994, 2010.
32. **van Rooij E, Liu N, Olson EN.** MicroRNAs flex their muscles. *Trends Genet* 24: 159–166, 2008.
33. **van Rooij E, Olson EN.** Searching for miR-acles in cardiac fibrosis. *Circ Res* 104: 138–140, 2009.
34. **Wang Q, Wang Y, Minto AW, Wang J, Shi Q, Li X, Quigg RJ.** MicroRNA-377 is up-regulated and can lead to increased fibronectin production in diabetic nephropathy. *FASEB J* 22: 4126–4135, 2008.
35. **Woodcock EA, Grubb DR, Filtz TM, Marasco S, Luo JT, McLeod-Dryden TJ, Kaye DM, Sadoshima J, Du XJ, Wong C, McMullen JR, Dart AM.** Selective activation of the "b" splice variant of phospholipase Cβ1 in chronically dilated human and mouse atria. *J Mol Cell Cardiol* 47: 676–683, 2009.
36. **Xiao J, Liang D, Zhang Y, Liu Y, Zhang H, Li L, Liang X, Sun Y, Chen YH.** MicroRNA expression signature in atrial fibrillation with mitral stenosis. *Physiol Genomics* 43: 655–664, 2011.
37. **Yeh YH, Burstein B, Qi XY, Sakabe M, Chartier D, Comtois P, Wang Z, Kuo CT, Nattel S.** Funny current downregulation and sinus node dysfunction associated with atrial tachyarrhythmia: a molecular basis for tachycardia-bradycardia syndrome. *Circulation* 119: 1576–1585, 2009.

Influence of atrial fibrillation on microRNA expression profiles in left and right atria from patients with valvular heart disease

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