Journal of the American College of Cardiology
© 2014 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 63, No. 9, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2013.09.041

Biomarkers

Circulating miR-29a, Among Other Up-Regulated MicroRNAs, Is the Only Biomarker for Both Hypertrophy and Fibrosis in Patients With Hypertrophic Cardiomyopathy

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Objectives The purpose of this paper was to determine whether microRNAs (miRNAs) involved in myocardial remodeling were differentially expressed in the blood of hypertrophic cardiomyopathy (HCM) patients, and whether circulating miRNAs correlated with the degree of left ventricular hypertrophy and fibrosis.

Background miRNAs—small, noncoding ribonucleic acids (RNAs) that regulate gene expression by inhibiting RNA translation—modulate cellular function. Myocardial miRNAs modulate processes such as cardiomyocyte (CM) hypertrophy, excitation–contraction coupling, and apoptosis; non–CM-specific miRNAs regulate myocardial vascularization and fibrosis. Recently, the possibility that circulating miRNAs may be biomarkers of cardiovascular disease has been raised.

Methods Forty-one HCM patients were characterized with conventional transthoracic echocardiography and cardiac magnetic resonance. Peripheral plasma levels of 21 miRNAs were assessed by quantitative real-time polymerase chain reaction and were compared with levels in a control group of 41 age- and sex-matched blood donors.

Results Twelve miRNAs (miR-27a, -199a-5p, -26a, -145, -133a, -143, -199a-3p, -126-3p, -29a, -155, -30a, and -21) were significantly increased in HCM plasma. However, only 3 miRNAs (miR-199a-5p, -27a, and -29a) correlated with hypertrophy; more importantly, only miR-29a correlated also with fibrosis.

Conclusions Our data suggest that cardiac remodeling associated with HCM determines a significant release of miRNAs into the bloodstream: the circulating levels of both cardiac- and non–cardiac-specific miRNAs are significantly increased in the plasma of HCM patients. However, correlation with left ventricular hypertrophy parameters holds true for only a few miRNAs (i.e., miR-199a-5p, -27a, and -29a), whereas only miR-29a is significantly associated with both hypertrophy and fibrosis, identifying it as a potential biomarker for myocardial remodeling assessment in HCM. (J Am Coll Cardiol 2014;63:920–7) © 2014 by the American College of Cardiology Foundation

Hypertrophic cardiomyopathy (HCM) is a common inherited heart disease with a prevalence of approximately 1:500 in the general population (1–3). More than 1,400 distinct mutations have been identified in cardiac sarcomere genes, most of which are unique to individual families. This genetic heterogeneity seems related to independent mutational events

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Manuscript received July 30, 2013; revised manuscript received September 18, 2013, accepted September 23, 2013.

(4). One of the hallmarks of HCM is myocardial remodeling, characterized by cardiomyocyte hypertrophy, sarcomeric disarray, and fibrosis (1–3).

Several studies have demonstrated a functional role of microribonucleic acid (miRNA) in myocardial hypertrophy (5–8). These small, noncoding ribonucleic acids (RNAs) function as post-transcriptional regulators of gene expression, modulating several physiological and pathological processes. In the heart, they have a crucial role in physiological development, hypertrophy, ischemia/reperfusion injury, angiogenesis, atherosclerosis, apoptosis, and fibrosis. In animal models of hypertrophy, miRNAs were found to be up-regulated, down-regulated, or unchanged in comparison with the levels measured in normal hearts (9).

Since the discovery of their presence also in the bloodstream (10), miRNAs have attracted interest as putative circulating biomarkers of cardiovascular disease (11): distinctive profiles of circulating miRNAs have been reported for acute myocardial infarction (12), heart failure (13), coronary artery disease (14), and diabetes mellitus (15). However, whether HCM is accompanied by a specific circulating miRNA signature is still not known.

We thus aimed to characterize the circulating miRNA profile of HCM, evaluating 21 miRNAs directly involved in angiogenesis, fibrosis, apoptosis, hypertrophy, and smooth muscle cell biology (Online Table 1). Moreover, we assessed the correlation with the degree of left ventricle (LV) hypertrophy, as evaluated by transthoracic echocardiography (TTE) and cardiac magnetic resonance (CMR). We found that 12 circulating miRNAs were significantly increased in HCM patients with respect to healthy age- and sex-matched subjects, but only 3 of these miRNAs (miR-27a, -29a, and -199a-5p) correlated with cardiac hypertrophy. Notably, only miR-29a also correlated with myocardial fibrosis.

Methods

Further details regarding the methods of this study can be found in the Online Appendix.

Patient population. Forty-one unrelated patients diagnosed with HCM were recruited at the Division of Cardiology, "Federico II" University of Naples, Naples, Italy. The diagnosis of HCM was based on echocardiographic demonstration of a hypertrophied, nondilated LV (wall thickness >15 mm) in the absence of any other cardiac or systemic disorder producing a comparable grade of hypertrophy (16). Classification parameters defining HCM status were those established by the American Heart Association guidelines (16). No patients were in heart failure. In addition, 41 healthy age- and sex-matched subjects, recruited from the Italian blood donor organization AVIS, were enrolled in the study to serve as controls. None of the selected healthy subjects had an abnormal blood pressure response to exercise or a family history of cardiovascular disease.

To determine potential differences between HCM and left ventricular hypertrophy (LVH) consequent to pressure overload, the current study included 12 high-risk patients with severe symptomatic aortic stenosis (aortic valve area [AVA] <1 cm^2; body surface-indexed AVA [iAVA] <0.6 cm^2/m^2) (17) recruited at the "Federico II" University of Naples.

Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the institution's human research committee. All subjects underwent physical examination, electrocardiogram (ECG), 24-h ECG Holter monitoring, TTE and Doppler studies, and CMR. Patient characteristics are summarized in Table 1; TTE and CMR parameters are presented in Table 2.

Abbreviations and Acronyms

AUC = area under the curve
BNP = brain natriuretic peptide
CMR = cardiac magnetic resonance
Ct = threshold cycle
ECG = electrocardiogram
HCM = hypertrophic cardiomyopathy
hs-cTnT = high-sensitivity cardiac troponin T
LV = left ventricle/ventricular
LVH = left ventricular hypertrophy
miRNA = microribonucleic acid
MWT = maximum wall thickness
ROC = receiver-operating characteristic
TTE = transthoracic echocardiography

Blood collection and RNA isolation. A 5-ml sample of peripheral blood was collected in ethylenediaminetetraacetic acid-containing Vacutainer tubes, processed to eliminate all blood cells, and aliquots were stored at −80°C. Total RNA was extracted with miRNeasy Mini Kit, following the manufacturer's instructions. Each RNA sample was quantified with a spectrophotometer (NanoDrop 1000, Thermo Scientific, Wilmington, Delaware).

Blood and plasma sampling and storage techniques were the same for patients and controls.

Assessment of circulating miRNAs. Reverse transcription reactions were performed using the EXIQON miRNA Reverse Transcription Mercury Universal cDNA synthesis kit (Exiqon A/S, Vedbaek, Denmark). A PCR System 9700 (Applied Biosystems, Foster City, California) was used to carry out the reverse transcription PCR reactions. A total of 21 miRNAs previously associated with cardiovascular disease were selected for investigation: miR-1, -16, -21, -26a, -27a, -29a, -30a, -126-3p, -126-5p, -133a, -199a-3p, -199a-5p, -143, -145, -155, -195, -208a, -208b, -214, -499-3p, and -499-5p. To generate the customized panel of miRNAs, specific primer sequences were obtained from the Exiqon website. miR-208a was also measured with a TaqMan assay (Applied Biosystems).

Quantitative real-time polymerase chain reactions were performed in triplicate for all samples; a ±0.2 difference between detected threshold cycle (Ct) values was considered acceptable. Ct values were computed with Sequence Detection System software version 2.4 (Applied Biosystems). The data were analyzed with automatic settings for assigning the

Table 1 Clinical Characteristics of HCM Patients

	HCM Patients		Controls		
	n	Value	n	Value	p Value*
Age (yrs)	41	50 ± 13	41	50 ± 10	0.687
Male	41	71%	41	73%	0.797
Significant (>30 mm Hg) left ventricular outflow tract gradient	10	24.4%	0	0%	—
Family history of HCM	11	27%	0	0%	—
Family history of sudden death	7	17%	0	0%	—
History of syncope	3	7.3%	0	0%	—
History of atrial fibrillation	3	7.3%	0	0%	—
ICD implant	5	12.2%	0	0%	—
Maximum wall thickness >30 mm	4	9.8%	0	0%	—
End-stage HCM	1	2.4%	0	0%	—
Nonsustained tachycardia	7	17%	0	0%	—
Abnormal blood pressure response to exercise	10	24.4%	0	0%	—
Pharmacological therapy					
No therapy	7	17%	40	97.5%	<0.001
Beta-blockers	25	61%	1	2.4%	<0.001
Sotalol	2	5%	0	0%	0.479
Verapamil	5	12.2%	0	0%	0.073
Disopyramide	4	9.8%	0	0%	0.134
Mirapexin	1	2.4%	0	0%	1.0
Ivabradine	1	2.4%	0	0%	1.0
ACEIs/AIIRAs	9	22%	1	2.4%	0.013
Diuretics	10	24.4%	1	2.4%	0.016
Statins	11	26.8%	0	0%	0.003
Aspirin/clopidogrel	7	17%	0	0%	0.023
Warfarin	5	12.2%	0	0%	0.074
New York Heart Association functional class					
I	27	65.8%	ND	ND	—
II	14	34.2%	ND	ND	—
Mitral regurgitation					
Absent	7	17%	ND	ND	—
Mild	20	49%	ND	ND	—
Moderate	12	29%	ND	ND	—
Severe	2	5%	ND	ND	—

Values are mean ± SD or %, as indicated. *p Value computed with paired *t* test for continuous variables and McNemar's test for categorical variables.
ACEI = angiotensin-converting enzyme inhibitor; AIIRA = angiotensin II receptor antagonist; HCM = hypertrophic cardiomyopathy; ICD = implantable cardioverter-defibrillator.

baseline. The Ct was defined as the fractional cycle number at which the fluorescence exceeded the given threshold. miRNA expression levels were normalized to a nonendogenous synthetic miRNA.

Relative quantification was obtained using the $2^{-\Delta\Delta Ct}$ method (18), by which the normalized fold change was determined as follows: 1) normalization of the Ct of the target miRNA to that of the reference (spike) for all HCM patients and healthy individuals (baseline group); and 2) $\Delta Ct^{HCM} = Ct^{Target\ miRNA} - Ct^{Spike\text{-}in\ miRNA}$, and $\Delta Ct^{Healthy} = Ct^{Target\ miRNA} - Ct^{Spike\text{-}in\ miRNA}$ for all samples. Then, differential expression ($\Delta\Delta Ct$) of each considered miRNA was calculated (and expressed as a $2^{-\Delta\Delta Ct}$ ratio) by subtracting mean ΔCt^{HCM} from mean $\Delta Ct^{Healthy}$ (18).

Assessment of hypertrophy. TTE. All echocardiographic studies were performed using a commercial ultrasound machine (IE 33, Philips, Andover, Massachusetts) equipped with an S5-1 5- to 1-MHz phased array transducer. LV end-diastolic and -systolic diameters were measured in M-mode images and indexed to the body surface area, according to American Society of Echocardiography guidelines (19). The extent of LVH (hypertrophy index) (20) was calculated from the short-axis view at the level of the mitral valve and papillary muscles by dividing the LV wall into 4 segments (anterior septum, posterior septum, lateral free wall, and posterior free wall) and by adding the maximal wall thickness measured (at both the mitral valve and papillary muscle level) in each of the 4 ventricular segments (20). As an additional simple estimate of LVH, the maximal wall thickness (MWT) measured at any level in the LV wall was also considered (3). Color Doppler flow imaging was used for semiquantitative assessment of mitral regurgitation, which was graded from mild to severe according to EAE criteria (21). The LV outflow tract gradient was recorded at rest with a 1.9-MHz nonimaging transducer and was calculated with the simplified Bernoulli's

Table 2 TTE and CMR Parameters in HCM Patients

TTE and CMR Parameters	HCM Patients	
	n	Value
Left atrial diameter (mm)	41	47.9 ± 7.2
Left atrial indexed volume (ml/m^2)	41	41 ± 19
Left ventricular end-diastolic diameter indexed (mm/m^2)	41	25 ± 3
Left ventricular ejection fraction, TTE (%)	41	63.2 ± 7.3
Left ventricular outflow tract gradient (mm Hg)	41	18 ± 21
Maximum wall thickness, TTE (mm)	31	21 ± 5
Hypertrophy index, TTE (mm)	41	6 ± 3
Maximum wall thickness, CMR (mm)	21	20 ± 5
Left ventricular mass, CMR (g)	21	158 ± 39
Left ventricular mass index, CMR (g/m^2)	21	82 ± 17
Myocardial fibrosis score,* CMR	21	15 ± 13

Values are mean ± SD. *Total score of 10 sections/heart.
CMR = cardiac magnetic resonance; HCM = hypertrophic cardiomyopathy; TTE = transthoracic echocardiography.

equation. The LV outflow tract obstruction was considered significant at ≥30 mm Hg. All parameters were measured on 3 consecutive cycles (selected based on image quality) and averaged.

CMR. Patients with claustrophobia, arrhythmias (interfering with ECG gating), or a pacemaker/implanted cardioverter-defibrillator were excluded from this part of the study (n = 20, 49%). The CMR studies were performed using a 1.5-T magnetic resonance imaging system (Gyroscan Intera, Philips Medical System, Best, the Netherlands) equipped with high-performance gradients. LV short-axis images were obtained using 10 slices covering the LV from the apex to the base for evaluation of LV mass (19). LV mass was normalized to body surface area. Late gadolinium-enhancement images in short-axis orientation were acquired for quantification of myocardial fibrosis using a 3-dimensional T1-weighted inversion recovery turbo gradient echo sequence. To assess the presence of myocardial fibrosis, a semiquantitative evaluation was performed by giving a score from 0 to 4, where 0 = no evidence of fibrosis; 1 = fibrosis between 0% and 25%; 2 = fibrosis between 25% and 50%; 3 = fibrosis between 50% and 75%; and 4 = transmural fibrosis (22).

Statistical methods. CLINICAL DATA. Data were analyzed with SYSTAT version 12.0 (SYSTAT Software Inc., San Jose, California) and Stata version 11/SE program (College Station, Texas). Normality assumption was verified graphically (i.e., Q plot) and was confirmed using the Kolmogorov-Smirnov test. The Pearson's test was performed to evaluate potential correlation between clinical variables and miRNA levels. Receiver-operating characteristic (ROC) curves were established for discriminating patients with or without HCM.

EXPRESSION DATA. Statistical analyses were undertaken with the Stata version 11/SE program and GenEx software version 5.0 (Exiqon). Initially, miRNAs with a Ct cutoff ≥39 were filtered out to minimize any potential instrument background noise that could have interfered with the measurements. Quantitative variables are given as means ± SD and interquartile ranges. Normality assumption was verified graphically (i.e., Q plot) and was confirmed using the Kolmogorov-Smirnov test. TaqMan quantitative real-time polymerase chain reaction assays were done in triplicate with the synthetic spiked-in miRNA across all samples. A mean Ct for the synthetic miRNA and all samples was calculated. A 2-sided *t* test was conducted to determine which miRNAs were differently expressed. Paired *t* test and McNemar's test were used for continuous and categorical variables, respectively, to compare cases and control in Table 1. Statistical significance was defined as $p < 0.05$ for all tests. All p values are 2-sided.

Results

Circulating miRNA levels in HCM patients. Plasma miRNA levels were assessed in 41 HCM patients and 41 age- and sex-matched healthy controls. Among the 21 miRNAs analyzed, 12 were found significantly increased in HCM plasma. These were: miR-27a, -199a-5p, -26a, -145, -133a, -143, -199a-3p, -126-3p, -29a, -155, -30a, and -21 (Fig. 1). No significant differences were observed for miR-499-5p, -195, or -126-5p, whereas miR-214, -16, and -1 tended to be decreased, although not significantly. The cardiac-specific miR-208b was detected in only 13 HCM patients, whereas miR-499-3p was not detected in any of the samples. miR-208a was detected in only 12 HCM patients with both Exiqon and TaqMan analyses.

To determine the validity of the results obtained in HCM patients, we measured the same miRNAs in another type of cardiac hypertrophy: concentric LVH induced by severe aortic valve stenosis. We found a similar trend for only 5 miRNA (miR-21, -26a, -27a, -30a, and -133a) (Online Fig. 1), suggesting specific miRNA signatures for the 2 pathological conditions.

ROC analysis. ROC curves for each of the miRNAs analyzed were generated. Eight of the 12 significantly increased miRNAs had an optimal area value under the curve (AUC) >0.70 (Fig. 2).

Correlation of miRNA level with LV hypertrophy and myocardial fibrosis. After we had determined the differential miRNA expression pattern, we examined whether any miRNA was correlated (either directly or inversely) with clinical prognostic variables of HCM (Tables 1 and 2). To be noted, the clinical variables considered and the measured miRNAs were all normally distributed. We found significantly positive correlation coefficients for miR-29a and hypertrophy, as defined by 4 of the parameters tested (MWT determined by both TTE and CMR, LV mass, and the hypertrophy index) (Table 3, Online Fig. 2). miR-27a and -199a-5p correlated positively with hypertrophy when defined as LV mass or as LV mass index evaluated by CMR; however, no correlation with TTE parameters was found for either of these 2 miRNAs.

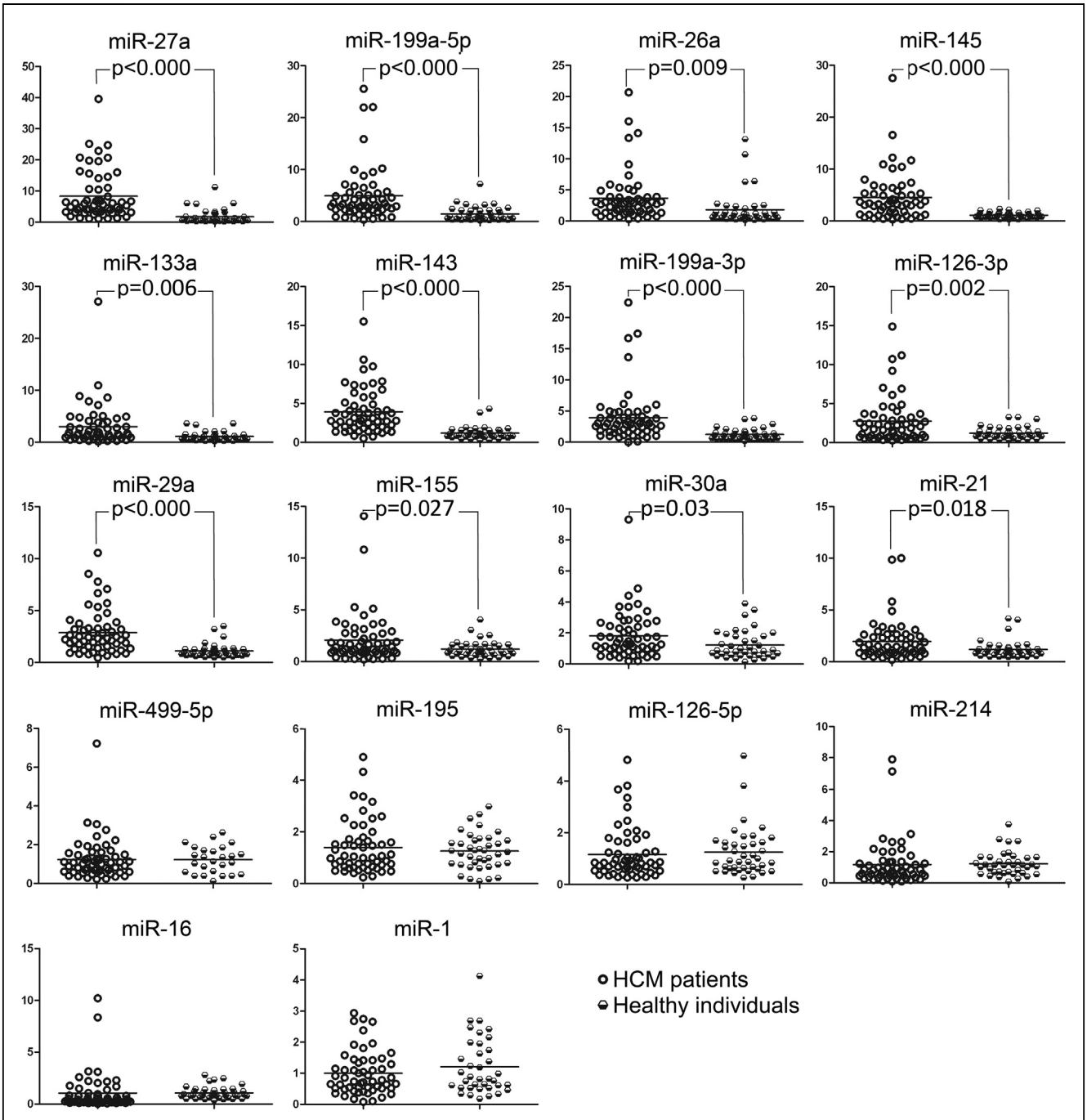

Figure 1 **Relative Expression of Circulating miRNAs**

Individual value plots of the variability and differences in the plasma level of each microribonucleic acid (miRNA) in healthy individuals **(right of each plot)** and patients with hypertrophic cardiomyopathy (HCM) **(left of each plot)**. Numbers represent p values obtained with 2-sided *t* test of miRNA expression in the HCM versus healthy groups. A **crossbar on each plot** indicates the mean expression level for each group.

In addition, significant correlations were found for miR-21 and -155 with the parameters MWT (as assessed by CMR) and hypertrophy index. Importantly, among miRNA associated with LV mass (i.e., miR-27a, -29a, and -199a-5p), only miR-29a correlated positively with myocardial fibrosis, as assessed with CMR ($r = 0.691$, $p = 0.003$) (Online Fig. 3).

To evaluate potential synergic effects between miR-21 and -29a—2 fibroblast-associated miRNAs that were significantly altered in HMC—an estimated model was used, but no significant results were found. In addition, no significant interactions were found between circulating levels of miRNAs and any of the drugs administered to the

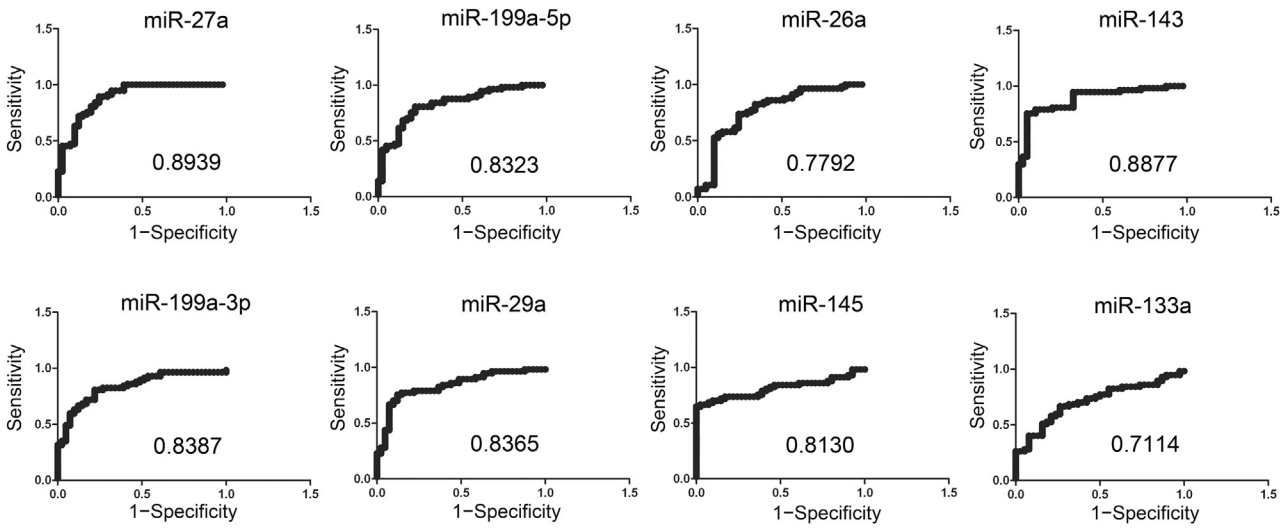

Figure 2 AUC Analysis of Receiver-Operating Characteristics

The area under the curve (AUC) (values given on the **graphs**) for miRNAs with significantly increased circulating levels were computed for the HCM group. Abbreviations as in Figure 1.

patients (including beta-blockers, verapamil, disopyramide, angiotensin-converting enzyme inhibitors/angiotensin II receptor antagonists, diuretics, statins, and aspirin/clopidogrel), which could also be affected by the small sample size.

Discussion

We have assessed the circulating levels of 21 miRNAs involved mostly in cardiac remodeling in patients with HCM, a primary form of cardiomyopathy. Our aim was to characterize the circulating miRNA profile in HCM patients and identify the determinants of any differential expression versus age- and sex-matched healthy individuals.

We found that levels of the majority of the analyzed miRNAs (12 of 21) were significantly increased in the plasma of HCM patients; moreover, 8 of these 12 miRNAs had an ROC curve that distinguished the HCM group from the healthy group (AUC >0.70). In particular, among the "myo-miRNAs" (23), miR-133a was increased, miR-499 could not be measured at all, and miR-208a and -208b were increased in only a subset of patients. The miRNAs that increased the most, however, were those involved in the regulation of angiogenesis and vascular cell differentiation. Within this group, the increased level of the miR-143/145

Table 3 Pearson Correlation Coefficients of Significantly Increased miRNAs and Prognostic Clinical Parameters of HCM

	LVEDI, TTE		MWT, TTE		MWT, CMR		LVMI, CMR		Hypertrophy Index, TTE*	
	r Value	p Value	r Value	p Value	r Value	p Value	r Value	p Value	r Value	p Value
miR-29a	−0.197	0.146	**0.463**	**0.005**	**0.412**	**0.021**	0.325	0.069	**0.475**	**0.001**
miR-27a	−0.030	0.824	0.126	0.469	0.281	0.125	**0.380**	**0.032**	0.186	0.227
miR-199a-5p	0.012	0.932	0.117	0.504	0.151	0.419	**0.421**	**0.017**	0.087	0.576
miR-21	−0.143	0.294	0.291	0.090	**0.406**	**0.023**	0.176	0.334	**0.346**	**0.021**
miR-155	−0.088	0.519	0.294	0.086	**0.356**	**0.049**	0.325	0.069	**0.308**	**0.042**
miR-126-3p	−0.078	0.565	0.048	0.783	0.255	0.167	0.294	0.102	0.086	0.425
miR-133a	−0.103	0.451	0.164	0.346	0.181	0.329	0.041	0.822	0.175	0.230
miR-30a	0.107	0.432	0.006	0.973	0.157	0.398	0.302	0.093	−0.030	0.848
miR-26a	−0.019	0.889	0.321	0.060	0.315	0.085	0.292	0.105	0.252	0.099
miR-199a-3p	0.029	0.839	0.172	0.347	−0.113	0.552	0.080	0.673	0.150	0.348
miR-145	−0.170	0.211	0.106	0.546	0.218	0.238	0.199	0.276	0.161	0.296
miR-143	−0.115	0.397	0.207	0.233	0.266	0.148	0.217	0.232	0.242	0.114

Values are Pearson correlation coefficient (r) and relative computed p value. Significant values are highlighted in **bold**. *Sum of maximum wall thickness (MWT) measured in each of 4 left ventricular segments in short-axis view at the mitral valve and papillary muscle level.

LVEDI = left ventricular end-diastolic diameter index; LVMI = left ventricular mass index; miRNA = microribonucleic acid; other abbreviations as in Table 2.

cluster—a miRNA family mostly expressed in smooth muscle cells (24–26)—is surprising. These miRNAs have been shown to be expressed also during cardiac differentiation in vitro and cardiac development, although only transiently (27). Thus, the surge in plasma of these miRNAs reflects either an activation in cardiomyocytes of the fetal gene program—a hallmark of cardiac hypertrophy (28)—or significant vasculogenesis in the HCM heart (29). We tend to favor the second hypothesis, because in a model of miR-143/145 transgenesis generated in our laboratory (25), we could not detect expression of this cluster in cardiomyocytes during development.

The most striking finding of this study is the correlation between miR-29a and cardiac hypertrophy, as measured by assessing 4 parameters with 2 independent techniques, and fibrosis, as measured with CMR, in patients with HCM. miR-29 is produced mostly by fibroblasts, and its family members are key regulators of fibrosis, modulating mRNA levels of collagen and other extracellular matrix genes (30–32). In addition, this miRNA was found to be a key player in liver (33), pulmonary (34), and kidney fibrosis (35) and in systemic sclerosis (36). Moreover, miR-29a was up-regulated along with miR-29c in an animal model of physiological cardiac adaptation to exercise training (37), in contrast with a previous pattern reported for miRNAs associated with pathological hypertrophy (38). To date, no data are available on miR-29a in HCM, and our study is, to our knowledge, the first to point out an association between circulating miR-29 and the presence of fibrosis in HCM. Interestingly, the association of miR-29a with myocardial fibrosis seems to be specific for HCM in that its circulating level in patients with severe aortic stenosis was not increased relative to the control group.

We also found that in contrast with miR-29a, another miRNA involved in cardiac fibrosis, miR-21 (39), although also significantly increased in HCM plasma, did not correlate with the degree of hypertrophy or with the extension of myocardial fibrosis.

High-sensitivity cardiac troponin T (hs-cTnT) and brain natriuretic peptide (BNP) have been recently proposed as biomarkers of fibrosis in HCM patients (40). In comparison with hs-cTnT and BNP, miRNAs show a more significant AUC. Also, although hs-cTnT is correlated with the extent of fibrosis, BNP is associated with LV overload rather than myocardial fibrosis, and therefore does not seem to be a direct marker of fibrosis (40).

Another significant result is that myo-miRNAs, as well as other cardiovascular miRNAs, can be released into the bloodstream even when there is no ongoing myocardial damage. Myo-miRNAs have already been measured in the plasma of patients with myocardial infarction (12). In the present study, we found that the circulating level of some of these miRNAs can increase also when there is cardiac hypertrophy and fibrosis without any clinically evident acute cellular damage. In this case, the circulating levels reflect mostly miRNAs secreted from intact cells rather than released from damaged cells. This holds true also for nonmuscle-specific miRNAs, and in our view, this indicates that an organ undergoing stress tends to release miRNAs into the bloodstream from all cell types of which it is composed.

Finally, the circulating levels of miRNAs transcribed as an isocluster, such as miR-1 and miR-133a, were not necessarily up-regulated together, indicating that the members of multicistronic miRNA genes can be subject to different intracellular metabolic pathways: some may be released from cells, whereas others have an intracellular fate.

Study limitations. Hitherto, there are no studies in the literature on the mechanisms of miRNA release and on their metabolism once secreted into the extracellular space. This lack of knowledge on miRNA biology hinders the assessment of circulating miRNAs. Moreover, the method used in this study for assessing circulating miRNA levels does not distinguish between miRNAs transported by exosomes and those transported by plasma proteins; it is thus possible that we did not efficiently measure exosome-bound miRNAs. Future studies are undoubtedly needed to clarify these points. In addition, we assumed that the miRNAs measured in the bloodstream were produced mainly by the heart. It is in theory possible that secondary effects on other organs or tissues induced by primary cardiomyopathy are reflected in an increased production of miRNAs. For instance, the surge in miR-29a could be due to damage of the aortic arch (31,32). However, our patient population was selected on the basis of being free from major complications in organs other than the heart. Thus, it is highly probable that plasma levels mirrored the extent of miRNA biogenesis within the heart.

Conclusions

A circulating miRNA profile distinguishes HCM patients from healthy individuals. Three significantly up-regulated miRNAs—miR-27a, -29a, and -199a-5p—correlated with LV mass, whereas only miR-29a correlated with fibrosis. The circulating miRNA profile of this type of cardiomyopathy was also different from that of hypertrophy because of aortic stenosis, indicating that circulating profiles may be disease specific. This is the first instance in which a circulating miRNA has been shown to correlate with myocardial fibrosis in a clinical setting, identifying miR-29a as a potential biomarker for myocardial remodeling assessment in HCM.

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Key Words: circulating microRNAs ■ fibrosis ■ hypertrophic cardiomyopathy ■ myocardial remodeling.

APPENDIX

For supplemental methods information as well as a supplemental table and figures, please see the online version of this article.