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Review Article

miRNAS in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges

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Abstract

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the world. Although considerable progress has been made in the diagnosis, treatment and prognosis of CVD, there is still a critical need for novel diagnostic biomarkers and new therapeutic interventions to decrease the incidence of this disease. Recently, there is increasing evidence that circulating miRNAs (miRNAs), *i.e.* endogenous, stable, single-stranded, short, non-coding RNAs, can be used as diagnostic biomarkers for CVD. Furthermore, miRNAs represent potential novel therapeutic targets for several cardiovascular disorders. In this review we provides an overview of the effects of several CVD; including heart failure, acute myocardial infarction, arrhythmias and pulmonary hypertension; on levels of circulating miRNAs. In addition, the use of miRNA as therapeutic targets is also discussed, as well as challenges and recommendations in their use in the diagnosis of CVD.

Keywords: microRNA; cardiovascular diseases; biomarker; therapeutic targets

Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in the world, especially in developed countries. As such, there is an urgent need to identify new prognostic and diagnostic biomarkers for the prevention and treatment of CVD. With the advance of precision medicine and next generation sequencing, microRNAs (miRNAs) have become potential markers for this disease. miRNAs are endogenous, conserved, single-stranded, small (~22 nucleotides) non-coding RNAs that influence most, if not all biological processes. miRNAs are critical regulators of cardiovascular function and play important roles in almost all aspects of cardiovascular biology[1-4]. This review provides an overview of the biology, therapeutic and diagnostic potential, as well as the limitations of miRNAs in the diagnosis and treatment of CVD.

Basic biology and stability of circulating miRNAs

The first miRNA, lin-4, was discovered in *C elegans* in 1993. Lin-4 regulates *C elegans* development by binding to the lin-14 mRNA to inhibit LIN-14 protein expression[5, 6]. miRNAs regulate gene expression at the post-transcriptional level by binding to 3'- untranslated regions of target mRNAs[7]. An individual miRNA can target several to hundreds of distinct mRNAs[8]. miRNAs inhibit translation and/or induce degradation of its target mRNA, depending on the degree of complementarity, and the number and the accessibility of the binding sites[9]. The greater the complementarity between the miRNA and its target(s), the more likely the miRNA will promote degradation[10].

It is estimated that the human genome encodes approximately 1000 miRNAs. Among them, more than 100 have been identified in sera from healthy subjects and are designated circulating miRNAs[11]. Unlike intracellular mRNAs, circulating miRNAs show remarkable stability and resistance to degradation by endogenous RNase activity[12, 13]. The majority of the circulating miRNAs are derived from blood cells with others from various tissues such as heart, lung, liver and kidney[14, 15]. It has been proposed that circulating miRNAs reside

in microvesicles including exosomes, microparticles and apoptotic bodies, which may provide protection from RNase activity and account for the shedding of miRNAs into the circulation (Figure 1)[14]. The stability of circulating miRNAs has stimulated interest in their use as biomarkers for the diagnosis and prognosis of various diseases including CVD.

miRNAs in the diagnosis and prognosis of cardiovascular disease
Heart failure
Heart failure (HF) is a clinical diagnosis when the heart fails to provide sufficient circulatory force to meet the body's metabolic requirements[16]. It is one of the major causes of mortality in the US, responsible for ~30% of patient deaths annually[17]. Heart failure is the final manifestation of CVD and cardiac injury, as well as less common but important etiologies including cardiomyopathies, valvular heart disease, prolonged

arrhythmias, myocarditis, infections and exposure to cardio-toxic drugs[18]. Circulating miRNAs have been identified as potential biomarkers of HF. Mounting evidence suggests that miRNAs are involved in the development and progression of HF.

Changes, both increases and decreases in the levels of almost 30 circulating miRNAs have been associated with HF and comorbid pathologies (Table 1, Figure 2). Declining levels of circulating miRNAs, including miR-18a, miR-27a, miR-30e, miR-26b, miR-199a, miR-106a and miR-652, are found in patients with HF. Reductions in circulating miRNAs let-7i, miR-18b, miR-18a, miR-223, miR-301a, miR-652 and miR-423 have been reported within 48 h after acute HF admission, and are associated with an increased risk of 180-day mortality[19]. miR-21 is upregulated and miR-1 downregulated in patients with symptomatic HF. Additionally, miR-1 levels decrease with the severity of New York Heart Association Class, and is
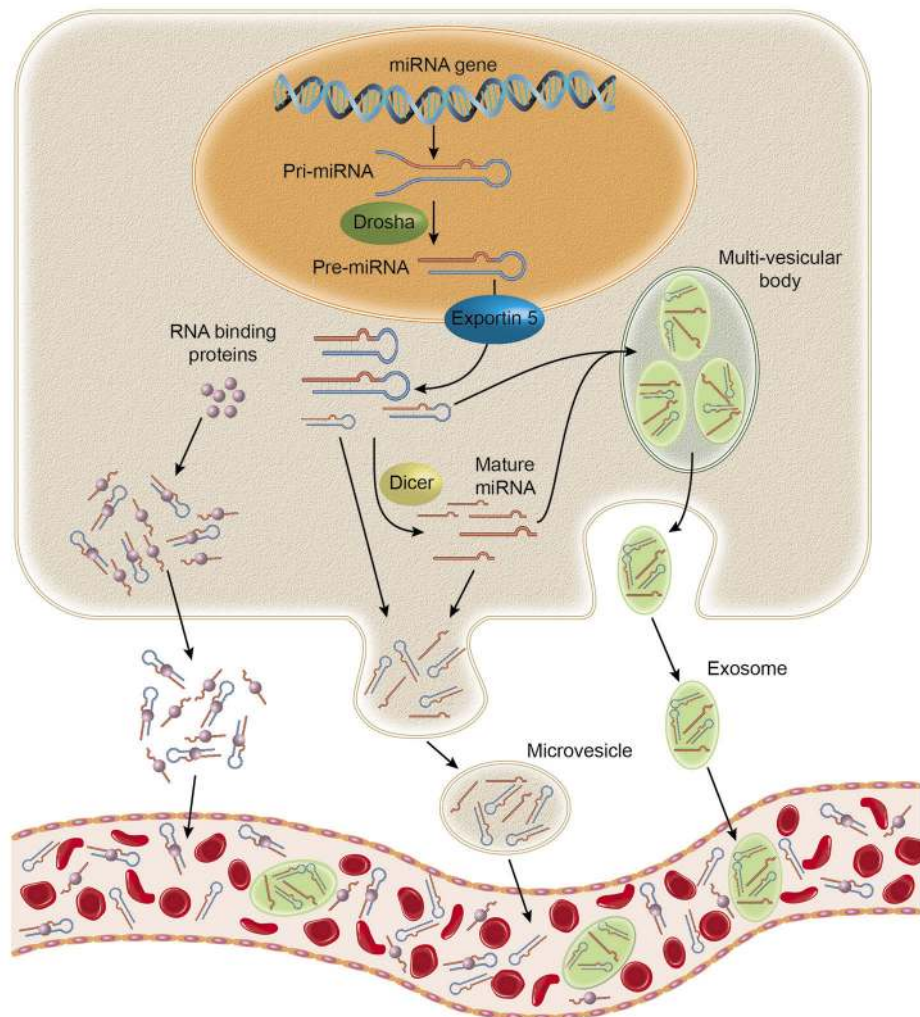

Figure 1. Biogenesis of circulating miRNAs. miRNAs are transcribed in the nucleus as pri-miRNAs with 5'-caps and 3'-polyA tails. Drosha removes the cap and polyA tail to generate pre- miRNAs, which are exported from the nucleus via Exportin 5. In the cytoplasm Dicer processes the pre-miRNA to mature miRNAs. Pre-miRNA and mature miRNA can (a) bind to RNA-binding proteins to be directly excreted from the cell, (b) packaged into microvesicles or (c) packed into exosomes and multi-vesicular bodies where the exosomes are then released. Pre-miRNA and mature miRNAs are taken into the bloodstream by endocytosis, binding to receptors or via membrane fusion.

inversely related to the N-terminal pro-brain natriuretic peptide concentration in patients in Class II/III[20]. There is also increasing evidence that miR-210 levels are positively correlated with NYHA functional classifications. Similarly, patients with improved HF, as represented by reduced B-type natriuretic peptide (BNP), show decreased levels of plasma miR-210[21]. miR-126 and miR-423 levels are low in HF patients, with lower levels of miR-423 predicting >1-year mortality[22-24]. In two independent cohorts of 2203 total subjects, miR-1254 and miR-1306 were associated with increased risk of death and hospitalization in chronic HF patients[25]. Additionally, circulating miR-1306 was positively associated with adverse clinical outcome in acute HF patients[26]. Increased levels of miR-208b and miR-499 are strongly associated with increased risk of HF or death.

The diagnostic performance of BNP was improved when used in combination with circulating miR-30c, miR-221, miR-328, miR-146a or miR-375, alone or as part of a diagnostic panel. Additionally, combinations of two or more miRNAs with BNP was able to significantly improve the predictive value of models to distinguish HF with preserved ejection fraction from HF with reduced ejection fraction compared to using BNP alone[27].

Medical interventions are also associated with changes in miRNA levels. Compared to stable HF patients, individuals with advanced HF with left ventricular (LV) assist device implantation express higher cardiac myomirs; muscle-specific miRNAs; miR-208b, miR-208a and miR-499; and myomirs miR-1 and miR-133b[28]. miR-208b and miR-499 are released in the coronary sinus after cardioplegia and reperfusion to markedly higher levels than that present prior to surgery[29]. A prospective, non-randomized self-control trial was performed with 81 HF and dyssynchrony patients to determine if LV reverse remodeling after cardiac resynchronization therapy was associated with changes in circulating miRNAs. Responding subjects had higher levels of circulating miR-26b, miR-145, miR-92a, miR-30e and miR-29a, compared to non-responders[30]. Similar to miR-1306, baseline levels of circulating miR-30d was associated with LV remodeling in response to cardiac resynchronization therapy in advanced chronic HF patients, as well as a 1-year all-cause mortality in acute HF patients[31, 32].

Combined these observations identify several miRNAs as potential candidates that could be used as diagnostic biomarkers for HF to provide valuable clinical information. Additionally, they may be important tools in monitoring the progress of therapeutic interventions. Additional studies will be needed to validate any candidate, as described below under Challenges (Figure 2).

Acute myocardial infarction

Acute myocardial infarction (AMI), a major cause of morbidity and mortality in humans, is the result of coronary artery occlusion leading to myocardial tissue damage[33]. Myocardial remodeling after an AMI; heart chamber dilation and ventricular wall thinning; is caused by apoptosis and fibrosis within cells and tissue[34]. Although percutaneous coronary intervention and surgical revascularization have improved clinical outcomes of AMI, ischemia/reperfusion injury induced during the revascularization procedure can produce clinical complications leading to additional cardiac injury[35, 36].

Changes in the levels of circulating miRNAs have been reported in AMI patients with ischemia-related HF, including increases in miR-1, miR-133, miR-21, miR-29b, miR-192, miR-194, miR-34a, miR-208, miR-499, miR-423, miR-126, miR-134,
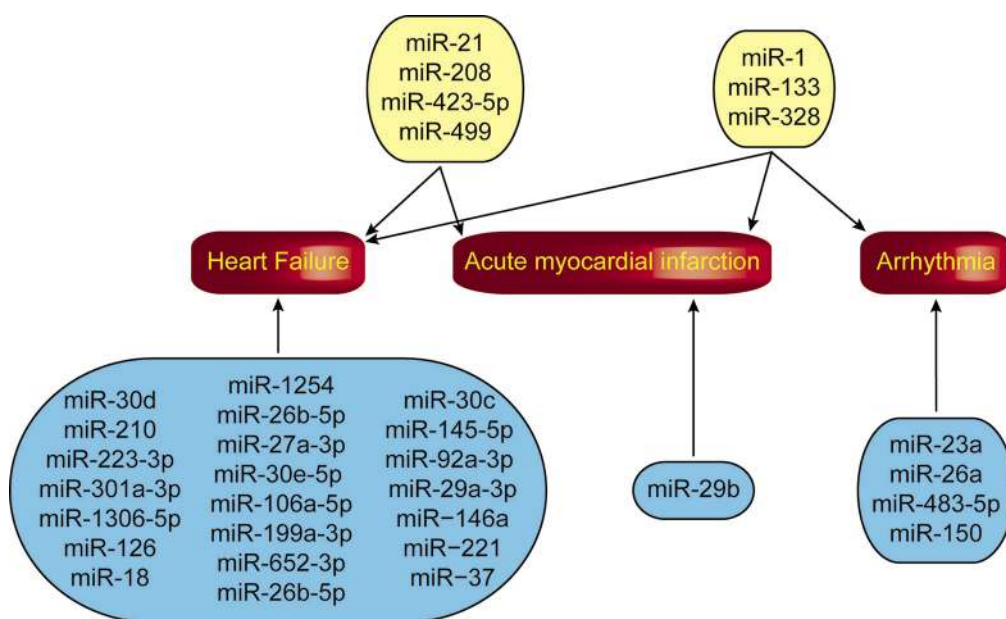

Figure 2. miRNAs associated with the diagnosis and prognosis of heart failure, acute myocardial infarction and arrhythmia. miRNAs in blue boxes are associated with a single pathology, while those in yellow boxes with multiple pathologies.

Table 1. miRNAs in heart failure.

miRNA ID	Change in expression	Purpose	Pathology (number of subjects)*	Reference
miR-1254	↑	Diagnosis/Death/HF hospitalization	CHF (2203)	[25]
miR-1306	↑			
	↑		AHF (496)	[26]
miR-30d	↓	Diagnosis/response to CRT	CHF (766)	[32]
	↓	Diagnosis/Death	AHF (96)	[31]
miR-21	↑	Diagnosis	HF (61)	[20]
miR-1	↓			
miR-210	↑	Diagnosis	HF (39)	[21]
miR-126	↓	Diagnosis	AHF (236)	[22]
miR-423	↓	Diagnosis/Death	CHF (44)	
	↓	Diagnosis/Death	AHF (137)	[19]
	↑	Diagnosis	HF (30)	[23]
	↑	Diagnosis	CHF (30)	[24]
miR-1	↑	Diagnosis	HF (39)	[28]
miR-133a/b	↑			
miR-208a/b	↑			
miR-499	↑			
	↑	Diagnosis/Predict: HF and death	MI (319)	[29]
miR-18a	↓	Diagnosis/Death	AHF (137)	[19]
miR-18b	↓			
miR-223	↓			
miR-301a	↓	Predict: Death		
miR-26b	↓			
miR-27a	↓			
miR-30e	↓			
miR-106a	↓			
miR-199a	↓	Diagnosis		
miR-652	↓	Diagnosis/Death		
miR-26b	↑	Diagnosis/Predict: CRT	HF (81)	[30]
miR-145	↑			
miR-92a	↑			
miR-30e	↑			
miR-29a	↑			
miR-30c	↓	Diagnosis	HfpEF (90)	[27]
miR−146a	↓		HfrEF (90)	
miR−221	↓			
miR−328	↓			
miR−37	↓			

*HF, heart failure; CHF, chronic HF; AHF, acute HF; MI, myocardial infarction; HfpEF, heart failure with preserved ejection fraction; HfrEF, heart failure with reduced ejection fraction; CRT, cardiac resynchronization therapy.

miR-328 and miR-486, and decreases in miR-106, miR-197 and miR-223 (Table 2, Figure 2)[29, 37-55]. Temporal changes in miR-NAs related to myocardial growth, fibrosis and viability are associated with post-infarct volume LV structural remodeling[56-58]. Thus, circulating miRNAs can be employed as diagnostic biomarkers for AMI.

In animal models of AMI, serum levels of miR-1, a regulator of cardiac muscle development and differentiation, peaked 6 h post AMI and returned to basal levels after 3 days[59]. Levels of serum miR-1 were also positively associated with myocardial infarct size[52]. In post-AMI patients, miR-1 was significantly correlated with (a) the absolute change in infarct volume, (b) showed a trend for positive correlation with LV ejection frac-

tion and (c) was associated with AMI mortality[46, 49].

Similar to miR-1, miR-133 is crucial regulator of cardiac and skeletal muscle development, and involved in vascular smooth muscle cell biology[60, 61]. The relationship between miR-133 and AMI however, is not defined. It has been reported that AMI patients had a 4.4-fold higher plasma level of miR-133 that returned to basal levels after 7 days, compared to control subjects. Additionally, miR-133 was significantly correlated with all-cause mortality[37, 46]. In contrast, another study found no significant difference in miR-133 plasma levels between AMI and healthy patients[53]. Additionally, circulating miR-133 levels did not significantly change in AMI patients with bradyarrhythmia or tachyarrhythmia, and was not associated

Table 2. miRNAs in acute myocardial infarction (AMI).

miRNA ID	Change in expression	Purpose	Pathology (number of subjects)*	Reference
miR-1	↑	Diagnosis/Predict: LVEF	AMI (44)	[49]
	↑	Diagnosis	AMI (31)	[52]
	↑		STEMI (33)	[41]
	↑		ACS (444)	[46]
	↑		AMI (70)	[42]
	↑		AMI (93)	[53]
miR-21	↑	Diagnosis/Predict: LVEF	AMI (198)	[51]
	↑	Diagnosis	AMI (44)	[49]
	↑		AMI (17)	[50]
miR-29b	↑	Diagnosis	AMI (44)	[49]
miR-133	↑	Diagnosis/Death	ACS (444)	[46]
	↑	Diagnosis	STEMI (33)	[41]
	↑		AMI (51)	[37]
	↑		AMI (246)	[38]
	-		AMI (93)	[53]
miR-208	↑	Diagnosis/Predict: HF and death	AMI (359)	[48]
	↑	Diagnosis	ACS (444)	[46]
	↑		AMI (70)	[42]
	↑		STEMI (19)	[47]
miR-328	↑	Diagnosis	AMI (51)	[37]
miR-499	↑	Diagnostic/predict: AMI	CABG (30)	[45]
	↑	Diagnosis	STEMI (33)	[41]
	↑		AMI (70)	[42]
	↑		AMI (32)	[43]
	↑		UA (37)	[44]
			NSTEMI (48)	
miR-423	↑	Diagnosis	AMI (246)	[38]
	↑		STEMI (12)	[39]
	↑		AMI (17)	[40]

*AMI, acute myocardial infarction; STEMI; ST-elevation myocardial infarction; ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; UA, unstable angina; NSTEMI, non-ST elevation myocardial infarction; LVEF, left ventricular ejection fraction; HF, heart failure.

with prediction of LV ejection fraction and BNP within 1-year post-AMI[37, 38].

Acute myocardial infarction patients had significantly higher levels of plasma miR-21, compared to healthy controls. miR-21 was shown to be a novel biomarker that was predictive of LV remodeling after AMI[51]. In addition, levels of miR-21 correlated with several traditional markers of AMI; creatine kinase-MB (CK-MB), creatine kinase (CK) and cardiac troponin I (cTnI), with comparable diagnostic accuracy[49, 50].

A higher level of circulating miR-208a was observed in patients with AMI that peaked 3 h after reperfusion, compared with unstable angina-patients[47]. Elevated miR-208a was significantly associated with increased risk of mortality or HF within 6 months after the AMI[46, 48]. Although miR-208b was not independently associated with the AMI clinical outcome after adjustment for cTnT, circulating miR-208a levels strongly correlated with cTnI and CK-MB released from the infarcted area[46, 47].

Levels of plasma cardiac myocyte-associated miR-499 was highly elevated and correlated with cTnI in AMI patients, which suggests its release from injured cardiomyocytes[43].

Compared to miR-1 or miR-208, miR-499 had a more accurate predictive value that was significantly greater than the most reliable biomarkers of AMI; cTnI and CK-MB[42]. Changes in the levels of circulating miR-499 were associated with unstable angina and non-ST elevation myocardial infarction (MI) in patients presenting within 3 h of symptom onset. This supports a role for serum miR-499 as a potentially novel biomarker to accelerate the diagnosis of acute coronary syndrome patients[44]. The sensitivity and specificity of miR-499 were greater than cTnI, suggesting that miR-499 could be an independent risk factor for perioperative MI. These findings also suggest that circulating miR-499 could be an early biomarker for the identification of perioperative MI in cardiac surgery[45].

It has been reported there is a significant elevation of miR-423 at 1, 3, and 12 months after MI, compared to baseline levels[38]. The level of miR-423 in AMI patients decreased 6, 12 and 24 h following percutaneous coronary intervention. Eventually, miR-423 levels were comparable to the control group and lower than baseline levels[40]. There are no significant correlations however, between miR-423 expression and indices of LV function and remodeling; echocardiographic

parameters, levels of cTn I or BNP; at any time-point during follow-up[38-40]. miR-29b plasma levels correlate with infarct volume changes in post-AMI patients. In addition, miR-29b levels associate with the alteration of LV end-diastolic volume over time[49].

Although variations in the levels of several miRNAs are associated with AMI, the specificity of the change to AMI versus comorbidities can be problematic. Many of the miRNAs affected by AMI regulate non-cardiac protein expression. For example, the levels of muscle specific miR-1 are affected by pathologies that lead to muscle damage. Thus, it may be general marker for muscle or tissue damage, but not specific for AMI[62-65]. Among the miRNAs associated with AMI, significant evidence supports miR-208a as an AMI-specific diagnostic biomarker[66, 67]. First, miR-208a is expressed in cardiomyocytes where it regulates myosin heavy chain expression, thus it is heart specific and its level of expression is significantly affected in a majority of AMI patients[68]. miR-208 is rapidly detected in AMI patients (<4 h post-AMI)[69]. Similarly, in animal models using experimentally induced myocardial infarction, miR-208 is detected within 1 h of AMI. The level begins to decrease after 3 h and is not significantly different that controls after 24 h[69]. These observations support the use of miR-208 as a biomarker for early AMI detection, but it would be unreliable as a long term biomarker. In this situation, other miRNAs could be used such miR-499, miR-1 or miR-133.

Arrhythmia
Arrhythmia defines a group of symptoms where the heart beat changes from its normal pattern. The symptomatic pattern can be irregular (dysrhythmia), too fast (tachycardia) or too slow (bradycardia). Changes in the levels of several circulating miRNAs have been associated with arrhythmia (Table 3, Figure 2).

Atrial fibrillation (AF) is a chronic arrhythmia affecting the majority of patients with CVDs and is a precipitating risk factor for HF. Atrial fibrillation may cause ventricular arrhythmia, increase the risk of stroke or lead to death[70]. The incidence rate of AF in aging populations increases 5%–15% in people over 80[71].

Changes in the levels of several miRNAs have been linked to AF. Deregulation of miR-29; which targets mRNAs encoding fibrosis-promoting proteins; has been found to contribute to AF, via regulating the genes involved in cardiac fibrosis and apoptosis[72-74]. miR-208b upregulation was documented in cardiac tissue from human and animal AF samples[75]. The risk of post-operative AF can be predicted from elevated serum levels of miR483[76]. Circulating miR-23a and miR-26a may be involved in the underlying biology of post-operative AF development[77].

There is evidence suggesting that miRNAs may regulate atrial remodeling via controlling Ca^{2+} channel protein expression. Significant up-regulation of miR-328, a regulator of cardiac hypertrophy[78], was observed in the atrial tissues of AF patients and in animal models of AF. Sequence analysis of miR-328 identified cardiac L-type Ca^{2+} channel α1c- and β1 subunits (CACNA1C and CACNB2) as its potential targets. Over expression of miR-328 confirmed its role as an effector of AF[79, 80]. Ling and colleagues found that miR-499 was significantly higher in atrial tissues of AF patients. miR-499 may play a role in the electrical remodeling by affecting the expression of small conductance, Ca^{2+}-activated K^+ channel isoform 3[81]. Overexpression of miR-208b in HL-1 atrial myocytes or primary cardiomyocytes isolated from chronic AF patients reduced the expression and function of CACNA1C and CACNB2, and the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2)[82]. Cardiomyocytes from AF patients showed significant upregulation of miR-30d with reduced levels of CACNA1C, and K^+-inwardly-rectifying channel, subfamily J3 (KCNJ3) mRNA and protein. *In vitro*, overexpression of miR-30 downregulated expression of KCNJ3 with a concomitant reduction of the acetylcholine-sensitive inward-rectifier K^+ current, while knockdown of miR-30d enhanced KCNJ3 expression[83].

Both clinical and animal studies have shown increase in levels of miR-21 in atria, leading to structural remodeling of the atrial myocardium[84]. In cardiac fibroblasts, from a transgenic mouse model of cardiac dysfunction[85], miR-21 levels selectively increase. miR-21 affects the activity of the extracellular signal-regulated/mitogen-activated protein kinase pathway, which ultimately affects cardiac structure and function, growth factor secretion and fibroblast survival to control the extent of interstitial fibrosis and cardiac hypertrophy[86].

Table 3. miRNAs in arrhythmia.

miRNA ID	Change in expression	Purpose	Pathology (number of subjects)*	Reference
miR-1	↓	Diagnosis: SVT	TACH (24)	[38]
miR-133	↑	Diagnosis: VT	TACH (24)	[39]
miR-328	↑	Diagnosis: AF		[80]
	↑	Diagnosis: AF	AF (122)	[79]
miR-23a	↓	Diagnosis: POAF	POAF (24)	[77]
miR-26a	↓	Diagnosis: POAF	POAF (24)	[77]
miR-483	↑	Predict: POAF	CABG (34)	[76]
miR-150	↓	Diagnosis: AF	HF (41)	[87]

*SVT, supraventricular tachycardia; VT, ventricular tachycardia; AF, atrial fibrillation; POAF, postoperative atrial fibrillation; TACH, Tachycardia; CABG, coronary artery bypass grafting; HF, heart failure.

Analysis of serum from AF patients identified a 3.2-fold decrease in the level of miR-150 in platelets and a 1.5-fold decrease in serum, compared cardiac disease-free controls[87]. It is possible that lower platelet miR-150 may be associated with many of the mechanisms pathways related with the development of AF, such as inflammation, platelet function and fibrosis[87, 88].

In pediatric patients with arrhythmias, paroxysmal or persistent tachycardia is common, and can lead to cardiac remodeling and HF. The incorporation of miR-1 into diagnostic models was able to increase the sensitivity and specificity in the prediction of supraventricular tachycardia. miR-133 was a biomarker of greater accuracy in evaluating ventricular tachycardia. While patients with supraventricular tachycardia showed low miR-1 level, those with ventricular tachycardia had higher miR-133 levels. Elevated miR-1 had been documented in AMI rat models and was generally associated with ischemic arrhythmia[89].

Pulmonary hypertension

Pulmonary arterial hypertension is characterized by an increase in the resistive and reactive components of pulmonary vascular impedance, which ultimately leads to right ventricular failure[90, 91]. Over 30 circulating miRNAs have been associated with the development and progression of pulmonary arterial hypertension[92]. The levels of many of these miRNAs correlate with pulmonary arterial dispensability and pulmonary vascular resistance index, and decrease response to oxygen and/or inhaled nitric oxide.

miRNAs as therapeutic targets for cardiovascular diseases

Changes in the circulating serum levels of many miRNAs are associated with several CVDs, suggesting that they may be potential therapeutic targets. In recent years, numerous miRNA mimics and anti-miRs; synthetic oligonucleotides that block miRNA function; have been evaluated in animal models for the treatment of various CVDs by targeting different aspects of cardiac pathology; apoptosis and autophagy or hypertrophy (Figure 3)[93-95].

In older animals, miR-22 is elevated and suppresses cardiac autophagy. Consequently, administration of miR-22 anti-miRs activates cardiac autophagy to prevent post-infarction remodeling and improve cardiac function in older mice[2]. miR-99a targets the mTOR/p70 ribosomal protein S6 kinase signaling pathway to prevent apoptosis and increase autophagy. Overexpression of miR-99a in a murine model of MI improved both cardiac function and survival by increasing these activities. In addition, overexpression of miR-99a ameliorated hypoxia-mediated apoptosis to improve cardiac function in ischemic heart of mice undergoing MI. Intra-myocardial injection of mice with miR-99a improved LV function and survival 4 weeks after the MI[96]. Similarly, adenovirus-delivered miR-214 or miR-21 improved LV remodeling and decreased myocardial apoptosis in a rat model of AMI or ischemia-reperfusion injury, respectively[97, 98]. In a rat model of myocardial ischemia/reperfusion injury, the levels of miR-320 significantly increase. The administration of miR-320 anti-miRs reduced the degree of myocardial fibrosis and apoptosis in LV remodeling[99].

The magnitude of cardiac hypertrophy and autophagy in cardiomyocytes is regulated by the miR-212/132 family, which targets the anti-hypertrophic and pro-autophagic FoxO3 transcription factor. While hypertrophic stimuli increase the levels of miR-212 and miR-132 expression, inhibition of miR-132 with anti-miRs rescues cardiac hypertrophy and HF in mice[100]. In a mouse model of hypertrophy and cardiac dysfunction, inhibition of Jagged1/Notch signaling by the administration of a locked nucleic acid anti-miR-652 resulted in attenuation of cardiac hypertrophy. Improved heart function was associated with reduced cardiac fibrosis[101]. These studies suggest that anti-miR-212, anti-miR-132 or anti-miR-652 may be promising agents for the treatment of pathological remodeling during HF and could be used as part of cardiac failure therapies.

miRNA-based treatments have also shown positive results in adult porcine models of CVD. In a model of reperfused AMI, a single intracoronary administration of encapsulated anti-miR-92a prevented LV remodeling without adverse effects[102]. Regional delivery of locked nucleic acid-modified anti-miR-92a reduced infarct size and post-ischemic loss of
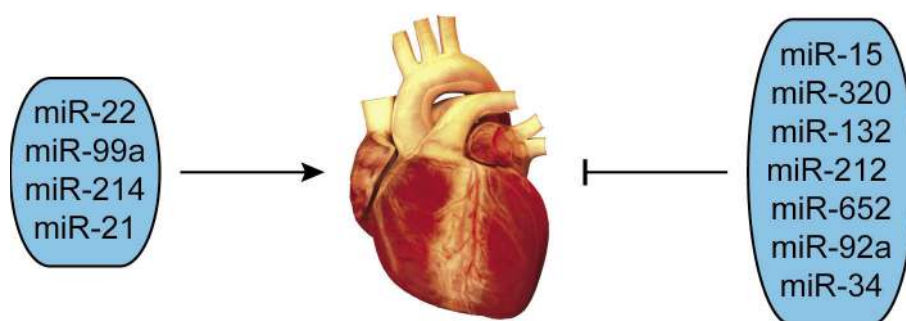

Figure 3. miRNAs as therapeutic targets for cardiovascular diseases. Increased expression (arrow head) or decreased expression (bar-head) provides beneficial or protective effects in the treatment of CVD.

function in a model of percutaneous ischemia/reperfusion[103]. Similarly, systemic delivery of locked nucleic acid modified anti-miR-15 effectively rendered cardiomyocytes resistant to hypoxia-induced cardiomyocyte cell death[104]. Combined these studies suggest that miRNA-based therapies using modified oligonucleotides are promising therapeutic agents for patients who suffer a large AMI or to affect cardiac remodeling and preserve cardiac function after ischemic injury.

Although promising, several limitations of miRNA-based therapeutics have been identified[105]. Recent data have shown some limitations of miRNAs-based therapies for cardiovascular diseases. For example, anti-miR-34a has shown sex preference, where it is more effective in females with moderate dilated cardiomyopathy than in males[106]. This suggests that there needs to be a better understanding of the mechanism of any miRNA-based therapy for cardiac disease before it can be moved into the clinic. Inhibition of the miR-34 family attenuates cardiac remodeling and improves heart function following pressure overload, a model chronic MI[107]. Administration of a single miRNA isoform, anti-miR-34a however, did not significantly affect cardiac fibrosis in mice with moderate cardiac pathology. It did however, attenuate atrial enlargement and maintained cardiac function[108]. These results suggest that inhibition of miR-34a based therapies may have limited potential.

Challenges

miRNAs regulate many biological processes and their levels of expression are affected in many human diseases. There is little doubt that miRNAs are critical regulators of cardiovascular function and play important roles in many aspects of cardiovascular biology. The roles of miRNAs in cancer have been extensively investigated and numerous miRNAs are established biomarkers for the diagnosis and prognosis of various types of cancers[109, 110]. Additionally, many miRNAs are under human clinic trials for the treatment of cancer[111, 112]. Although our knowledge of the roles of miRNAs in the biology of CVD has greatly improved, additional research is needed to identify and validate miRNAs as biomarkers for the diagnosis and prognosis of this class of diseases.

Several challenges need to be addressed to identify miRNAs that can be reliably used as diagnostic markers or therapeutic targets for CVD. As discussed above, there can be conflicting observation regarding changes in miRNA levels with specific cardiac pathologies. These differences can be attributed to potential differences in sample/patient number (i.e., low *n* values), sampling time, methods for miRNA quantification, miRNA normalization parameters and co-morbidities[113, 114]. As additional clinical studies are performed, the issue of sample number can be resolved by combining the results from multiple studies via meta analyses, but only if standard protocols for material acquisition and storage, and miRNA isolation and quantification are established. The establishment of standard operating procedures for miRNA quantification has been shown to reduce experimental variability. To better identify miRNAs linked to CVD it may be necessary to acquire

data for multiple time points after symptom onset and detailed medical histories, which could provide essential information on potential confounders[114]. Several methods have been developed to quantify circulating miRNAs: qRT-PCR, droplet digital PCR, quantitative stem-loop RT-PCR) and chip-based digital PCR, as well as RNAseq and microarrays[115-118]. However, the establishment of a common set of normalization miRNAs, statistical tools for analyses, internal spike miRNA controls, as well as primer sets and amplification conditions will also be necessary to reduce inter-study variability.

Changes in the levels of dozens of circulating miRNAs have been associated with several cardiac pathologies, however; there are limited numbers that can currently be used as CVD diagnostic markers. This limitation is a consequence of miRNA expression being affected by non-cardiac conditions (*eg*, cancer, infection, drug use, *etc*) and a lack of mechanistic association to the heart. As discussed above, several miRNAs show promise as markers based on their association with specific or several CVD. For a miRNA to be considered a diagnostic marker of CVD or a potential therapeutic target however, it should be predominantly expressed in cardiac tissue and/or essential for heart development, function, or repair of heart-specific damage, for example, miR-1, miR133a, miR-208a/b, and miR-499[119, 120].

Although promising, several limitations of miRNA-based therapeutics have been identified. Thus, how to avoid these limitations will be a major challenge in applying miRNAs for diagnostic and therapeutic purposes. Therefore, there is an urgent need for further development of miRNAs for the therapy of CVD in both animal models and human clinic trials for the treatment of CVD.

Acknowledgments

References
1   Elia L, Contu R, Quintavalle M, Varrone F, Chimenti C, Russo MA, *et al*. Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation 2009; 120: 2377–85.

2   Gupta SK, Foinquinos A, Thum S, Remke J, Zimmer K, Bauters C, *et al*. Preclinical development of a microRNA-based therapy for elderly patients with myocardial infarction. J Am Coll Cardiol 2016; 68: 1557–71.

3   Marques FZ, Campain AE, Tomaszewski M, Zukowska-Szczechowska E, Yang YH, Charchar FJ, *et al*. Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs. Hypertension 2011; 58: 1093–8.

4   Barwari T, Joshi A, Mayr M. MicroRNAs in cardiovascular disease. J Am Coll Cardiol 2016; 68: 2577–84.

5   Lee RC, Feinbaum RL, Ambros V. The c-elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843–54.

6   Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of

the heterochronic gene lin-14 by lin-4 mediates temporal pattern-formation in c-elegans. Cell 1993; 75: 855–62.

7 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–97.

8 Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15–20.

9 Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, *et al*. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 2005; 122: 553–63.

10 Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003; 115: 787–98.

11 Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, *et al*. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008; 18: 997–1006.

12 Tsui NB, Ng EK, Lo YM. Stability of endogenous and added RNA in blood specimens, serum, and plasma. Clin Chem 2002; 48: 1647–53.

13 Wang K, Zhang S, Marzolf B, Troisch P, Brightman A, Hu Z, *et al*. Circulating microRNAs, potential biomarkers for drug-induced liver injury. Proc Natl Acad Sci U S A 2009; 106: 4402–7.

14 Zampetaki A, Willeit P, Drozdov I, Kiechl S, Mayr M. Profiling of circulating microRNAs: from single biomarkers to re-wired networks. Cardiovasc Res 2012; 93: 555–62.

15 Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem 2010; 285: 17442–52.

16 Leonard BL, Smaill BH, LeGrice IJ. Structural remodeling and mechanical function in heart failure. Microsc Microanal 2012; 18: 50–67.

17 Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, *et al*. Executive summary: Heart disease and stroke statistics--2012 update: A report from the american heart association. Circulation 2012; 125: 188–97.

18 Schulte C, Westermann D, Blankenberg S, Zeller T. Diagnostic and prognostic value of circulating microRNAs in heart failure with preserved and reduced ejection fraction. World J Cardiol 2015; 7: 843–60.

19 Ovchinnikova ES, Schmitter D, Vegter EL, Ter Maaten JM, Valente MA, Liu LC, *et al*. Signature of circulating microRNAs in patients with acute heart failure. Eur J Heart Fail 2016; 18: 414–23.

20 Sygitowicz G, Tomaniak M, Blaszczyk O, Koltowski L, Filipiak KJ, Sitkiewicz D. Circulating microribonucleic acids mir-1, mir-21 and mir-208a in patients with symptomatic heart failure: preliminary results. Arch Cardiovasc Dis 2015; 108: 634–42.

21 Endo K, Naito Y, Ji X, Nakanishi M, Noguchi T, Goto Y, *et al*. MicroRNA 210 as a biomarker for congestive heart failure. Biol Pharm Bull 2013; 36: 48–54.

22 Seronde MF, Vausort M, Gayat E, Goretti E, Ng LL, Squire IB, *et al*. Circulating microRNAs and outcome in patients with acute heart failure. PLoS One 2015; 10: e0142237.

23 Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, *et al*. Mir423-5p as a circulating biomarker for heart failure. Circ Res 2010; 106: 1035–9.

24 Goren Y, Kushnir M, Zafrir B, Tabak S, Lewis BS, Amir O. Serum levels of microRNAs in patients with heart failure. Eur J Heart Fail 2012; 14: 147–54.

25 Bayes-Genis A, Lanfear DE, de Ronde MWJ, Lupon J, Leenders JJ, Liu Z, *et al*. Prognostic value of circulating microRNAs on heart failure-related morbidity and mortality in two large diverse cohorts of general heart failure patients. Eur J Heart Fail 2018; 20: 67–75.

26 van Boven N, Kardys I, van Vark LC, Akkerhuis KM, de Ronde MWJ, Khan MAF, *et al*. Serially measured circulating microRNAs and adverse clinical outcomes in patients with acute heart failure. Eur J Heart Fail 2018; 20: 89–96.

27 Watson CJ, Gupta SK, O'Connell E, Thum S, Glezeva N, Fendrich J, *et al*. MicroRNA signatures differentiate preserved from reduced ejection fraction heart failure. Eur J Heart Fail 2015; 17: 405–15.

28 Akat KM, Moore-McGriff D, Morozov P, Brown M, Gogakos T, Correa Da Rosa J, *et al*. Comparative RNA-sequencing analysis of myocardial and circulating small RNAs in human heart failure and their utility as biomarkers. Proc Natl Acad Sci U S A 2014; 111: 11151–6.

29 Gidlof O, Smith JG, Miyazu K, Gilje P, Spencer A, Blomquist S, *et al*. Circulating cardio-enriched microRNAs are associated with long-term prognosis following myocardial infarction. BMC Cardiovasc Disord 2013; 13: 12.

30 Marfella R, Di Filippo C, Potenza N, Sardu C, Rizzo MR, Siniscalchi M, *et al*. Circulating microRNA changes in heart failure patients treated with cardiac resynchronization therapy: responders vs non-responders. Eur J Heart Fail 2013; 15: 1277–88.

31 Xiao J, Gao R, Bei Y, Zhou Q, Zhou Y, Zhang H, *et al*. Circulating mir-30d predicts survival in patients with acute heart failure. Cell Physiol Biochem 2017; 41: 865–74.

32 Melman YF, Shah R, Danielson K, Xiao J, Simonson B, Barth A, *et al*. Circulating microRNA-30d is associated with response to cardiac resynchronization therapy in heart failure and regulates cardiomyocyte apoptosis: A translational pilot study. Circulation 2015; 131: 2202–16.

33 Fox CS, Coady S, Sorlie PD, D'Agostino RB, Pencina MJ, Vasan RS, *et al*. Increasing cardiovascular disease burden due to diabetes mellitus - the framingham heart study. Circulation 2007; 115: 1544–50.

34 Sun Y. Myocardial repair/remodelling following infarction: Roles of local factors. Cardiovasc Res 2009; 81: 482–90.

35 Takemura G, Nakagawa M, Kanamori H, Minatoguchi S, Fujiwara H. Benefits of reperfusion beyond infarct size limitation. Cardiovasc Res 2009; 83: 269–76.

36 Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, *et al*. Role of apoptosis in reperfusion injury. Cardiovasc Res 2004; 61: 414–26.

37 Wang R, Li N, Zhang Y, Ran Y, Pu J. Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. Intern Med 2011; 50: 1789–95.

38 Bauters C, Kumarswamy R, Holzmann A, Bretthauer J, Anker SD, Pinet F, *et al*. Circulating mir-133a and mir-423-5p fail as biomarkers for left ventricular remodeling after myocardial infarction. Int J Cardiol 2013; 168: 1837–40.

39 Eryilmaz U, Akgullu C, Beser N, Yildiz O, Kurt Omurlu I, Bozdogan B. Circulating microRNAs in patients with ST-elevation myocardial infarction. Anatol J Cardiol 2016; 16: 392–6.

40 Nabialek E, Wanha W, Kula D, Jadczyk T, Krajewska M, Kowalowka A, *et al*. Circulating microRNAs (mir-423-5p, mir-208a and mir-1) in acute myocardial infarction and stable coronary heart disease. Minerva Cardioangiol 2013; 61: 627–37.

41 D'Alessandra Y, Devanna P, Limana F, Straino S, Di Carlo A, Brambilla PG, *et al*. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur Heart J 2010; 31: 2765–73.

42 Liu X, Fan Z, Zhao T, Cao W, Zhang L, Li H, *et al*. Plasma mir-1, mir-208, mir-499 as potential predictive biomarkers for acute myocardial infarction: an independent study of Han population. Exp Gerontol 2015; 72: 230–8.

43 Corsten MF, Dennert R, Jochems S, Kuznetsova T, Devaux Y, Hofstra

www.nature.com/aps
1082
Zhou SS *et al*

L, *et al*. Circulating microRNA-208b and microRNA-499 reflect myocardial damage in cardiovascular disease. Circ Cardiovasc Genet 2010; 3: 499–506.

44 Shalaby SM, El-Shal AS, Shoukry A, Khedr MH, Abdelraheim N. Serum miRNA-499 and miRNA-210: a potential role in early diagnosis of acute coronary syndrome. IUBMB Life 2016; 68: 673–82.

45 Yao Y, Du J, Cao X, Wang Y, Huang Y, Hu S, *et al*. Plasma levels of microRNA-499 provide an early indication of perioperative myocardial infarction in coronary artery bypass graft patients. PLoS One 2014; 9: e104618.

46 Widera C, Gupta SK, Lorenzen JM, Bang C, Bauersachs J, Bethmann K, *et al*. Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. J Mol Cell Cardiol 2011; 51: 872–5.

47 Bialek S, Gorko D, Zajkowska A, Koltowski L, Grabowski M, Stachurska A, *et al*. Release kinetics of circulating mirRNA-208a in the early phase of myocardial infarction. Kardiol Pol 2015; 73: 613–9.

48 Lv P, Zhou M, He J, Meng W, Ma X, Dong S, *et al*. Circulating mir-208b and mir-34a are associated with left ventricular remodeling after acute myocardial infarction. Int J Mol Sci 2014; 15: 5774–88.

49 Grabmaier U, Clauss S, Gross L, Klier I, Franz WM, Steinbeck G, *et al*. Diagnostic and prognostic value of mir-1 and mir-29b on adverse ventricular remodeling after acute myocardial infarction–the sitagrami-mir analysis. Int J Cardiol 2017; 244: 30–36.

50 Zhang Y, Liu YJ, Liu T, Zhang H, Yang SJ. Plasma microRNA-21 is a potential diagnostic biomarker of acute myocardial infarction. Eur Rev Med Pharmacol Sci 2016; 20: 323–9.

51 Liu X, Dong Y, Chen S, Zhang G, Zhang M, Gong Y, *et al*. Circulating microRNA-146a and microRNA-21 predict left ventricular remodeling after ST-elevation myocardial infarction. Cardiology 2015; 132: 233–41.

52 Cheng Y, Tan N, Yang J, Liu X, Cao X, He P, *et al*. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin Sci (Lond) 2010; 119: 87–95.

53 Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, *et al*. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem Biophys Res Commun 2010; 391: 73–7.

54 Danowski N, Manthey I, Jakob HG, Siffert W, Peters J, Frey UH. Decreased expression of mir-133a but not of mir-1 is associated with signs of heart failure in patients undergoing coronary bypass surgery. Cardiology 2013; 125: 125–30.

55 Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, *et al*. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. Circ Res 2013; 113: 322–6.

56 Zile MR, Mehurg SM, Arroyo JE, Stroud RE, DeSantis SM, Spinale FG. Relationship between the temporal profile of plasma microRNA and left ventricular remodeling in patients after myocardial infarction. Circ Cardiovasc Genet 2011; 4: 614–19.

57 Pan Z, Sun X, Shan H, Wang N, Wang J, Ren J, *et al*. MicroRNA-101 inhibited postinfarct cardiac fibrosis and improved left ventricular compliance via the FBJ osteosarcoma oncogene/transforming growth factor-beta1 pathway. Circulation 2012; 126: 840–50.

58 van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, *et al*. Dysregulation of microRNAs after myocardial infarction reveals a role of mir-29 in cardiac fibrosis. Proc Natl Acad Sci U S A 2008; 105: 13027–32.

59 Townley-Tilson WH, Callis TE, Wang D. MicroRNAs 1, 133, and 206: Critical factors of skeletal and cardiac muscle development, function, and disease. Int J Biochem Cell Biol 2010; 42: 1252–5.

60 Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM,

et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 2006; 38: 228–33.

61 Kondkar AA, Abu-Amero KK. Utility of circulating microRNAs as clinical biomarkers for cardiovascular diseases. Biomed Res Int 2015; 2015: 821823.

62 Han C, Shen JK, Hornicek FJ, Kan Q, Duan Z. Regulation of microRNA-1 (mir-1) expression in human cancer. Biochim Biophys Acta 2017; 1860: 227–32.

63 Parkes JE, Day PJ, Chinoy H, Lamb JA. The role of microRNAs in the idiopathic inflammatory myopathies. Curr Opin Rheumatol 2015; 27: 608–15.

64 Coenen-Stass AML, Wood MJA, Roberts TC. Biomarker potential of extracellular miRNAs in duchenne muscular dystrophy. Trends Mol Med 2017; 23: 989–1001.

65 Horak M, Novak J, Bienertova-Vasku J. Muscle-specific microRNAs in skeletal muscle development. Dev Biol 2016; 410: 1–13.

66 Creemers EE, Tijsen AJ, Pinto YM. Circulating microRNAs: Novel biomarkers and extracellular communicators in cardiovascular disease? Circ Res 2012; 110: 483–95.

67 Sun T, Dong YH, Du W, Shi CY, Wang K, Tariq MA, *et al*. The role of microRNAs in myocardial infarction: from molecular mechanism to clinical application. Int J Mol Sci 2017; 18. pii: E745. doi: 10.3390/ijms18040745.

68 Malizia AP, Wang DZ. MicroRNAs in cardiomyocyte development. Wiley Interdiscip Rev Syst Biol Med 2011; 3: 183–90.

69 Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, *et al*. Circulating microRNA: A novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur Heart J 2010; 31: 659–66.

70 Sistino JJ. Epidemiology of cardiovascular disease in the last decade: Treatment options and implications for perfusion in the 21st century. Perfusion 2003; 18: 73–7.

71 den Hoed M, Eijgelsheim M, Esko T, Brundel BJ, Peal DS, Evans DM, *et al*. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat Genet 2013; 45: 621–31.

72 Dawson K, Wakili R, Ordog B, Clauss S, Chen Y, Iwasaki Y, *et al*. MicroRNA29: A mechanistic contributor and potential biomarker in atrial fibrillation. Circulation 2013; 127: 1466–75, 75e1–28.

73 Hale CS, Levis WR. MicroRNA-29 and an integrated understanding of atrial fibrillation. J Drugs Dermatol 2013; 12: 1083.

74 Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, *et al*. Mir-29 is a major regulator of genes associated with pulmonary fibrosis. Am J Respir Cell Mol Biol 2011; 45: 287–94.

75 Oliveira-Carvalho V, Carvalho VO, Bocchi EA. The emerging role of mir-208a in the heart. DNA Cell Biol 2013; 32: 8–12.

76 Harling L, Lambert J, Ashrafian H, Darzi A, Gooderham NJ, Athanasiou T. Elevated serum microRNA 483-5p levels may predict patients at risk of post-operative atrial fibrillation. Eur J Cardiothorac Surg 2017; 51: 73–78.

77 Feldman A, Moreira DAR, Gun C, Wang HL, Hirata MH, de Freitas Germano J, *et al*. Analysis of circulating mir-1, mir-23a, and mir-26a in atrial fibrillation patients undergoing coronary bypass artery grafting surgery. Ann Hum Genet 2017; 81: 99–105.

78 Li C, Li X, Gao X, Zhang R, Zhang Y, Liang H, *et al*. MicroRNA-328 as a regulator of cardiac hypertrophy. Int J Cardiol 2014; 173: 268–76.

79 Lu Y, Hou S, Huang D, Luo X, Zhang J, Chen J, *et al*. Expression profile analysis of circulating microRNAs and their effects on ion channels in Chinese atrial fibrillation patients. Int J Clin Exp Med 2015; 8: 845–53.

80 Lu Y, Zhang Y, Wang N, Pan Z, Gao X, Zhang F, *et al*. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation.

www.chinaphar.com
Zhou SS *et al*
1083

Circulation 2010; 122: 2378–87.

81 Ling TY, Wang XL, Chai Q, Lau TW, Koestler CM, Park SJ, *et al*. Regulation of the sk3 channel by microRNA-499--potential role in atrial fibrillation. Heart Rhythm 2013; 10: 1001–9.

82 Canon S, Caballero R, Herraiz-Martinez A, Perez-Hernandez M, Lopez B, Atienza F, *et al*. Mir-208b upregulation interferes with calcium handling in hl-1 atrial myocytes: Implications in human chronic atrial fibrillation. J Mol Cell Cardiol 2016; 99: 162–73.

83 Morishima M, Iwata E, Nakada C, Tsukamoto Y, Takanari H, Miyamoto S, *et al*. Atrial fibrillation-mediated upregulation of mir-30d regulates myocardial electrical remodeling of the G-protein-gated K$^+$ channel, $I_{K.Ach}$. Circ J 2016; 80: 1346–55.

84 Adam O, Lohfelm B, Thum T, Gupta SK, Puhl SL, Schafers HJ, *et al*. Role of mir-21 in the pathogenesis of atrial fibrosis. Basic Res Cardiol 2012; 107: 278.

85 Engelhardt S, Hein L, Wiesmann F, Lohse MJ. Progressive hypertrophy and heart failure in beta1-adrenergic receptor transgenic mice. Proc Natl Acad Sci U S A 1999; 96: 7059–64.

86 Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, *et al*. MicroRNA-21 contributes to myocardial disease by stimulating map kinase signalling in fibroblasts. Nature 2008; 456: 980–4.

87 Goren Y, Meiri E, Hogan C, Mitchell H, Lebanony D, Salman N, *et al*. Relation of reduced expression of mir-150 in platelets to atrial fibrillation in patients with chronic systolic heart failure. Am J Cardiol 2014; 113: 976–81.

88 Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. Physiol Rev 2011; 91: 265–325.

89 Lu Y, Zhang Y, Shan H, Pan Z, Li X, Li B, *et al*. MicroRNA-1 downregulation by propranolol in a rat model of myocardial infarction: A new mechanism for ischaemic cardioprotection. Cardiovasc Res 2009; 84: 434–41.

90 O'Rourke MF. Vascular impedance in studies of arterial and cardiac function. Physiol Rev 1982; 62: 570–623.

91 Wang Z, Chesler NC. Pulmonary vascular wall stiffness: An important contributor to the increased right ventricular afterload with pulmonary hypertension. Pulm Circ 2011; 1: 212–23.

92 Sucharov CC, Sucharov J, Karimpour-Fard A, Nunley K, Stauffer BL, Miyamoto SD. Micro-RNA expression in hypoplastic left heart syndrome. J Card Fail 2015; 21: 83–8.

93 Bernardo BC, Ooi JY, Lin RC, McMullen JR. MiRNA therapeutics: A new class of drugs with potential therapeutic applications in the heart. Future Med Chem 2015; 7: 1771–92.

94 Nouraee N, Mowla SJ. MiRNA therapeutics in cardiovascular diseases: Promises and problems. Front Genet 2015; 6: 232.

95 Poller W, Dimmeler S, Heymans S, Zeller T, Haas J, Karakas M, *et al*. Non-coding RNAs in cardiovascular diseases: Diagnostic and therapeutic perspectives. Eur Heart J 2017. doi: 10.1093/eurheartj/ehx165.

96 Li Q, Xie J, Li R, Shi J, Sun J, Gu R, *et al*. Overexpression of microRNA-99a attenuates heart remodelling and improves cardiac performance after myocardial infarction. J Cell Mol Med 2014; 18: 919–28.

97 Qin Y, Yu Y, Dong H, Bian X, Guo X, Dong S. MicroRNA 21 inhibits left ventricular remodeling in the early phase of rat model with ischemia-reperfusion injury by suppressing cell apoptosis. Int J Med Sci 2012; 9: 413–23.

98 Yang X, Qin Y, Shao S, Yu Y, Zhang C, Dong H, *et al*. MicroRNA-214 inhibits left ventricular remodeling in an acute myocardial infarction rat model by suppressing cellular apoptosis via the phosphatase and tensin homolog (PTEN). Int Heart J 2016; 57: 247–50.

99 Song CL, Liu B, Diao HY, Shi YF, Li YX, Zhang JC, *et al*. The protective effect of microRNA-320 on left ventricular remodeling after myocardial ischemia-reperfusion injury in the rat model. Int J Mol Sci 2014; 15: 17442–56.

100 Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, *et al*. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. Nat Commun 2012; 3: 1078.

101 Bernardo BC, Nguyen SS, Winbanks CE, Gao XM, Boey EJ, Tham YK, *et al*. Therapeutic silencing of mir-652 restores heart function and attenuates adverse remodeling in a setting of established pathological hypertrophy. FASEB J 2014; 28: 5097–110.

102 Bellera N, Barba I, Rodriguez-Sinovas A, Ferret E, Asin MA, Gonzalez-Alujas MT, *et al*. Single intracoronary injection of encapsulated antagomir-92a promotes angiogenesis and prevents adverse infarct remodeling. J Am Heart Assoc 2014; 3: e000946.

103 Hinkel R, Penzkofer D, Zuhlke S, Fischer A, Husada W, Xu QF, *et al*. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. Circulation 2013; 128: 1066–75.

104 Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM, *et al*. Inhibition of mir-15 protects against cardiac ischemic injury. Circ Res 2012; 110: 71–81.

105 Chen Y, Zhao H, Tan Z, Zhang C, Fu X. Bottleneck limitations for microRNA-based therapeutics from bench to the bedside. Pharmazie 2015; 70: 147–54.

106 Bernardo BC, Ooi JY, Matsumoto A, Tham YK, Singla S, Kiriazis H, *et al*. Sex differences in response to miRNA-34a therapy in mouse models of cardiac disease: Identification of sex-, disease- and treatment-regulated miRNAs. J Physiol 2016; 594: 5959–74.

107 Bernardo BC, Gao XM, Winbanks CE, Boey EJ, Tham YK, Kiriazis H, *et al*. Therapeutic inhibition of the mir-34 family attenuates pathological cardiac remodeling and improves heart function. Proc Natl Acad Sci U S A 2012; 109: 17615–20.

108 Bernardo BC, Gao XM, Tham YK, Kiriazis H, Winbanks CE, Ooi JY, *et al*. Silencing of mir-34a attenuates cardiac dysfunction in a setting of moderate, but not severe, hypertrophic cardiomyopathy. PLoS One 2014; 9: e90337.

109 Drusco A, Croce CM. MicroRNAs and cancer: A long story for short RNAs. Adv Cancer Res 2017; 135: 1–24.

110 Lujambio A, Lowe SW. The microcosmos of cancer. Nature 2012; 482: 347–55.

111 Tyagi N, Arora S, Deshmukh SK, Singh S, Marimuthu S, Singh AP. Exploiting nanotechnology for the development of microRNA-based cancer therapeutics. J Biomed Nanotechnol 2016; 12: 28–42.

112 Catela Ivkovic T, Voss G, Cornella H, Ceder Y. MicroRNAs as cancer therapeutics: a step closer to clinical application. Cancer Lett 2017; 407: 113–22.

113 Tijsen AJ, Pinto YM, Creemers EE. Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases. Am J Physiol Heart Circ Physiol 2012; 303: H1085–95.

114 Navickas R, Gal D, Laucevicius A, Taparauskaite A, Zdanyte M, Holvoet P. Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systematic review. Cardiovasc Res 2016; 111: 322–37.

115 de Planell-Saguer M, Rodicio MC. Detection methods for microRNAs in clinic practice. Clin Biochem 2013; 46: 869–78.

116 Robinson S, Follo M, Haenel D, Mauler M, Stallmann D, Heger LA, *et al*. Chip-based digital pcr as a novel detection method for quantifying microRNAs in acute myocardial infarction patients. Acta Pharmacol Sin 2018; 39: 1217–27.

117 Sourvinou IS, Markou A, Lianidou ES. Quantification of circulating miRNAs in plasma: effect of preanalytical and analytical parameters on their isolation and stability. J Mol Diagn 2013; 15: 827–34.

118 Miotto E, Saccenti E, Lupini L, Callegari E, Negrini M, Ferracin M. Quantification of circulating miRNAs by droplet digital pcr: Comparison of evagreen- and taqman-based chemistries. Cancer Epidemiol Biomarkers Prev 2014; 23: 2638–42.

119 Yu P, Wang H, Xie Y, Zhou J, Yao J, Che L. Deregulated cardiac specific microRNAs in postnatal heart growth. Biomed Res Int 2016; 2016: 6241763.

120 Chistiakov DA, Orekhov AN, Bobryshev YV. Cardiac-specific miRNA in cardiogenesis, heart function, and cardiac pathology (with focus on myocardial infarction). J Mol Cell Cardiol 2016; 94: 107–21.