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REVIEW

Advances in exploring the role of microRNAs in the pathogenesis, diagnosis and therapy of cardiac diseases in China

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Cardiovascular disease has become the most serious health threat and represents the major cause of morbidity and mortality in China, as in other industrialized nations. During the past few decades, China's economic boom has tremendously improved people's standard of living but has also changed their lifestyle, increasing the prevalence of cardiovascular disease, the so-called 'disease of modern civilization'. This new trend has attracted a significant amount of research. Many of the studies conducted by Chinese investigators are orientated towards understanding the molecular mechanisms of cardiovascular disease. At the molecular level, the long-standing consensus is that cardiovascular disease is associated with a sequence mutation (genetic anomaly) and expression deregulation (epigenetic disorder) of protein-coding genes. However, new research data have established the non-protein-coding genes microRNAs (miRNAs) as a central regulator of the pathogenesis of cardiac disease and a potential new therapeutic target for cardiovascular disease. These small non-coding RNAs have also been subjected to extensive, rigorous investigations by Chinese researchers. Over the years, a large body of studies on miRNAs in cardiovascular disease has been conducted by Chinese investigators, yielding fruitful research results and a better understanding of miRNAs as a new level of molecular mechanisms for the pathogenesis of cardiac disease. In this review, we briefly summarize the current status of research in the field of miRNAs and cardiovascular disease in China, highlighting the advances made in elucidating the role of miRNAs in various cardiac conditions, including cardiac arrhythmia, myocardial ischaemia, cardiac hypertrophy and heart failure. We have also examined the potential of miRNAs as novel diagnostic biomarkers and therapeutic targets.

LINKED ARTICLES

This article is part of a themed section on Chinese Innovation in Cardiovascular Drug Discovery. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-23

Abbreviations

AF, atrial fibrillation; AMI, acute myocardial infarction; APD, action potential duration; Cx43, connexin 43 (also known as GJA1, gap junction protein, α1); Drp1, dynamin-related protein-1; E-C, excitation-contraction; HSP60, heat shock protein 60; I_{CaL}, L type calcium current; JP2, junctophilin-2; *KCNJ2*, gene for potassium inwardly rectifying channel, subfamily J, member 2 also known as $K_{ir}2.1$, inwardly rectifying potassium channel 2.1; LNA, locked nucleic acid; MI, myocardial infarct; miRNAs, microRNAs; NFAT, nuclear factor of activated T-cells; SERCA2a, sarcoplasmic reticulum calcium ATPase; $K_{Ca}2.3$ (also known as SK3) small conductance calcium-activated potassium channels 3; TGF-β1, transforming growth factor-β1; TGFBR2, transforming growth factor-β receptor II

Tables of Links

TARGETS		
GPCRs[a]	**Ion channels**[c]	**Enzymes**[e]
β-adrenoceptors	Connexin 43 (Cx43)	Caspase 8
Ligand-gated ion channels[b]	$Ca_v1.2$	Furin
Ryanodine receptor	$Ca_v1.3$	PKA
Catalytic receptors[d]	$K_{Ca}2.1$	PKCε
CCK4 (serum response factor)	$K_{Ca}2.3$ (SK3)	SERCA2
TGFBR2	$K_{ir}2.1$	

LIGANDS	
Aldosterone	HSP60
Angiotensin II	Isoprenaline
cAMP	Propranolol
H_2O_2	TGF-β1

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d,e]Alexander *et al.*, 2013a,b,c,d,e).

Introduction

In China, cardiovascular disease has become the most prevalent disease and the major cause of morbidity that accounts for 40% of total death. Currently, the total number of patients with cardiovascular diseases, including coronary disease, stroke, heart failure and hypertension, is approximately 290 million (Research Center for Cardiovascular Diseases, 2013), and this uptrend is expected to continue in the future. The reality is that cardiovascular disease has become a major challenge to both clinicians and fundamentalists, and the main social economic burden in China. One of the causes of the situation is our current lack of thorough understanding of the molecular mechanisms of cardiovascular disease, which hampers optimal or even satisfactory therapeutic outcomes. There remains an urgent need for a better understanding of the molecular mechanisms of cardiovascular disease and rational approaches for the development of novel therapeutic strategies.

MicroRNAs (miRNAs) are small single-stranded non-coding RNA molecules (about 22 nucleotides long), which can generally inhibit protein translation by binding inexactly to the 3′-untranslated regions of target mRNAs, leaving mRNAs either degraded or intact (Lee *et al.*, 2002; Liu *et al.*, 2005; Peters and Meister, 2007). In the past few decades, miRNAs have become one of the main focuses of researchers in the cardiovascular field worldwide. A large body of evidence has confirmed the regulatory role of miRNAs in the pathogenesis and progression of cardiovascular disease, highlighting the possibility that miRNAs can be used as targets for the treatment of cardiovascular diseases (Hata, 2013; Quiat and Olson, 2013; Dangwal and Thum, 2014). Chinese researchers have also provided significant contributions to elucidating the role of miRNAs in various cardiovascular conditions, including arrhythmia, ischaemia, fibrosis, hypertrophy and heart failure, as well as to the potential development of miRNA-based therapies for cardiovascular disease. In this review, we will summarize the milestones of the investigations conducted by Chinese researchers exploring the role of miRNAs in cardiovascular diseases.

miRNAs and cardiac arrhythmias

The occurrence of arrhythmias, manifested as irregular beats of the heart, is often associated with various cardiovascular diseases, such as cardiac ischaemia, cardiac hypertrophy and heart failure. Cardiac electrophysiological disturbances are the major cause of arrhythmia, and the basic elements governing cardiac electrophysiological function are ion channel proteins that are located on the cell membrane. Under disease conditions, the expression of these proteins is altered and the electrophysiological balance disturbed, which then leads to arrhythmias (Nattel *et al.*, 2007). The current or conventional therapy for cardiac arrhythmias is the use of pharmacological agents to primarily block ion channels. Unfortunately, the limited efficacy and pro-arrhythmic potential of anti-arrhythmic agents limit their application in clinical practice. Our group revealed that miRNAs are key regulators of the expression of cardiac ion channel genes, with the potential to control arrhythmogenicity (Yang *et al.*, 2007; Luo *et al.*, 2013; Li *et al.*, 2014).

Ventricular arrhythmias, which occur during periods of cardiac ischaemia, are often very severe and sometimes fatal. In the first publication linking miRNAs to arrhythmias, we described the aberrant up-regulation of miR-1 in the cardiac tissue obtained from patients with cardiac ischaemic disease (Yang *et al.*, 2007). We successfully induced ventricular arrhythmias by delivering miR-1 into the myocardium of healthy normal hearts in rats. In a rat model of cardiac ischaemia, administration of the specific inhibitor of miR-1 (antisense oligonucleotide-1) to knockdown endogenous miR-1 markedly reduced the occurrence of ventricular arrhythmias. We then verified that the genes of connexion 43 (Cx43; previously known as gap junction protein, alpha 1) *GJA1* and the potassium inwardly rectifying channel $K_{ir}2.1$, *KCNJ2*, are the direct targets of miR-1, which were negatively regulated by miR-1. As decreased expression of Cx43 and $K_{ir}2.1$ during cardiac ischaemia are the factors that cause ischaemic arrhythmias, we concluded that the up-regulation of miR-1 during cardiac ischaemia is pro-arrhythmic and the mechanism involves the direct post-transcriptional repressions of

GJA1 and *KCNJ2* (Yang *et al*., 2007). In the same issue, Anderson and Mohler (2007) commented that 'the work of Yang *et al*. is an exciting step in the dissection of new molecular signalling pathways for arrhythmias and sudden death'. In a transgenic mouse line with miR-1 overexpression, we also observed atrioventricular block of varying degrees, which further highlights the pro-arrhythmic property of miR-1 (Zhang *et al*., 2013b).

Atrial fibrillation (AF) is the most common sustained arrhythmia characterized by electrical and structural remodelling of the atrium. Although it can be non-symptomatic and normally not fatal by itself, it is closely associated with the considerable morbidity and mortality of cardiovascular patients. It seems that there is still no ideal pharmacological intervention. A series of studies from our group revealed the roles of miR-328 (Lu *et al*., 2010), miR-26 (Luo *et al*., 2013), miR-133 and miR-590 (Shan *et al*., 2009b) in AF vulnerability. miR-328 is the first miRNA that was identified to be involved in the induction and perpetuation of AF. We found that the levels of miR-328 robustly increased in atrial tissues of a canine AF model, established by rapid pacing, and in AF patients. Transgenic overexpression of miR-328 in mice enhanced the occurrence of AF, whereas knockdown of miR-328 did the opposite. Moreover, overexpression of miR-328 inhibited L-type calcium current (I_{CaL}) and shortened atrial action potential duration (APD), which underlies its pro-AF action. Furthermore, we established that the genes encoding I_{CaL} channel protein α1c- and β1 subunits, *CACNA1C* and *CACNB1*, are the cognate targets of miR-328 (Lu *et al*., 2010). Different from the pro-AF property of miR-328, we identified miR-26 as an endogenous anti-arrhythmic miRNA, the first anti-arrhythmic miRNA identified thus far: up-regulation of miR-26 decreases the propensity of the heart for arrhythmias. This is in contrast to other miRNAs. In particular, miR-26 is an anti-AF miRNA and loss of this anti-AF effect due to down-regulation of miR-26 increases the vulnerability of the heart to AF induction and maintenance. Furthermore, our study identified miR-26 as an important regulator of the expression of inward rectifier K^+ channel $K_{ir}2.1$: down-regulation of miR-26a results in the overexpression of $K_{ir}2.1$, leading to excessive shortening of atrial APD in favour of AF. Down-regulation of miR-26a occurs as a result of an enhanced Ca^{2+}-dependent activation of nuclear factor of activated T-cells (NFAT), a key signalling pathway for the perpetuation of AF (Luo *et al*., 2013).

In an earlier study, we showed that miR-133 and miR-590 participate in the structural remodelling of atria (Shan *et al*., 2009b), a major pathological alteration accounting for the pathogenesis of AF. We found that both miR-133 and miR-590 are reduced in the atrium of nicotine-treated dogs. Transforming growth factor-β1 (TGF-β1) and transforming growth factor, β receptor II (TGFBR2) are the potential targets of miR-133 and miR590 respectively. As expected, as opposed to those of miR-133 and miR-590, the protein levels of TGF-β1 and TGFBR2 are increased in the atrium of nicotine-treated dogs. Furthermore, we validated that TGF-β1 and TGFBR2 are the targets of miR-133 and miR-590, as overexpression of miR-133 and 590 inhibited the expression of TGF-β1 and TGFBR2 in cultured atrial fibroblasts, and the luciferase activity of the chimeric vectors containing the 3′-UTR of TGF-β1 and TGFBR2 respectively (Shan *et al*., 2009b). In the same issue, Andreas Goette commented that 'the elegant study by Shan *et al*. extends our knowledge about the impact of nicotine and miRNAs on pro-arrhythmic atrial remodelling. The present study will stimulate and expand further research in this highly interesting and innovative field. In addition, interference with miRNAs using small silencing RNAs or antisense oligonucleotides has the potential to be developed as a novel therapeutic approach to AF' (Goette, 2009).

A number of studies from other Chinese researchers have also demonstrated the involvement of miRNAs in AF. Ling *et al*. discovered that the expression of miR-499 increased by 2.33-fold in the atria of the AF patients, while small conductance calcium-activated potassium channels 3 ($K_{Ca}2.3$ also known as SK3) protein expression was reduced by 46% (Ling *et al*., 2013). Overexpression of miR-499 in cultured HL-1 cells suppressed the protein expression of $K_{Ca}2.3$. These data indicated that miR-499 may be a causal factor of the electrical remodelling that occurred in AF (Ling *et al*., 2013). A few studies performed by Chinese investigators profiled miRNA expression in the atrial tissue of AF patients. Chen's group screened the miRNA profile in the right atrial appendages of mitral stenosis patients with AF and without AF, and found 28 aberrantly expressed miRNAs (Xiao *et al*., 2011). In a recent study, Liu *et al*. found that 22 miRNAs were differentially expressed in the atria of mitral stenosis patients with AF (Liu *et al*., 2014a). They also identified the differentially expressed miRNAs in the right and left atria from patients with rheumatic mitral valve disease (Liu *et al*., 2014b). Liu *et al*. analysed the miRNA expression profile in the plasma of paroxysmal AF, and less miR-150 was detected in the plasma of AF patients than that of the non-AF control group. Moreover, the level of miR-150 was correlated with AF (Liu *et al*., 2012). The arrhythmia-related miRNAs are summarized in Figure 1.

It appears from the published studies that miRNAs control arrhythmogenic potential by being either anti-arrhythmic or pro-arrhythmic through their targeting of electrical determinants/ion channels and structural determinants/fibrosis regulators, to directly or indirectly alter the electrical activities of the heart and regulate its arrhythmogenicity. Moreover, changes in the same ion channel induced by miRNAs in different regions of the heart might lead to different outcomes as regards arrhythmogenesis. For example, down-regulation of $K_{ir}2.1$ due to miR-1 overexpression promotes ischaemic arrhythmias, whereas up-regulation of $K_{ir}2.1$ due to miR-26a down-regulation induces AF. This property may be explained by the different underlying cellular contexts and arrhythmogenic substrates.

miRNAs and cardiac hypertrophy/heart failure

Cardiac hypertrophy is a common pathological process that occurs in various cardiovascular diseases, such as hypertension, cardiac infarction and aortic stenosis. At the early stage, the heart undergoes hypertrophic growth to compensate for the impaired cardiac function. However, the heart will finally enter a decompensation stage as the disease keeps progressing, which is called heart failure. The characteristic

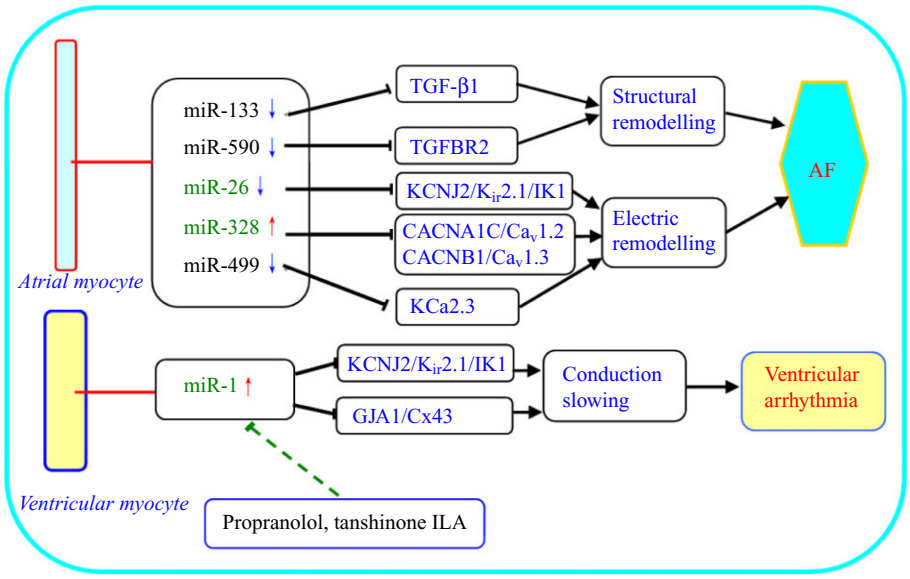

Figure 1

Schematic summary of miRNAs involved in the pathogenesis of arrhythmia. miRNAs in green have been experimentally validated to be pro- or anti-arrhythmic. miRNAs in black have been demonstrated to participate in electrical and/or structural remodelling, but lack *in vivo* evidence. Propranolol and tanshinone IIA were shown to produce anti-arrhythmic effects by inhibiting miR-1 expression.

alterations in cardiac hypertrophy include the re-initiation of fetal gene expression and the enlargement of cardiac myocytes, induced thorough the modulation of a series of biological signalling pathways (Frey and Olson, 2003). The significant role of miRNA in cardiac hypertrophy and heart failure has been extensively explored, and a host of miRNAs has been shown to be critical in the pathogenesis of cardiac hypertrophy and heart failure (Gladka *et al.*, 2012). Moreover, harnessing the deregulation of certain miRNAs is able to alleviate the progress of cardiac hypertrophy and heart failure (Seok *et al.*, 2014), implying the therapeutic potential of miRNAs.

Several Chinese research groups have documented the important role of a number of miRNAs in the development of cardiac hypertrophy and heart failure. Our group clarified the role of miR-133 (Dong *et al.*, 2010) and miR-328 (Li *et al.*, 2014) in cardiac hypertrophy through different but related signalling pathways. Calcineurin is a calcium/calmodulin-activated serine/threonine phosphatase that can dephosphorylate NFAT and lead to its nucleus translocation to regulate gene expression. The calcineurin NFAT axis has been well-established as a key signalling pathway in mediating cardiac hypertrophic responses (Molkentin *et al.*, 1998). We found that miR-133 regulates the expression of calcineurin through post-transcriptional repression (Dong *et al.*, 2010). Conversely, calcineurin/NFAT signalling also inhibits miR-133 expression (Dong *et al.*, 2010). These data indicate that miR-133 and calcineurin regulate cardiac hypertrophy via their reciprocal repression. Intriguingly, in our miR-328 transgenic mouse model, the animals are not only more vulnerable to spontaneous AF but also are more prone to the development of cardiac hypertrophy induced by aortic artery banding (Li *et al.*, 2014). Administration of locked nucleic acid–miR-328 (LNA-miR-328, a specific inhibitor of miR-328) *in vivo* is able to prevent this pathological alteration, indicating the potential of miR-328 as a therapeutic target in cardiac hypertrophy. We validated sarcoplasmic reticulum calcium ATPase (SERCA2a) as a direct target for miR-328, the down-regulation of which is known to be the key event in the development of cardiac hypertrophy and transition into heart failure. Targeting SERCA2a is a mechanism for cardiac hypertrophy induced by miR-328 overexpression (Li *et al.*, 2014).

Li's group demonstrated that miR-23a (Lin *et al.*, 2009) promotes cardiac hypertrophy, whereas miR-9 (Wang *et al.*, 2010b) and miR-489 (Wang *et al.*, 2014) do the opposite. miR-23a is up-regulated in cultured neonatal cardiac myocytes when exposed to hypertrophic stimulus, isoprenaline or aldosterone. This up-regulation is necessary for the induction of hypertrophy both *in vitro* and *in vivo* (Lin *et al.*, 2009). Furthermore, they discovered that the transcription of miR-23a is directly activated by the transcription factor NFATc3, and the muscle-specific ring finger protein 1, an anti-hypertrophic protein, is the downstream target of miR-23a. These data indicate that miR-23a is a component of the calcineurin-NFATc3 signalling pathway, which regulates cardiac hypertrophy (Lin *et al.*, 2009). In contrast, the hypertrophic stimulation with isoprenaline or aldosterone decreases the expression level of miR-9 (Wang *et al.*, 2010b). Administration of miR-9 alleviates cardiac hypertrophy and improves cardiac function. They demonstrated that myocardin is transcriptionally regulated by NFATc3 and functions as a downstream signalling molecule of the NFATc3-mediated hypertrophic response, whereas miR-9 negatively regulates the expression of myocardin to exert its anti-hypertrophic activity (Wang *et al.*, 2010b). In another study, they found that miR-489 is substantially reduced in response to angiotensin II stimulation. Overexpression of miR-489 in cardiomyocytes *in vitro* and mice *in vivo* by transgenic manipulation

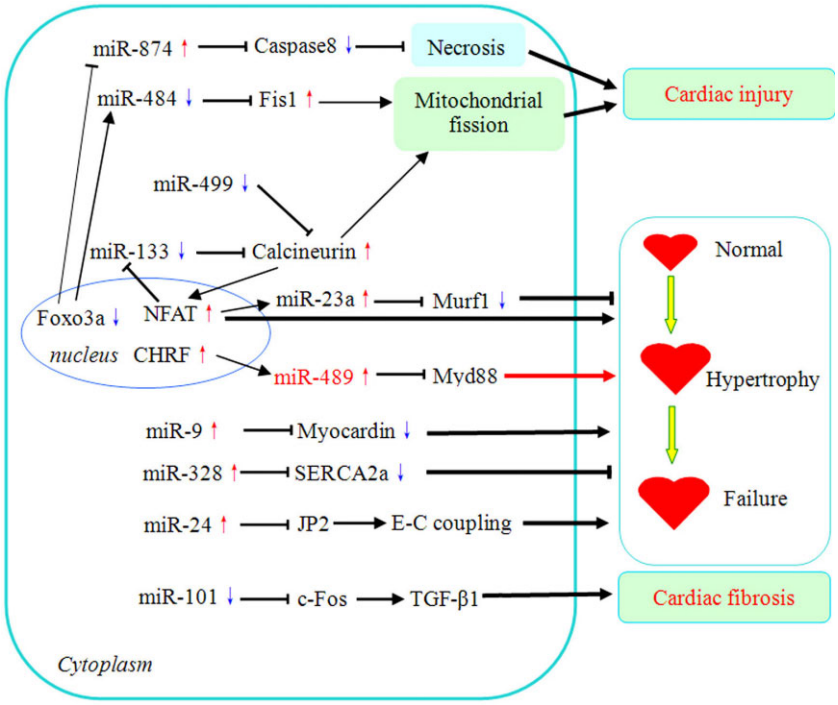

Figure 2

Schematic summary of miRNAs involved in the pathogenesis of cardiac ischaemia, hypertrophy, fibrosis and failure. With the exception of miR-489, the dys-regulation of all the other miRNAs is detrimental to the heart. c-Fos, FBJ osteosarcoma oncogene.

both alleviate the hypertrophic response to angiotensin II stress (Wang *et al.*, 2014). Myeloid differentiation primary response gene 88 (*Myd88*) was identified as a miR-489 target to mediate the anti-hypertrophic action of miR-489. Moreover, a long non-coding RNA CHRF was found to act as an endogenous sponge of miR-489, which mediates the down-regulation of miR-489 (Wang *et al.*, 2014).

Normal cardiac excitation–contraction (E-C) coupling is necessary to maintain the basic contractile function of the heart. E-C coupling may be affected under various disease conditions. Failing cardiomyocytes exhibit a decreased efficiency of E-C coupling. Xu *et al.* for the first time revealed the regulatory effect of miRNAs on E-C coupling (Xu *et al.*, 2012; Li *et al.*, 2013; Zhang *et al.*, 2013a). Junctophilin-2 (JP2) is an anchoring protein that links T-tubules and the sarcoplasmic reticulum to form the structural units for E-C coupling (Takeshima *et al.*, 2000). In the failing heart, the expression of JP2 is decreased, which leads to the impairment of signal transduction from L-type Ca^{2+} channels to the ryanodine receptor and thus compromises the normal function of E-C coupling (Minamisawa *et al.*, 2004; Xu *et al.*, 2007; Wei *et al.*, 2010; van Oort *et al.*, 2011). Xu *et al.* discovered that JP2 is a direct target of miR-24, which was found to be up-regulated in hypertrophic myocytes. Forced expression of miR-24 in cardiomyocytes reduces the expression of JP2 and fully reproduces the defective Ca^{2+} signalling in failing cardiomyocytes (Xu *et al.*, 2012). More interestingly, Xu *et al.* found that *in vivo* silencing of miR-24 blocks the transition to decompensation hypertrophy while allowing compensated hypertrophy to persist in mice subjected to transaortic constriction. These findings indicate that miR-24 may be a potential target in the treatment of heart failure (Li *et al.*, 2013). The relationship between the dys-regulation of miR-24 and JP2 and a malfunction of E-C coupling was also observed in the human samples of heart failure caused by dilated cardiomyopathy and ischaemic cardiomyopathy (Zhang *et al.*, 2013a), which adds weight to the potential of targeting miR-24 to treat human heart failure in the future. The miRNAs involved in the regulation of cardiac hypertrophy and heart failure are summarized in Figure 2.

These studies suggest that miRNAs are critically involved in the development of cardiac hypertrophy either as anti-hypertrophic or pro-hypertrophic mediators. Also, the ability of these miRNAs to regulate the intracellular Ca^{2+} handling process is their common and main mechanism of action.

miRNAs and cardiac interstitial fibrosis

Interstitial fibrosis is a pathological change characteristically observed in diseased hearts of caused by various conditions, such as hypertension and infarction. The development of interstitial fibrosis is caused by excessive deposition of collagen, which increases the stiffness of the ventricular wall and impairs the electrical and mechanical properties of the heart. Interstitial fibrosis is closely related to the susceptibility to arrhythmia and cardiac diastolic dysfunction. Recently, microRNAs were shown to be critically involved in the development of interstitial fibrosis and considered as a promising target in alleviating fibrosis.

The pathological role of miRNAs, such as miR-29 (van Rooij *et al.*, 2008), miR-21 (Thum *et al.*, 2008), miR-133 (Duisters *et al.*, 2009) and miR-30, in cardiac fibrosis has been reported by several groups. However, before our study, there has not been any *in vivo* evidence that manipulating miRNA expression is able to alleviate cardiac fibrosis. We, for the first time, demonstrated that forced overexpression of miR-101 carried by adenovirus significantly reduces cardiac fibrosis and improves cardiac function in a rat model of chronic myocardial infarction (Pan *et al.*, 2012b). We found that the level of miR-101 is reduced in both the infarct heart of rats and the angiotensin II-treated cardiac fibroblasts. Overexpression of miR-101 in cultured cardiac fibroblasts decreases the production of collagen I and III. In a rat model of myocardial infarction, myocardial infection of the adenovirus carrying miR-101 ameliorates the impaired cardiac function, probably by alleviating interstitial collagen deposits. We found that miR-101 acts directly on FBJ osteosarcoma oncogene and inhibits the expression of its downstream signalling molecule TGF-β1, a typical pro-fibrotic factor (Figure 2). Dr Thomas Thum and Johan M. Lorenzen have written an editorial on this work. They commented that, 'Pan *et al*. present a detailed and innovative analysis of miR-101 deregulation in cardiac fibrosis after myocardial infarct (MI). Unresolved points remain to be elucidated, but interfering with miR-101 expression in this setting carries future therapeutic potential' (Thum and Lorenzen, 2012). miR-24 is another miRNA that has been shown to inhibit cardiac fibrosis (Wang *et al.*, 2012a). Wang *et al*. observed that the expression of miR-24 is reduced in MI hearts, and overexpression of miR-24 produced anti-fibrotic effects in a mouse model of myocardial infarction and in a cellular model of fibrogenesis. They identified furin, a protease regulating the activation of latent TGF-β, as a direct target for miR-24, and demonstrated that miR-24 regulates cardiac fibrosis through the furin-TGF-β pathway (Wang *et al.*, 2012a).

miRNAs and cardiac ischaemic disease

Li's group reported the participation of miR-499 (Wang *et al.*, 2011), miR-484 (Wang *et al.*, 2012b) and miR-874 (Wang *et al.*, 2013) in myocardial ischaemic injury. Under physiological conditions, the level of miR-499 is substantial in the heart (Kloosterman *et al.*, 2006). However, under pathological conditions, the level of miR-499 was reduced, and less miR-499 was detected in the cardiac region at risk of ischaemia and in cultured neonatal rat cardiomyocytes exposed to anoxia (Wang *et al.*, 2011). Transgenic overexpression of miR-499 in mice heart significantly alleviated apoptosis and infarct injury of mice subjected to cardiac ischaemia–reperfusion in comparison with controls. In contrast, knockdown of endogenous miR-499 by i.v. administration of antagomir-499, a specific inhibitor of miR-499, exacerbated ischaemia–reperfusion induced cardiac injury. In addition, in a chronic cardiac infarction model, miR-499 transgenic overexpression significantly alleviated cardiac remodelling, as manifested by a reduced heart/body weight ratio, cell area, interstitial collagen deposition and thus improved cardiac function. Both the α- (CnAα) and β- (CnAβ) isoforms of the calcineurin catalytic subunit have been identified as the direct targets of miR-499, and they have been shown to be involved in the cell death process induced by anoxia (Wang *et al.*, 2011). Furthermore, dynamin-related protein-1 (Drp1) has been identified as the downstream molecule in mediating cardiac injury induced by miR-499 down-regulation and calcineurin up-regulation. It has been shown that calcineurin can phosphorylate Drp1 and lead to its translocation to the mitochondria, which then promotes mitochondrial fission (Frank *et al.*, 2001; Cereghetti *et al.*, 2008). In this study, it was found that mitochondrial Drp1 accumulation and fission were attenuated by miR-499 overexpression in the presence of wild-type CnAα, but not mutant CnAα, indicating that the calcineurin/Drp1 pathway is involved in miR-499-regulated cardiac myocyte injury. Moreover, the authors found that miR-499 is transcriptionally regulated by p53 (Wang *et al.*, 2011). The results of this study suggest that miR-499 may exert certain therapeutic potential in treating apoptosis-related cardiac diseases. In another study, the same group observed that overexpression of miR-484 alleviated fission and apoptosis in cardiomyocytes, and the mechanism involved the direct inhibition of the mitochondrial fission protein Fis1 (Wang *et al.*, 2012b). Moreover, they also revealed that the transcription of miR-484 is transactivated by forkhead transcription factor Foxo3a (Wang *et al.*, 2012b). Li's group demonstrated that knockdown of miR-874 by antagomir miR-874 attenuates the necrotic morphological alterations in cardiomyocytes treated with H_2O_2 (Wang *et al.*, 2013). Consistent with this, in an *in vivo* ischaemia–reperfusion mouse model, knockdown of miR-874 also reduced myocardial necrosis. Furthermore, the suppression of caspase-8 was found to mediate this anti-necrotic effect of miR-874. Moreover, it has been shown that miR-874 is transcriptionally regulated by Foxo3a (Wang *et al.*, 2013). These findings indicate that manipulation of miR-874 expression may represent a novel approach in the treatment of cardiac ischaemic diseases.

Our group tested the therapeutic potential of a miR-1-based approach in cardiac ischaemia–reperfusion injury (Pan *et al.*, 2012a). We administered the LNA–anti-miR-1 to suppress endogenous miR-1 in the tail vein of rats, and observed apparent inhibition of miR-1 levels in the ischaemia–reperfusion heart. Meanwhile, LNA–anti-miR-1 administration significantly attenuated cardiac ischaemic–reperfusion injury as manifested by small infarct area and less apoptosis (Pan *et al.*, 2012a). In addition, we found that miR-1 overexpression significantly inhibited the expression of PKCε and heat shock protein 60 (HSP60), two proteins involved in cardiac injury. A luciferase assay verified that miR-1 was able to directly target the 3′-UTR of PKCε and HSP60 (Pan *et al.*, 2012a). These data imply that knockdown of miR-1 expression is effective in the treatment of cardiac ischaemic injury, and has the potential to be applied as a therapy to patients with ischaemic cardiac diseases.

miRNA as a signalling mediator of agents used in cardiovascular diseases

In addition to being directly involved in the pathogenesis of various cardiac diseases, miRNAs also participate in mediat-

ing the classical effects of agents used to treat cardiovascular diseases. β-Adrenoceptor blockers are a class of agents that are widely used in the clinic to treat various cardiovascular diseases (Lohse *et al.*, 2003). Studies have shown that the β-blocker exerts its beneficial effects by affecting a host of biological processes, such as cardiac myocyte apoptosis, metabolism, oxygen free-radical scavenging and calcium handling (Reiken *et al.*, 2003). However, the involvement of miRNAs in the beneficial effects of β-blockers in various cardiac diseases remains unclear. We found that miR-1 plays an important role in mediating the beneficial effects of the classical β-blocker, propranolol (Lu *et al.*, 2009). Administration of propranolol is able to reverse the aberrant increase in miR-1 in the cardiac tissue of a rat MI model (Lu *et al.*, 2009). Consistent with its miR-1-suppressing effect, propranolol restored the expression of $K_{ir}2.1$ channels and the gap junction channel Cx43, and thus mitigated membrane depolarization and the slowing of cardiac conduction. We also found that propranolol inhibited the expression of CCK4 (also known as serum response factor), one of the known transcriptional enhancers of miR-1 (Zhao *et al.*, 2005). Moreover, we clarified that activation of the classical cAMP/PKA signalling pathway participates in the regulating effect of propranolol on miR-1 (Lu *et al.*, 2009).

Tanshinone IIA is a compound extracted from the traditional Chinese medicine Danshen, which has been used in the treatment of ischaemic cardiac diseases for thousands of years in China (Cheng, 2007). Our study showed that miR-1 expression is regulated by tanshinone IIA to exert its anti-arrhythmic effect (Shan *et al.*, 2009a). Tanshinone IIA treatment reduced the occurrence of ventricular arrhythmias in a rat model of acute cardiac infarction. Furthermore, tanshinone IIA down-regulated the up-regulation of miR-1 and prevented the reduction in $K_{ir}2.1$ protein in the ischaemic heart, which may underlie its anti-arrhythmic action (Shan *et al.*, 2009a).

Circulating miRNAs as biomarkers for cardiac disease

Based upon the data that miR-1 is up-regulated in the myocardium during cardiac ischaemia injury and participates in the occurrence of ischaemic arrhythmia (Yang *et al.*, 2007), we speculated that miR-1 is released into the plasma during acute cardiac infarction and can be used as a biomarker for cardiac injury. Our study indeed provided the first evidence for this idea (Ai *et al.*, 2010). As expected, a higher level of miR-1 was detected in the plasma of acute myocardial infarction (AMI) patients than non-AMI controls. The level of miR-1 was correlated with the augmentation of QRS complex duration, but not with age, gender, blood pressure, blood glucose and other known biomarkers for AMI. Upon discharge, the increased circulating miR-1 was reduced to its normal level. These observations indicate that the circulating miR-1 has the potential to be a biomarker for AMI, which will provide useful guidance for the proper management of AMI (Ai *et al.*, 2010). Another Chinese group also explored the usefulness of circulating miRNAs as novel biomarkers in AMI (Wang *et al.*, 2010a). They found that miR-208a is only present in the plasma from the AMI patients but not from the non-AMI subjects, indicating that plasma miR-208a may be a novel biomarker for the clinical diagnosis of AMI (Wang *et al.*, 2010a).

Opportunities and challenges for microRNAs as a potential treatment option

This study indicated that correcting the deregulation of a particular miRNA is able to prevent the pathogenesis of cardiovascular diseases, which highlights the potential of targeting miRNAs as therapeutic entities for cardiac disease treatment. The general strategy in harnessing the miRNA dys-regulation contains two parts: miRNA inhibitors such as synthesized anti-miRs to suppress the up-regulated miRNAs and miRNA mimics or mimetics to restore the down-regulated miRNAs. There are several advantages of miRNAs as a new class of drug target. The small size and conserved sequence of miRNAs make them attractive candidates as drug targets. Some of them demonstrate the property of tissue-specific expression, which makes them ideal targets to improve drug selectivity. Moreover, miRNAs are natural multi-target candidates, as they have the ability to directly target a series of genes of a specific signalling pathway or a pathological cellular process. Considering the significant advantages of miRNA-based therapy, researchers are trying to translate the exciting experimental findings into clinical therapeutics. In China, several miRNAs are in the pre-clinical study stages, including miR-328 in AF, miR-24 in heart failure and miR-499 in cardiac ischaemia.

However, there is still a long way to go before the application of miRNA-based therapy in cardiac diseases. We still face some unanswered questions and unresolved problems. The clinically applicable delivery methods of miRNA mimics or inhibitors seem to be one of the biggest obstacles in accomplishing miRNA-based therapy. One exciting advance is the synthesis of short anti-miRs, which can easily enter the target organ and are fairly stable, showing drug-like properties. Yet, there are still no clinical data showing the therapeutic efficacy of anti-miRs in cardiac diseases. Off-target effects are also avserious issue that needs to be deeply taken into account when developing miRNA-based agents. As a single miRNA can target hundreds of downstream genes involved in many cellular processes and tissue types, off-target effects would seem to be inevitable. Some unknowns remain about the mode of the biological action of miRNAs. Some unexpected problems may emerge, which may hinder the development of miRNA-based therapies. Therefore, rigorous fundamental and clinical studies are needed to fully clarify these problems before an miRNA-based therapy for cardiac diseases can become a reality.

In summary, tremendous advances have been made by Chinese researchers in the pathophysiological roles and the diagnostic and therapeutic potential of miRNAs in various cardiac diseases, which will surely improve our understanding of the molecular mechanisms for cardiac diseases and facilitate the development of novel diagnostic and therapeutic strategies.

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Conflict of interest

None.

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