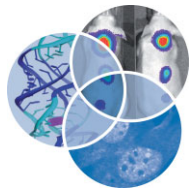
Advanced Review



# MicroRNAs in cardiac hypertrophy: angels or devils

Su-Ling Ding,† Lu-Yu Zhou† and Pei-Feng Li*

MicroRNAs (miRNAs) are short noncoding RNA molecules that can regulate gene expression via affecting mRNA stability or translation efficiency. miRNAs mediate many important cellular processes and emerge as a newly discovered regulator of gene expression. In cardiac hypertrophy, miRNAs expression is aberrantly altered. Some of these miRNAs can promote cardiac hypertrophy, whereas others can inhibit the process. In this review, we summarize the up- and downregulated miRNAs during cardiac hypertrophy and discuss about their roles in cardiac hypertrophy. The studies on miRNAs shed new light on the mechanism of cardiac hypertrophy and suggest that they may be promising therapeutic targets in tackling cardiac hypertrophy.  *WIREs RNA* 2011 2 124–134 DOI: 10.1002/wrna.61

## INTRODUCTION

Heart failure (HF) is a phenomenon in which the function of the heart is severely impaired and the life quality and longevity are greatly worsened or reduced. HF is one of the leading causes of hospitalization and death worldwide.[1] Cardiac hypertrophy is a common response to a variety of physiological as well as pathophysiological stimuli. Although physiological hypertrophy shows an enhancement of cardiac function, the pathological hypertrophy will eventually lead to HF. Because of the maladaptive consequence of pathological hypertrophy, many scientific works endeavor to figure out the underlying mechanism of pathological hypertrophy and hope to reverse its deleterious aspect. Thus, hypertrophy is considered to be a therapeutic target for HF.[2,3] However, those underlying molecular mechanisms of cardiac hypertrophy are still poorly understood. Elucidating new molecular mechanisms is essential for discovering novel impactful therapeutic targets suppressing maladaptive hypertrophy.[4,5]

MicroRNAs (miRNAs) are 21–23 nt single-stranded noncoding RNA molecules that are found to be gene expression regulators and can affect mRNA stability or translation efficiency.[6,7] Like other kinds of RNAs, miRNAs are mainly transcribed from DNA by RNA polymerase II, only a few miRNAs are transcribed by polymerase III, and they are processed into noncoding RNAs. The classical miRNA biosynthesis process involves Drosha/Pasha, Exportin-5, and RNA-induced silencing complex (RISC). In general, RNA polymerase II or III produces the hairpin-structured pri-miRNA, which is comprised of a double-stranded stem, 5′ and 3′ single-stranded flanking regions, and a terminal loop region.[8] Pri-miRNAs are further processed into pre-miRNA by Drosha in the nucleus. In this process, the 5′ and 3′ single-stranded flanking regions are cleaved by the RNase domains of Drosha, whereas Pasha, the partner of Drosha, determines the site of cleavage.[9] Then, pre-miRNAs are exported to cytoplasm through Exportin-5. In the cytoplasm, Dicer, TAR RNA binding protein (TRBP), and argonaute2 (Ago2) assemble the RISC loading complex. After the assembly is completed, the pre-miRNAs are loaded into the complex. Then, Dicer cleaves away the loop of the pre-miRNA to generate an miRNA duplex. One strain in the duplex will be maintained in the mature RISC, whereas the abandoned strain, which is denoted as miRNA*, will be cleaved by Ago2 and finally degraded. The selected strain is also called the guide strain. Hereafter, the guide miRNA and Ago2 form the mature RISC, the latter will combine the target mRNA and induce target cleavage, translational repression, and mRNA deadenylation.[10] Usually, the guide strand has a less thermodynamic stability at the 5′ end.[11] Bases 2–8 of the guide miRNA (numbered from the 5′ end) have perfect Watson–Crick complementarity to the target mRNA.[7] It has been found that the biosynthesis pathway of miRNA is not

†These two authors contributed equally to this work.

*Correspondence to: peifli@ioz.ac.cn

Division of Cardiovascular Research, National Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

DOI: 10.1002/wrna.61

limited to the classical pathway. miRNAs can also be generated from introns. During mRNA processing, the spliced intron can also form the hairpin structure which has a similar structure of a pre-miRNA. In addition, it has been proved that these introns can be processed through the miRNA biosynthesis pathway to form an miRNA. These miRNAs that are generated from introns are also called mitrons.[12] As miRNAs have been first discovered in *Caenorhabditis elegans*,[13] more than 1000 miRNAs have been so far discovered, and they have been found to get involved in many cellular processes and exhibit powerful biological functions. Strikingly, the growing evidence has shown that miRNAs are able to regulate cardiac hypertrophy, and they light a new way for us to study and tackle it.

## ABERRANT EXPRESSION OF miRNAs IN CARDIAC HYPERTROPHY

miRNAs play pivotal roles in many physiological and pathological processes, and under different cellular stress they show a differential expression profile.[14] Many studies have revealed that the expression levels of some miRNAs are changed in diseased human heart.[15] Ikeda et al.[15] has compared miRNAs expression patterns in ischemic cardiomyopathy, dilated cardiomyopathy, and aortic stenosis and found that seven miRNAs show similar expression pattern in all three diseases. There have been lots of research studies demonstrating that miRNA expression profile changes during the process of HF.[16–18] As been well verified,[19] genes which normally express only during fetal development have been observed to increase on RNA transcription and/or protein synthesis level during cardiac hypertrophy. Thum et al.[17] have demonstrated that the miRNAs expression pattern in the failing heart is also similar to that of coding gene mRNAs.

Generally, Dicer is essential for normal development. The study in traditional Dicer knockout mice reveals that miRNAs may play important roles in cardiac development and function. Knockout of Dicer leads to postnatal lethality and the mice die before heart formation.[20,21] Also, angiogenesis was severely impaired.[21] Heart-specific Dicer knockout mice live to embryonic day 18.5 and show a significant decrease in the expression of mature miRNA.[22] At the same time, it suffers from rapid progressive dilated cardiomyopathy and dies of HF. The research in the conditional Dicer knockout mice further demonstrates the pivotal role of Dicer in the cardiac pathological process. The heart-specific conditional Dicer knockout in 3-week-old mice results in sudden death, whereas the conditional knockout of Dicer in 8-week-old mice provokes pathological hypertrophy.[23] Another research work on muscle hypertrophy denoted that Drosha and Exportin-5 transcript levels are increased during hypertrophy-associated muscle functional overload.[24] Besides Dicer, Ago2 is another miRNA genesis member that is broadly expressed in the embryo. The Ago2 knockout mice not only suffer from a defect in neural tube closure but also suffer from cardiac failure. Specifically, the Ago2 knockout embryos show an enlarged pericardial membrane at embryonic day 10.5.[25] Recent research work by Kalsotra et al. also confirmed the importance of Dicer for the maintenance of normal cardiac function. They used Cre-loxP approach to generate tamoxifen (Tam)-inducible, heart-specific Dicer knockout mice and found that the cardiomyocyte-specific loss of Dicer in adult mice results in many abnormalities in cardiac structure and function such as dilation of the left ventricle, thinning of the left ventricular wall, and reduced fractional shortening, eventually leading to premature death within 10 weeks of Tam treatment.[26] Those results indicate that miRNA might play an important role in cardiac hypertrophy.

To further explore the role of miRNAs in cardiac hypertrophy, several laboratories have adopted miRNA microarray to analyze the global miRNA expression profile in pathological cardiac hypertrophy models.[17,27–29] In addition, these works provided lots of hints in the study of miRNAs and cardiac hypertrophy. In 2006, van Rooij et al. has reported the first microarray using two pathological cardiac hypertrophy animal models including thoracic aortic-banded hearts (TAB) and the calcineurin-overexpressed transgenic mice. Their results show that the miRNAs expression profiles that changed in two animal models are similar, suggesting that the two types of animal models share a similarity in hypertrophic mechanism controlled by miRNAs.[29] Nevertheless, more experimental results reveal that the expression profiles of miRNAs depend on the cardiac hypertrophic stimuli and the time course of treatment (Tables 1 and 2). This suggests that the different hypertrophic stimuli and the divergent phases of hypertrophic development may require the involvement of distinct miRNAs. In addition, new trusty methods for measuring miRNAs are required to be developed. As shown in Tables 1 and 2, although the function of many miRNAs in hypertrophy remains unclear until now, those including miR-1, miR-21, miR-23a, miR-26, miR-133, miR-195, and miR-208 have been demonstrated to play a functional role in cardiac hypertrophy. In particular, the molecular targets of miR-1, miR-23a, miR-26, miR-133, and miR-208 have been identified. There is no doubt that more miRNAs and their targets will be revealed in the hypertrophic machinery.

**TABLE 1** | The Aberrant Expressed miRNAs During Cardiac Hypertrophy

| Upregulated miRNAs | Downregulated miRNAs | Cardiac Hypertrophic Models |
|---|---|---|
| miR-21 | miR-29c | In 3-week TAB and calcineurin A transgenic mice[29] |
| miR-23a/b | miR-93 | |
| miR-24 | miR-133a | |
| miR-27a/b | miR-150 | |
| miR-125b | miR-181b | |
| miR-195 | | |
| miR-199a | | |
| miR-214 | | |
| miR-217 | | |
| miR-17-5p | miR-30b/c | In 2-week TAB hearts[28] |
| miR-18b | miR-150 | |
| miR-19b | | |
| miR-20b | | |
| miR-21 | | |
| miR-106a | | |
| miR-125b | | |
| miR-140 | | |
| miR-142-3p | | |
| miR-153 | | |
| miR-184 | | |
| miR-200a | | |
| miR-208 | | |
| miR-210 | | |
| miR-211 | | |
| miR-221 | | |
| miR-222 | | |
| miR-21 | miR-29a/b/c | In 1-week TAB hearts[27] |
| miR-27a/b | miR-30e | |
| miR-146 | miR-126-5p | |
| miR-214 | miR-133a/b | |
| miR-341 | miR-149 | |
| miR-424 | miR-150 | |
| | miR-185 | |
| | miR-451 | |
| | miR-486 | |
| miR-18a | miR-187 | In PE-treated neonatal cardiomyocytes[28] |
| miR-20b | miR-292-5p | |
| miR-21 | miR-373* | |
| miR-23a | miR-466 | |
| miR-106a | | |
| miR-125b | | |
| miR-133a | | |

Cardiac hypertrophy is a typical age-related event. It has been reported that young, middle-aged, and elderly patients with hypertrophic cardiomyopathy have different morphological and prognostic characteristics.[30] However, there has been so far no report studying the miRNAs expression profile and their roles in cardiac hypertrophy according to different life stages, although it is possible that cardiac hypertrophy in different ages may have distinct miRNA expression profiles. We mainly summarized the pathological pathway of cardiac hypertrophy in the following section.

## SPECIFIC FUNCTIONS OF miRNAs IN CARDIAC HYPERTROPHY

Based primarily on experimental results from overexpression and deletion, some miRNAs are anti-hypertrophic, whereas others are pro-hypertrophic. One miRNA can regulate multiple target genes, which form a sophisticated network to maintain normal functioning of a living organism. It is believed that with the constant study on the miRNA regulation, the global function of miRNA will be unveiled. By then, we can give a more accurate definition and classification of miRNAs.

In general, as shown in Figure 1, the current considered pro-hypertrophic miRNAs include miR-23a, miR-195, and miR-208, whereas miR-1, miR-26, and miR-133 are considered to be anti-hypertrophic. Although many miRNAs represented an altered expression profile during cardiac hypertrophy, and some even shows a pro- or anti-hypertrophic role, the detailed mechanism is still unknown. Table 2 summarizes the hitherto clarified miRNA targets during cardiac hypertrophy. miRNAs may have distinct targets even though they have analogous effect on cardiac hypertrophy. Unveiling the role of miRNAs in cardiac hypertrophy is of great importance in the development of hypertrophy therapies.

## ROLE OF miRNAs IN THE INITIATION OF HYPERTROPHY

miR-195 is the first characterized miRNA involved in inducing hypertrophic growth in the adult heart.[29] The miRNA array data show that miR-195 is upregulated in both human and mouse hypertrophic hearts.[29] Adenoviral transfection to increase the expression level of miR-195 is sufficient to induce hypertrophy in cultured rat cardiomyocytes and miR-195 overexpression leads to dilated cardiomyopathy and heart dysfunction in mouse model.[29,31] These results suggest that miR-195 is a pro-hypertrophic factor

**TABLE 2** | A Summary of miRNA Functions in Cardiac Hypertrophy

| miRNA | Expression | Functions | Target Genes | References |
|---|---|---|---|---|
| miR-1 | Downregulated | Anti-hypertrophy | Hand2 | 56,57 |
| | | | HDAC4 | |
| | | | CaM | |
| | | | Mef2A | |
| | | | Gata4 | |
| miR-21 | Upregulated | Controversial | Unknown | 27–29,51,65,67 |
| miR-23a | Upregulated | Pro-hypertrophy | MuRF1 | 42 |
| miR-26 | Downregulated | Anti-hypertrophy | Gata4 | 61 |
| miR-133 | Downregulated | Anti-hypertrophy | Cdc42 | 50,52,53 |
| | | | Rho-A | |
| | | | Nelf-A/WHSC2 | |
| miR-195 | Upregulated | Pro-hypertrophy | Unknown | 29 |
| miR-208 | Upregulated | Pro-hypertrophy | THRAP1 | 32 |

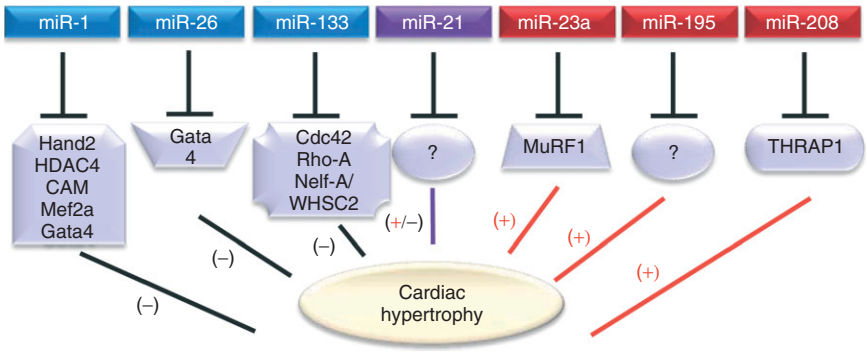



**FIGURE 1** | MicroRNAs (miRNAs) can regulate cardiac hypertrophy. miR-1, miR-26, and miR-133 play an anti-hypertrophic role and they can inhibit hypertrophy (–). miR-23a, miR-195, and miR-208a play a pro-hypertrophic role and they can promote hypertrophy (+). The exact role of miR-21 in cardiac hypertrophy remains to be further characterized.

and actively participates in the hypertrophic process (Figure 1). Nevertheless, no targets of miR-195 have been so far identified in the hypertrophic pathway.[29]

miR-208 is found to be a conserved cardiac-specific miRNA earlier which resides in the intron 27 of $\alpha$-myosin heavy chain ($\alpha$-MHC) gene and specifically expressed in both human and mouse hearts.[32] In addition, northern blot shows that it abundantly exists in heart, at trace level in lung, and not detectable in other tissues. miR-133 is another heart-specific miRNA, except for that it is also expressed in skeletal muscle. miR-208 is one of the miRNAs that are upregulated in cardiac hypertrophy in the TAB animal model.[28] Recently there are new researches revealing that miR-208 is actually an miRNA family that includes miR-208a and miR-208b. miR-208a is the previous verified miR-208 residing in the intron 27 of $\alpha$-MHC, whereas miR-208b is encoded within an intron of $\beta$-cardiac muscle myosin heavy chain gene ($\beta$-MHC).[33] miR-208a and miR-208b are of similar sequence with identical seed regions. $\alpha$- and $\beta$-MHC are both molecule motors of muscle, but $\alpha$-MHC has higher adenosine triphosphatase activity than $\beta$-MHC. During normal development, they are expressed in a developmental stage-specific manner with $\beta$-MHC expressed in mouse fetal heart and $\alpha$-MHC expressed mainly in the adult heart.[34] Although the switch from fetal isoform $\beta$-MHC to the adult isoform $\alpha$-MHC in the mouse occurs shortly after birth, a similar switch from miR-208b to miR-208a expression is also observed, implying they are cotranscribed with their MHC host genes.[33] Transgenic overexpression of miR-208a specifically in the heart induces hypertrophic growth in mice, with enlarged chambers and thickened ventricular walls appearing in the miR-208a transgenic hearts.[33] miR-208a can regulate $\beta$-MHC expression. It is reported that overexpression of miR-208a in the heart dramatically increases $\beta$-MHC protein levels.[32,33] Furthermore, miR-208a-deficient mice fail to upregulate $\beta$-MHC but instead increase $\alpha$-MHC expression, and

they have a blunted hypertrophic and fibrotic response to pressure overload. The level of miR-208a is also verified to be correlated with $\beta$-MHC mRNA levels in patients with dilated cardiomyopathy.[35] The balance of $\alpha$-MHC and $\beta$-MHC isoforms expression is correlated with contractile velocity and the balance is broken during cardiac diseases.[36–39] miR-208a upregulates $\beta$-MHC potentially by targeting thyroid hormone receptor associated protein 1 (THRAP1), which is a cofactor of the thyroid hormone nuclear receptor (TR).[40] miR-208a downregulates the expression of THRAP1. Thyroid hormone (T3) signaling leads to an upregulation of $\alpha$-MHC and a downregulation of $\beta$-MHC transcription after birth, which is similar to the phenotype occurring during cardiac diseases.[41] Thus, miR-208a executes its pro-hypertrophic function via inhibiting the TR–T3 signal pathway (Figure 1).

Recently, miR-23a is demonstrated to be a pro-hypertrophic miRNA in the heart.[42] miR-23a is reported to be upregulated in TAB-induced cardiac hypertrophy and its overexpression is sufficient to induce cardiomyocyte hypertrophy *in vitro*.[29] miR-23a expression is upregulated in cardiomyocytes treated with isoproterenol (Iso) or aldosterone (Aldo), both of which can induce cardiac hypertrophy.[43,44] Knockdown of miR-23a by delivering its antagomir through an osmotic minipump implanted under the mouse skin can attenuate hypertrophy. The underlying mechanism of miR-23a upregulation has also been unveiled. miR-23a locates in the miR-23a∼27a∼24-2 cluster. These three miRNAs are upregulated upon hypertrophic stimulation. Nevertheless, it appears that only miR-23a is the key factor in mediating the hypertrophic signal of Iso and Aldo. miR-23a acts as a downstream mediator of nuclear factor of activated T cells c3 (NFATc3), a transcriptional factor that is translocated into nuclear upon dephosphorylation in many different hypertrophic signaling pathways including calcineurin.[45] Inhibition of calcineurin–NFAT signaling usually attenuates hypertrophic responses and delays the progression from hypertrophy to HF. TAB-induced pressure overload or myocardial infarction can activate calcineurin–NFAT signaling pathway.[46,47] Muscle ring finger 1 (MuRF1) is revealed to be a downstream target of miR-23a, the latter predominantly suppresses MuRF1 protein but not mRNA levels (Figure 1). MuRF1 is a striated muscle-specific protein that can inhibit cardiac hypertrophy.[48]

Although the expression levels of many miRNAs are altered during hypertrophy (Table 1), it is expected that more pro-hypertrophic miRNAs will be identified in the future.

## miRNAs INHIBIT HYPERTROPHY

miR-133 family is comprised of three members, miR-133a-1, miR-133a-2, and miR-133b. The similarity of their sequences makes an obstacle in figuring out the expression profile of each member.[49] Care et al. has reported that miR-133 and miR-1 are anti-hypertrophic miRNAs and they belong to the same transcription unit. They are downregulated in the mice model of cardiac hypertrophy as well as in the left ventricular tissue of patients with cardiac hypertrophy.[50] Other reports also demonstrate that both miR-133 and miR-1 play a key role in cardiac hypertrophy[17] (see Figure 1). miR-133 overexpression can significantly inhibit hypertrophy not only *in vitro* but also *in vivo*. Knockdown of miR-1 or miR-133 can easily induce a marked hypertrophic response.[50,51] miR-133 exerts its effect through targeting Cdc42, Rho-A, and Nelf-A/WHSC2, which have been previously shown to take part in hypertrophic remodeling.[50,52,53] Rho-A and Cdc42 are related to myofilament organization assembly of sarcomere units.[47,49] miR-133b has been demonstrated to play an anti-hypertrophic role in Iso-induced hypertrophy.[54] It should be noted that miR-133 and miR-1 are reduced during physiological hypertrophy, suggesting that they may play important roles in a universal hypertrophic signal pathway.[55]

Other investigations further confirm the anti-hypertrophic function of miR-1. miR-1 knockout mice die at the embryonic stage with obvious sepal defects, whereas the survived ones show thickening of chamber walls in adulthood.[56] Heart and neural crest derivatives-expressed protein 2 (Hand2) and histone acetylase 4 (HDAC4) are identified as miR-1 targets.[56] Hand2 is a heart-specific transcription factor that promotes cardiomyocyte expansion. HDAC4 is a conserved transcriptional repressor of muscle gene expression among vertebrate species and contains two naturally miR-1-binding sites at its 3′ UTR.[49] It has recently been reported that miR-1 withstands hypertrophy through negatively regulating calmodulin (CaM), muscle enhancer factor-2a (Mef2a), and Gata4, all of which are components in calcium signaling and related to hypertrophy.[57] CaM transgenic mice suffer severe cardiomyocyte hypertrophy and HF.[58] Mef2a overexpression can induce cardiac hypertrophy, whereas inhibition of Mef2a signaling prevents cardiomyocyte hypertrophy.[59] Gata4 can regulate a subset of genes correlating with cardiac hypertrophy through protein–protein interactions.[60]

miR-26 has been recently shown to play an essential role in regulating myocyte survival and hypertrophy through targeting Gata4[61] (Figure 1). It is significantly downregulated by 40% after 1 week of pressure overload on the heart. Consequently, the

expression of Gata4 is increased during hypertrophy. Modulation of miR-26 expression levels with various doses of an adenovirus expressing miR-26 gene reveals that miR-26 can inhibit endothelin-1-mediated upregulation of Gata4 in a dose-dependent fashion. In contrast, knockdown of miR-26 using an adenovirus expressing a tandem repeat of antisense miR-26 induces a dose-dependent increase in cell size.

Hitherto, the exact roles of some miRNAs in cardiac hypertrophy are still hazy. For example, miR-21 is a versatile miRNA involving in many cellular processes. At first, miR-21 is explored to be involved in cell growth and apoptosis related to tumor and is upregulated in many forms of cancer.[62–64] miR-21 has been demonstrated to have an impact on cardiac structure and function by regulating ERK–MAPK signal pathway in cardiac fibroblasts.[65] Recently, it has been revealed that miR-21 is upregulated in cardiac hypertrophy induced by hypertrophic stimuli such as phenylephrine (PE), leukemia inhibitory factor, and fetal bovine serum.[27–29,51] miR-21 can inhibit PE-induced hypertrophy in cardiomyocytes, accompanying with repressed hypertrophic gene markers expression.[28] However, its genuine role in myocyte hypertrophy remains controversial according to the experimental results from different research groups. Cheng et al. have shown that the antisense oligonucleotide for miR-21 to suppress its expression can result in a blockage of the response to hypertrophic agonists, angiotensin II, and PE,[27] whereas Tatsuguchi et al. have reported that overexpression of miR-21 leads to a blunted hypertrophic response.[27,28] It seems that more studies should be performed on miR-21 to unmask its actual function and mechanism involved in hypertrophy. In particular, the direct targets of miR-21 in cardiac hypertrophy have not been characterized (Figure 1). miR-21 is also involved in other cardiac events such as cardiac fibroblast compartment.[66] In ischemia–reperfusion, miR-21 regulates metalloprotease-2 expression in cardiac fibroblasts of the infarct zone via a phosphatase and tensin homolog (PTEN) pathway.[67]

miR-100 and miR-92 are involved in the hypertrophic process. miR-100 is upregulated in the failing heart whereas miR-92 is downregulated. Studies using mimics or inhibitors of miR-100 and miR-92 in cardiomyocytes show that upregulation of miR-100 represses the expression of the adult genes $\alpha$-MHC and sarco(endo)plasmic reticulum ATPases (SERCA), and at the same time can increase isoproterenol-mediated upregulation of the fetal genes ANF and $\beta$-MHC. The direct targets of miR-100 have not been identified. miR-92 has a minimal effect on the expression of fetal or adult genes, but its role in hypertrophy remains unclear and awaits further study.[54]

## TRANSCRIPTIONAL REGULATION OF miRNAs IN CARDIAC HYPERTROPHY

miRNAs refine mechanisms of gene translation through regulating their targets, and we cannot keep from asking questions that how their expressions are regulated. Transcriptional factors modulate gene expression by binding to *cis* elements of target genes, thus it is necessary to ask whether the expression of miRNAs is also modulated by transcriptional factors. In this respect, miR-1 has been shown to be a direct transcriptional target of serum response factor (SRF)[18] (Figure 2). SRF is an important activator of numerous muscle-specific genes.[68–71] miR-1 genes can be transcriptionally regulated by SRF. Hand2 that promotes ventricular cardiomyocyte expansion is a target of miR-1.[18] SRF is able to promote hypertrophy,[72,73] whereas miR-1 can inhibit hypertrophy. Thus, it is required to elucidate whether the cross-talk between SRF and miR-1 is integrated into the hypertrophic machinery.

As mentioned above, miR-23a is an influential pro-hypertrophic miRNA in heart, and NFATc3 can directly activate miR-23a expression through the transcriptional machinery[42] (Figure 2). The promoter region of the miR-23a∼27a∼24-2 cluster contains one optimal and conservative NFAT-binding site. The study with chromatin immunoprecipitation analysis, luciferase reporter assay, and mutations in the NFAT-binding site of the promoter shows that NFATc3 can directly bind to the promoter region and influence the promoter activity. The data from NFATc3 RNAi also suggest that miR-23a

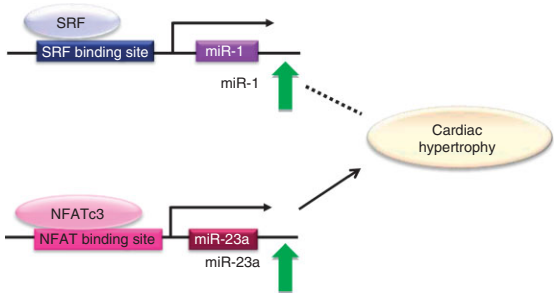



**FIGURE 2** | The expression of microRNAs (miRNAs) can be regulated by transcription factors. Serum response factor (SRF) can promote the expression of miR-1. However, it is not yet clear whether this regulation is integrated into the hypertrophic machinery. Nuclear factor of activated T cells c3 (NFATc3) can upregulate the expression of miR-23a that provokes hypertrophy.

is regulated by NFATc3, because knockdown of NFATc3 inhibits the expression of miR-23a and hypertrophic responses.

Taken together, while to profoundly explore the signaling pathways of cardiac hypertrophy in which miRNAs participate, it is also vital to investigate the transcriptional modulators of miRNAs in order to fully understand the machinery of miRNAs in cardiac hypertrophy.

## POTENTIAL miRNA-BASED CLINICAL APPLICATIONS

The apparent role of miRNAs in heart development and hypertrophy with their several special properties has aroused many ideas by which these miRNAs can be used in clinical applications for heart diseases that are adversely affected by hypertrophy. These include the following two aspects.

First, in different kinds of heart diseases, subsets of miRNAs are differentially expressed. Thus miRNAs expression levels can be served as sensitive biomarkers for cardiac diseases including hypertrophy. For example, miR-195 and miR-23a, which are upregulated during hypertrophy, may be used as diagnostic indicators for pathological hypertrophy at the early stage. But obtaining cardiac tissues at an early stage for diagnosis is an extremely difficult task. It is thus required to explore whether those aberrant expressed miRNAs in cardiac tissues are also deviated in blood. If these miRNAs can be served as circulating biomarkers for cardiac hypertrophy, its diagnosis will be exceedingly facilitated. Increasing evidence suggests that circulating miRNAs might be useful as stable biomarkers for HF. miR-1 and miR-208 are elevated in plasma following myocardial injury,[74,75] whereas miR-423-5p is newly verified to be upregulated in plasma of patients with HF.[76]

Second, the tactics of rescuing the up- or downregulated miRNAs in hypertrophy to the normal level also can be employed to develop new therapies.[15] For example, rescuing miR-1 or miR-133 or even both simultaneously to necessary levels potentially can be utilized to suppress pathological hypertrophy. miRNAs are small enough to achieve sufficient delivery efficiency *in vivo*.[77,78] Currently, miRNA mimics and antagomirs have already been applied to up- or downregulate specific miRNAs in cellular and animal models.[78] Mimics and antagomirs are both chemically engineered oligonucleotides, which are stable *in vivo* with sequence identically or reversely complemented to a given miRNA, respectively. But the approach for intravenous delivery of mimics or antagomirs directly to the body will create a marked alteration of the corresponding miRNAs in different organs. It is an intractable implement for miRNA-based therapy as to how to exclusively deliver these mimics and antagomirs to the heart. Local delivery can be an effective approach. For instance, the mimics or antagomirs can be locally delivered to the heart using a catheter through surgery. They will be driven into myocardial cells by the pharmacokinetics and biodistribution of the molecules. Alternatively, we can even consider the development of novel strategies such as conjugating mimics or antagomirs with specific antibodies or heart-specific homing signals. The clinical application of miRNA-based therapy will take a heavy burden and embark on a long road.

As every coin has two sides, miRNA-based therapy has advantages and disadvantages at the same time. Studies show single miRNA has multiple mRNA targets, which acts usually in concert to control a common pathway and/or biological function.[55] For example, miR-1 can inhibit hypertrophy by negatively regulating CaM, Mef2a, and Gata4, all of which are components in calcium signaling and related to hypertrophy as shown above. Thus, CaM, Mef2a, and Gata4 can be reregulated by just modulating miR-1 expression level. This feature of miRNA to target not only a single gene but also the whole gene networks regulating cardiac hypertrophy provides an opportunity to make it a more efficacious therapeutic method for the treatment of pathological hypertrophy than other conventional drugs just targeting one molecule. However, this feature can also bring out 'off-target' side effects. If miRNAs have numerous molecular targets involving in different cellular functions, the targeting of an miRNA may bring about perturbation on multiple cellular functions simultaneously, some pathological and others beneficial. For instance, miR-133 can not only regulate hypertrophic process but also additionally affect cardiac conductance, and miR-21 still has a role in tumor growth and apoptosis. If they were applied to treat hypertrophy, their side effects on cardiac conductance or tumor growth need to be avoided first. Future studies should aim at maximizing advantages and minimizing disadvantages of the feature of miRNAs. At last, we have to realize that miRNA-based clinical applications still have a long way to go, and further studies should be performed to improve the understanding of the potential toxicity of such miRNA-based applications before it can be credibly used in the clinic for patients.

## CONCLUSION AND PERSPECTIVES

miRNAs have reshaped our understanding of pathogenesis for many diseases. In the past few years,

studies on miRNAs and cardiac hypertrophy have been profound and inspiring. Significant achievements have been obtained in unveiling the signaling pathway for cardiac hypertrophy, and growing evidence has proved that miRNAs play a pivotal role in many cellular processes that contribute to cardiac hypertrophy. However, most of the miRNAs found in human have not yet been analyzed since they were discovered, and their functions await us to unveil. It is obvious that by analyzing these unstudied miRNA and their targets and showing their essential roles during cardiac hypertrophy, we probably can uncover novel cardiac hypertrophic mechanisms, improve our knowledge of cardiac hypertrophy, and create innovative therapeutic approaches. It is expected that, by halting and reversing the insalubrious consequences of cardiac hypertrophy, miRNA-based diagnostics and therapeutics will greatly improve life quality of patients and extend their longevity. Although, until now, the achievements are not sufficient to implement the ambition of miRNA therapy and many questions and problems remain to be answered and solved, advances are built brick by brick. Apparently, the strategies for combating cardiac hypertrophy are facing a rapid development. The expanding knowledge of miRNAs on the regulation of cardiac hypertrophy will probably lead to a novel treatment for HF in the near future.

## ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (30730045, 30871243) and the National Basic Research Program of China (973 Program, 2007CB512000).

## REFERENCES

1. Juenger J, Schellberg D, Kraemer S, Haunstetter A, Zugck C, Herzog W, Haass M. Health related quality of life in patients with congestive heart failure: comparison with other chronic diseases and relation to functional variables. *Heart* 2002, 87:235–241.
2. Meijs MFL, de Windt LJ, de Jonge N, Cramer MJM, Bots ML, Mali W, Doevendans PA. Left ventricular hypertrophy: a shift in paradigm. *Curr Med Chem* 2007, 14:157–171.
3. Thum T, Catalucci D, Bauersachs J. MicroRNAs: novel regulators in cardiac development and disease. *Cardiovasc Res* 2008, 79:562–570.
4. McKinsey TA, Olson EN. Toward transcriptional therapies for the failing heart: chemical screens to modulate genes. *J Clin Invest* 2005, 115:538–546.
5. van Rooij E, Marshall WS, Olson EN. Toward microRNA-based therapeutics for heart disease: the sense in antisense. *Circ Res* 2008, 103:919–928.
6. Pauley KM, Eystathioy T, Jakymiw A, Hamel JC, Fritzler MJ, Chan EKL. Formation of GW bodies is a consequence of microRNA genesis. *EMBO Rep* 2006, 7:904–910.
7. Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. *Cell* 2003, 115:787–798.
8. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Mol Cell Biol* 2009, 10:126–139.
9. Han JJ, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, Sohn SY, Cho YJ, Zhang BT, Kim VN, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell* 2006, 125:887–901.
10. Winter J, Jung S, Keller S, Gregory RI, Diederichs S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009, 11: 228–234.
11. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. *Cell* 2003, 115:505.
12. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in *Drosophila*. *Cell* 2007, 130:89–100.
13. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993, 75:843–854.
14. Marsit CJ, Eddy K, Kelsey KT. MicroRNA responses to cellular stress. *Cancer Res* 2006, 66:10843–10848.
15. Ikeda S, Kong SW, Lu J, Bisping E, Zhang H, Allen PD, Golub TR, Pieske B, Pu WT. Altered microRNA expression in human heart disease. *Physiol Genomics* 2007, 31:367–373.
16. Prasad SVN, Duan ZH, Gupta MK, Surampudi VSK, Volinia S, Calin GA, Liu CG, Kotwal A, Moravec CS, Starling RC, et al. Unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks. *J Biol Chem* 2009, 284:27487–27499.
17. Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, Doevendans PA, Mummery CL, Borlak J, Haverich A, et al. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation* 2007, 116:258–267.

18. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* 2005, 436: 214–220.

19. Wagner M, Mascareno E, Siddiqui MAQ. Cardiac hypertrophy: signal transduction, transcriptional adaptation, and altered growth control. *Ann N Y Acad Sci* 1999, 874:1–10.

20. Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV, Hannon GJ, et al. Dicer is essential for mouse development. *Nat Genet* 2003, 35:215–217.

21. Yang WJ, Yang DD, Na SQ, Sandusky GE, Zhang Q, Zhao GS. Dicer is required for embryonic angiogenesis during mouse development. *J Biol Chem* 2005, 280:9330–9335.

22. Chen JF, Murchison EP, Tang R, Callis TE, Tatsuguchi M, Deng Z, Rojas M, Hammond SM, Schneider MD, Selzman CH, et al. Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. *Proc Natl Acad Sci U S A* 2008, 105:2111–2116.

23. Martins P, Bourajjaj M, Gladka M, Kortland M, van Oort RJ, Pinto YM, Molkentin JD, De Windt LJ. Conditional Dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. *Circulation* 2008, 118:1567–1576.

24. McCarthy JJ, Esser KA. MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J Appl Physiol* 2007, 102:306–313.

25. Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ. Argonaute2 is the catalytic engine of mammalian RNAi. *Science* 2004, 305:1437.

26. Kalsotra A, Wang K, Li PF, Cooper TA. MicroRNAs coordinate an alternative splicing network during mouse postnatal heart development. *Genes Dev* 2010, 24:653–658.

27. Cheng YH, Ji RR, Yue JM, Yang J, Liu XJ, Chen H, Dean DB, Zhang CX. MicroRNAs are aberrantly expressed in hypertrophic heart—Do they play a role in cardiac hypertrophy? *Am J Pathol* 2007, 170:1831–1840.

28. Tatsuguchi M, Seok HY, Callis TE, Thomson JM, Chen JF, Newman M, Rojas M, Hammond SM, Wang DZ. Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J Mol Cell Cardiol* 2007, 42:1137–1141.

29. van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci U S A* 2006, 103:18255–18260.

30. Yalçin F, Muderrisoglu H, Korkmaz ME, Ozin B. Do variable age-related secondary factors affect ventricular geometry in hypertrophic cardiomyopathy? *Adv Ther* 2002, 19:253–257.

31. Harris KS, Zhang Z, McManus MT, Harfe BD, Sun X. Dicer function is essential for lung epithelium morphogenesis. *Proc Natl Acad Sci U S A* 2006, 103:2208–2213.

32. van Rooij E, Sutherland LB, Qi XX, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 2007, 316:575–579.

33. Callis TE, Pandya K, Seok HY, Tang RH, Tatsuguchi M, Huang ZP, Chen JF, Deng Z, Gunn B, Shumate J, et al. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J Clin Invest* 2009, 119: 2772–2786.

34. Cheng YH, Ji RR, Yue JM, Yang J, Liu XJ, Chen H, Dean DB, Zhang CX. MicroRNAs are aberrantly expressed in hypertrophic heart: Do they play a role in cardiac hypertrophy? *Am J Pathol* 2007, 170:1831–1840.

35. Satoh M, Minami Y, Takahashi Y, Tabuchi T, Nakamura M. Expression of microRNA-208 is associated with adverse clinical outcomes in human dilated cardiomyopathy. *J Card Fail* 2010, 16:404–410.

36. Abraham WT, Gilbert EM, Lowes BD, Minobe WA, Larrabee P, Roden RL, Dutcher D, Sederberg J, Lindenfeld JA, Wolfel EE, et al. Coordinate changes in myosin heavy chain isoform gene expression are selectively associated with alterations in dilated cardiomyopathy phenotype. *Mol Med* 2002, 8:750–760.

37. Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeyer TJ, Quaife RA, Roden RL, Dutcher DL, Robertson AD, Voelkel NF, et al. Changes in gene expression in the intact human heart—downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *J Clin Invest* 1997, 100:2315–2324.

38. Nakao K, Minobe W, Roden R, Bristow MR, Leinwand LA. Myosin heavy chain gene expression in human heart failure. *J Clin Invest* 1997, 100:2362–2370.

39. Weiss A, Leinwand LA. The mammalian myosin heavy chain gene family. *Annu Rev Cell Dev Biol* 1996, 12: 417–439.

40. Ito M, Roeder RG. The TRAP/SMCC/Mediator complex and thyroid hormone receptor function. *Trends Endocrinol Metab* 2001, 12:127–134.

41. Morkin E. Control of cardiac myosin heavy chain gene expression. *Microsc Res Tech* 2000, 50:522–531.

42. Lin Z, Murtaza I, Wang K, Jiao J, Gao J, Li PF. MiR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy. *Proc Natl Acad Sci U S A* 2009, 106: 12103–12108.

43. Takeda Y, Yoneda T, Demura M, Miyamori I, Mabuchi H. Sodium-induced cardiac aldosterone synthesis causes cardiac hypertrophy. *Endocrinology* 2000, 141: 1901–1904.

44. Tse J, Powell JR, Baste CA, Priest RE, Kuo JF. Isoproterenol-induced cardiac hypertrophy: modifications in characteristics of beta-adrenergic receptor, adenylate cyclase, and ventricular contraction. *Endocrinology* 1979, 105:246–255.
45. Clipstone NA, Crabtree GR. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nature* 1992, 357:695–697.
46. Molkentin JD. Calcineurin and beyond: cardiac hypertrophic signaling. *Circ Res* 2000, 87:731–738.
47. Wilkins BJ, Molkentin JD. Calcineurin and cardiac hypertrophy: Where have we been? Where are we going? *J Physiol* 2002, 541:1–8.
48. Willis MS, Ike C, Li L, Wang DZ, Glass DJ, Patterson C. Muscle ring finger 1, but not muscle ring finger 2, regulates cardiac hypertrophy *in vivo*. *Circ Res* 2007, 100: 456–459.
49. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006, 38:228–233.
50. Carè A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, et al. MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 2007, 13:613–618.
51. Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circ Res* 2007, 100:416–424.
52. Brown JH, Del Re DP, Sussman MA. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ Res* 2006, 98:730–742.
53. Ke Y, Wang L, Pyle WG, de Tombe PP, Solaro RJ. Intracellular localization and functional effects of P21-activated kinase-1 (Pak1) in cardiac myocytes. *Circ Res* 2004, 94:194–200.
54. Sucharov C, Bristow MR, Port JD. miRNA expression in the failing human heart: functional correlates. *J Mol Cell Cardiol* 2008, 45:185–192.
55. van Rooij E, Olson EN. MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J Clin Invest* 2007, 117:2369–2376.
56. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D, et al. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell* 2007, 129:303–317.
57. Ikeda S, He A, Kong SW, Lu J, Bejar R, Bodyak N, Lee KH, Ma Q, Kang PM, Golub TR, et al. MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes. *Mol Cell Biol* 2009, 29:2193–2204.
58. Obata K, Nagata K, Iwase M, Odashima M, Nagasaka T, Izawa H, Murohara T, Yamada Y, Yokota M. Overexpression of calmodulin induces cardiac hypertrophy by a calcineurin-dependent pathway. *Biochem Biophys Res Commun* 2005, 338:1299–1305.
59. Zhang CL, McKinsey TA, Chang SR, Antos CL, Hill JA, Olson EN. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* 2002, 110:479–488.
60. Morin S, Charron F, Robitaille L, Nemer M. GATA-dependent recruitment of MEF2 proteins to target promoters. *EMBO J* 2000, 19:2046–2055.
61. Han M, Sayed D, He M, Abdellatif M. miRNA-26 plays essential role in myocyte survival and hypertrophy by regulating GATA4. *Circulation* 2009, 120:S732.
62. Chan JA, Krichevsky AM, Kosik KS. microRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 2005, 65:6029–6033.
63. Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res* 2005, 33:1290–1297.
64. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene* 2007, 26: 2799–2803.
65. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* 2008, 456:980–984.
66. Haghikia A, Hilfiker-Kleiner D. miRNA-21: a key to controlling the cardiac fibroblast compartment? *Cardiovasc Res* 2009, 82:1–3.
67. Roy S, Khanna S, Hussain SRA, Biswas S, Azad A, Rink C, Gnyawali S, Shilo S, Nuovo GJ, Sen CK, et al. MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. *Cardiovasc Res* 2009, 82:21–29.
68. Miano JM, Ramanan N, Georger MA, Bentley KLD, Emerson RL, Balza RO, Qi X, Weiler H, Ginty DD, Misra RP, et al. Restricted inactivation of serum response factor to the cardiovascular system. *Proc Natl Acad Sci U S A* 2004, 101:17132–17137.
69. Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* 2003, 113:329–342.
70. Norman C, Runswick M, Pollock R, Treisman R. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. *Cell* 1988, 55:989–1003.
71. Wang ZG, Wang DZ, Hockemeyer D, McAnally J, Nordheim A, Olson EN. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* 2004, 428:185–189.
72. Zhang X, Azhar G, Chai J, Sheridan P, Nagano K, Brown T, Yang J, Khrapko K, Borras AM, Lawitts J,

et al. Cardiomyopathy in transgenic mice with cardiac-specific overexpression of serum response factor. *Am J Physiol Heart Circ Physiol* 2001, 280:H1782–H1792.

73. Zhang X, Chai J, Azhar G, Sheridan P, Borras AM, Furr MC, Khrapko K, Lawitts J, Misra RP, Wei JY, et al. Early postnatal cardiac changes and premature death in transgenic mice overexpressing a mutant form of serum response factor. *J Biol Chem* 2001, 276:40033–40040.
74. Ai J, Zhang R, Li Y, Pu JL, Lu YJ, Jiao JD, Li K, Yu B, Li ZQ, Wang RR, et al. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. *Biochem Biophys Res Commun* 2009, 391:73–77.
75. Ji X, Takahashi R, Hiura Y, Hirokawa G, Fukushima Y, Iwai N. Plasma miR-208 as a biomarker of myocardial injury. *Clin Chem* 2009, 55:1944–1949.
76. Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, Pinto YM. miR423-5p as a circulating biomarker for heart failure. *Circ Res* 2010, 106: 1035–1039.
77. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, et al. miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell Metab* 2006, 3:87–98.
78. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* 2005, 438:685–689.