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MicroRNAs and Cardiovascular Diseases

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Abstract

MicroRNAs (miRNAs) are a class of small noncoding RNAs that have gained status as important regulators of gene expression. Recent studies have demonstrated that miRNAs are aberrantly expressed in the cardiovascular system under some pathological conditions. Gain- and loss-of-function studies using *in vitro* and *in vivo* models have revealed distinct roles for specific miRNAs in cardiovascular development and physiological function. The implications of miRNAs in cardiovascular disease have recently been recognized, representing the most rapidly evolving research field. In the present article, the currently relevant findings on the role of miRNAs in cardiac diseases will be updated and the target genes of these miRNAs are summarized.

Introduction

MicroRNAs (miRNAs) are endogenous, single-stranded, small, ~22-nucleotide noncoding RNAs. miRNAs are generally regarded as negative regulators of gene expression by inhibiting translation and/or promoting mRNA degradation by base pairing to complementary sequences within the 3' untranslated region (3'UTR) of protein-coding mRNA transcripts [1-3]. However, recent studies have suggested that miR-binding sites are also located in 5' untranslated regions (UTRs) or open reading frames (ORFs), and the mechanism(s) of miR-mediated regulation from these sites has not been defined [4-7]. The first miRNA assigned to a specific function was lin-4, which targets lin-14 during temporal pattern formation in *C. elegans* [8]. Since then, a variety of miRNAs have been discovered. More than 500 miRNAs have been cloned and sequenced in humans, and the estimated number of miRNA genes is as high as 1,000 in the human genome [9]. Each miRNA regulates dozens to hundreds of distinct target genes, thus miRNAs are estimated to regulate the expression of more than a third of human protein-coding genes [10]. On the other hand, accumulating evidence suggests that miRNAs are regulated by various mechanisms, including epigenetic changes [11]. Thus, the full picture of miRNA-associated regulation is quite complex.

Cardiovascular disease is the leading cause of morbidity and mortality in developed countries. The pathological process of the heart is associated with an altered expression profile of genes that are important for cardiac function. Much of our current understanding of cardiac gene expression indicates that it is controlled at the level of transcriptional regulation, in which transcription factors associate with their regulatory enhancer/promoter sequences to activate gene expression [12]. The regulation of cardiac gene expression is complex, with individual genes controlled by multiple enhancers that direct very specific

expression patterns in the heart. miRNAs have reshaped our view of how cardiac gene expression is regulated by adding another layer of regulation at the posttranscriptional level.

The implications of miRNAs in the pathological process of the cardiovascular system have recently been recognized, and research on miRNAs in relation to cardiovascular disease has now become a rapidly evolving field. Here we review the published studies that show the involvement of miRNAs in different aspects of cardiovascular system.

miRNAs have been reviewed recently in several specific systems, including cardiovascular development, cardiac fibrosis, and arrhythmia [13-15]. As is common to all new and rapidly moving fields, it is relatively hard to obtain an overview of the available knowledge from reviews. In this review, we will summarize the current understanding of miRNA function in the heart and outline details of what is known about their putative targets. In addition, we will review several aspects of the regulation of miR expression and their roles in cell signaling that have not been addressed in a cardiovascular context in the accompanying manuscripts [11,16].

Cardiac development

One approach to study the comprehensive requirements of miRNAs during vertebrate development has been to create mutations in the miRNA processing enzyme Dicer. Several groups disrupted the Dicer gene in mice and reported that the loss of Dicer resulted in embryonic lethality at embryonic day (E)7.5, before body axis formation, due to either a loss of pluripotent stem cells [17] or impaired angiogenesis in the embryo [18]. Dicer1 hypomorphic expression mice also exhibited corpus luteum insufficiency and infertility due to impaired angiogenesis [19]. To understand the role of miRNAs in the developing heart, cardiac-specific deletion of Dicer was generated using Cre recombinase expressed under the control of endogenous Nkx2.5 regulator elements. Nkx2.5-Cre is active from E8.5, during heart patterning and differentiation, but only after the initial commitment of cardiac progenitors. These embryos showed cardiac failure due to a variety of developmental defects, including pericardial edema and underdevelopment of the ventricular myocardium, which resulted in embryonic lethality at E12.5. These phenotypes are consistent with the defects during heart development observed in zebrafish embryos devoid of Dicer function [20]. It will be important to determine whether Dicer is required for earlier stages of cardiogenesis before E8.5. Dicer activity is also required for normal functioning of the mature heart, as adult mice lacking Dicer in the myocardium have a high incidence of sudden death, cardiac hypertrophy, and reactivation of the fetal cardiac gene program [21].

Recently, Rao *et al.* generated mice with a muscle-specific deletion of the DiGeorge syndrome critical region gene 8 (DGCR8), which is another component of the miRNA biogenesis pathway, by the use of muscle creatine kinase (MCK)-Cre mice and a conditional floxed allele of the DGCR8 [22]. Since endogenous MCK expression reportedly peaks around birth and declines to 40 % of peak levels by day 10, these mice can be used to determine the importance of the miRNA pathway in muscle homeostasis. The phenotypic outcome was similar to the cardiac-specific dicer deficient mice, showing a critical role for miRNAs in maintaining cardiac function in mature cardiomyocytes. The authors also reported that miRNA(miR)-1 was quite enriched and accounted for nearly 40% of all known miRNAs in the adult heart by the deep sequencing of a small RNA library. Since this result is quite different from the reports by others, further experiments using a high-throughput analyzer are required.

Two widely conserved miRNAs that display cardiac- and skeletal-muscle-specific expression during development and in adults are miR-1 and miR-133, which are derived from a common precursor transcript [23,24]. miR-1 has been shown to regulate cardiac

differentiation [23,25-27] and control heart development in mice by regulation of the cardiac transcription factor Hand2 [23]. The importance of miR-1 in cardiogenesis was shown in mice lacking miR-1-2 [26]. Although miR-1-1, which targets the same sequences as miR-1-2, is still expressed in miR-1-2-deficient mice, these mice had a spectrum of abnormalities, including ventricular septal defects in a subset that suffer early lethality, cardiac rhythm disturbances in those that survive, and a striking myocyte cell-cycle abnormality that leads to hyperplasia of the heart with nuclear division persisting postnatally. With regard to miR-133, mice lacking either miR-133a-1 or miR-133a-2 are normal, whereas deletion of both miRNAs causes lethal ventricular-septal defects in approximately half of the double-mutant embryos or neonates [28]. miR-133a double mutant mice that survive to adult succumb to dilated cardiomyopathy and heart failure. Dysregulation of cell cycle control genes and aberrant activation of the smooth muscle gene program were observed in double-mutant mice, which may be attributable to the upregulation of the miR-133a mRNA targets, Cyclin D2 and serum response factor (SRF).

Previous experiments indicated that miRNAs are broadly important for proper organ development. However, their individual temporal and spatial functions during organogenesis are largely unknown. The heart has been a particularly informative model for such organ patterning, with numerous transcriptional networks that establish chamber-specific gene expression and function [29]. Zebrafish have a two-chambered heart containing a single atrium and ventricle separated by the atrioventricular canal (AVC)[30]. miR-138 is specifically expressed in the ventricular chamber of the zebrafish heart. Temporal-specific knockdown of miR-138 in zebrafish by morpholino and antagomiR led to expansion of AVC gene expression into the ventricular chamber and failure of ventricular cardiomyocytes to fully mature, indicating that miR-138 is required for cardiac maturation and patterning in zebrafish[31]. It is worth noting that miR-138 is required during a discrete developmental window, 24-34 hour post-fertilization. Transcriptional networks that establish chamber-specific gene expression are highly conserved and miR-138 is also conserved across species, ranging from zebrafish to humans, thus, it will also be interesting to determine whether miR-138 plays similar roles in patterning of the mammalian four-chambered heart.

Cardiac hypertrophy

Because cardiac hypertrophy, an increase in heart size, is associated with nearly all forms of heart failure, it is of clinical importance that we understand the mechanisms responsible for cardiac hypertrophy. It has two forms: 1) physiological, where the heart enlarges in healthy individuals following heavy exercise and is not associated with any cardiac damage, and 2) pathological, where the size of the heart initially increases to compensate for the damage to cardiac tissue, but later on leads to a decline in the left ventricular function [32].

In the model of physiological hypertrophy, only one report has demonstrated that rats subjected to exercise training and transgenic mice with selective cardiac overexpression of a constitutively active mutant of the Akt kinase [33] had reduced levels of the muscle-specific miRNAs, miR-1 and miR-133. In line with this finding, miR-1 and miR-133 were found to be downregulated in the plantaris muscle of mice in response to functional overload [34].

Pathological hypertrophy is mainly caused by hypertension, loss of myocytes following ischemic damage, and genetic alterations that cause cardiomyopathy. Moreover, metabolic abnormality or stress can also lead to hypertrophy [35]. Pathological hypertrophy is the phenotypic endpoint that has been mostly studied in relation to miRNAs of the heart to date. In animal models of cardiac hypertrophy, whole arrays of miRNAs have indicated that separate miRNAs are upregulated, downregulated, or remain unchanged with respect to their levels in a normal heart [36-42]. In these studies, some miRNAs have been more frequently reported as differentially expressed in the same direction than others, indicating the

possibility that these miRNAs might have common roles in hypertrophy pathogenesis. For example, miR-21, miR-23a, miR-24, miR-125, miR-129, miR-195, miR-199, miR-208, and miR-212 have often been found to be upregulated with hypertrophy, whereas miR-1, miR-133, miR-29, miR-30, and miR-150 have often been found to be downregulated. Interestingly, the forced expression of individual miRNAs, such as miR-23a, miR-23b, miR-24, miR-195, miR-199a, and miR-214, found to be upregulated with cardiac hypertrophy, was sufficient to induce hypertrophic growth. More specifically, miR-195 was sufficient to drive pathological cardiac growth when overexpressed in transgenic mice [36]. Despite the interesting phenotype of these mice, neither targets nor mechanisms underlying miR-195's way of action have been discovered. In contrast to miR-195, *in vitro* overexpression of miR-150 and miR-181b, which are downregulated in cardiac hypertrophy, resulted in reduced cardiomyocyte cell size [36]. The role of miR-21 in hypertrophy is controversial [43,44]. The ability of individual miRNAs to modulate cardiac phenotypes suggests that regulated expression of miRNAs is a cause rather than simply a consequence of cardiac remodeling.

Although the levels of many miRNAs have been demonstrated to be altered in cardiac hypertrophy by a series of high-throughput miRNA microarray analyses, the transcriptional machinery that regulates the expression of miRNAs during cardiac hypertrophy and the molecular mechanisms responsible for individual miRNA-mediated effects on cardiac hypertrophy need to be studied in detail.

Transcriptional regulation of miRNAs is well studied for miR-1/miR-133. SRF is a cardiac-enriched transcription factor responsible for the regulation of organized sarcomeres in the heart [45]. SRF interacts synergistically with myocardin to activate miR-1-1 and miR-1-2 by binding to the upstream SRF-binding consensus element known as CARG box [23]. Myocyte enhancer factor 2 (MEF2) also activates transcription of the bicistronic precursor RNA encoding miR-1-2 and miR-133a-1 via an intragenic muscle-specific enhancer [46]. It was reported that nuclear factor of activated T cells isoform 3 (NFATc3), which is well-documented as playing a key role in mediating the hypertrophic signal of calcineurin, as well as other stimuli [47], regulates the expression of miR-23a. NFATc3 can bind directly to the promoter region of miR-23a and activate its expression, which may convey the hypertrophic signal by suppressing the translation of muscle specific ring finger protein 1 (MuRF1) [48]. It appears that different miRNAs have distinct mechanisms in regulating hypertrophy. miR-1 negatively regulates expression of hypertrophy-associated Calmodulin, MEF2a, and GATA4, and attenuates calcium-dependent signaling through the calcineurin-NFAT pathway [49]. miR-133 inhibits hypertrophy through targeting RhoA and Cdc42 [33]. It was reported that targets of miR-208 include thyroid hormone receptor (THR)-associated protein 1 (THRAP1) [50,51], suggesting that miR-208 initiates cardiomyocyte hypertrophy by regulating triiodothyronine-dependent repression of β -myosin heavy chain (MHC). miR-27a also regulates β -MHC gene expression by targeting TRbeta1 in cardiomyocytes[52].

An miRNA may have multiple targets and the current results do not exclude the involvement of any other molecules and/or pathways that can be regulated by miRNAs with reported functions.

Myocardial infarction and cell death

It is well established that acute myocardial infarction (MI) is a complex process in which multiple genes have been found to be dysregulated [53]. Therefore, it is reasonable to hypothesize that miRNAs could be involved in MI.

Cardiomyocyte death/apoptosis is a key cellular event in ischemic hearts. Ren *et al.* applied a mouse model of cardiac ischemia and reperfusion (I/R) *in vivo* and *ex vivo* to determine the miRNA expression signature in ischemic hearts, and found that miR-320 expression was consistently dysregulated in ischemic hearts [54]. They identified heat-shock protein 20 (HSP20), a known cardioprotective protein, as a target of for miR-320. Knockdown of endogenous miR-320 provides protection against I/R-induced cardiomyocyte death and apoptosis by targeting HSP20. The miRNA expression signature in rat hearts at 6 hours after MI revealed that miR-21 expression was significantly downregulated in infarcted areas but was upregulated in border areas [55]. Adenoviral transfer of miR-21 *in vivo* decreased cell apoptosis in the border and infarcted areas through its target gene, programmed cell death 4 (PDCD4), and activator protein 1 (AP1) pathway.

In vitro experiments showed that miR-1 and miR-133 produced opposing effects on apoptosis induced by oxidative stress in H9c2 rat ventricular cells, with miR-1 being pro-apoptotic and miR-133 being anti-apoptotic. Post-transcriptional repression of HSP60 and HSP70 by miR-1 and of caspase-9 by miR-133 contributes significantly to their opposing actions. miR-1 is also associated with the cell death pathway by inhibiting the translation of insulin like growth factor 1 (IGF)-1 [56,57].

Early ischemia or hypoxia preconditioning is an immediate cellular reaction to brief hypoxia/reoxygenation cycles that involve *de novo* protein, but not mRNA synthesis [58]. It is described as a mechanism that protects the heart against subsequent prolonged ischemia or I/R induced damage [59]. A recent study by Rane-S *et al.* revealed a unique function of miR-199a, serving as a molecular switch that triggers an immediate drop in gene expression in response to a decline in oxygen tension, possibly through selective miRNA stability and processing of the stem-loop [60]. They showed that miR-199a directly targets and inhibits translation of hypoxia-inducible-factor (HIF)-1 α and Sirtuin1. Hif-1 α regulates hypoxia-induced gene transcription and is regulated by a post-transcriptional oxygen-sensitive mechanism that triggers its prompt expression following a drop in oxygen levels. These results indicate that miR-199a is a master regulator of a hypoxia-triggered pathway and can be utilized for preconditioning cells against hypoxic damage. Since this result demonstrates a functional link between 2 key molecules that regulate hypoxia preconditioning and longevity, it would be of interest to examine the precise regulatory mechanism of miR-199a.

Recent studies have shown that some miRNAs are present in circulating blood and that they are included in exosomes and microparticles [61,62]. The levels of circulating miRNAs have been reported with several disease conditions [63,64]. In the cardiovascular diseases, studies on circulating miRNAs have been shown in rat model of myocardial injury [65]. Recently, circulating miRNAs are reported in patients with myocardial infarction [15]. From these results, it has been hypothesized that miRNAs in systemic circulation may reflect tissue damage, and for this reason, they can be used as a biomarker of myocardial infarction [66-68].

Cardiac fibrosis

Cardiac fibrosis is an important contributor to the development of cardiac dysfunction in diverse pathological conditions, such as MI, ischemic, dilated, and hypertrophic cardiomyopathies, and heart failure and can be defined as an inappropriate accumulation of extracellular matrix (ECM) proteins in the heart [69-74]. Cardiac fibrosis leads to an increased mechanical stiffness, initially causing diastolic dysfunction, and eventually resulting in systolic dysfunction and overt heart failure. In addition, fibrosis causes electrical connection disruption between cardiac myocytes, and hence increases the chance of arrhythmias. Finally, the enhanced diffusion distance for cardiac substrates and oxygen to

the cardiac myocytes, caused by fibrosis, negatively influences the myocardial balance between energy demand and supply [71,72].

It was shown that the miR-29 family, which is fibroblast enriched, targets mRNAs encoding a multitude of ECM-related proteins involved in fibrosis, including multiple Collagens, Fibrillins, and Elastin [75]. miR-29 is dramatically repressed in the border zone flanking the infarcted area in the mouse model of MI. Downregulation of miR-29 would be predicted to counter the repression of these mRNAs and enhance the fibrotic responses. Therefore, it is tempting to speculate that upregulation of miR-29 may be a therapeutic option for MI.

miR-21 is among the most strongly upregulated miRNAs in response to a variety of forms of cardiac stress [16,36,75]. Recently, Thum *et al.* showed that miR-21 is upregulated in cardiac fibroblasts in the failing heart, where it represses the expression of Sprouty homolog1 (SPRY1), a negative regulator of the extracellular signal-regulated kinase/mitogen-activated protein (ERK-MAP) kinase signaling pathway [76]. Upregulation of miR-21 in response to cardiac injury was shown to enhance ERK-MAP kinase signaling, leading to fibroblast proliferation and fibrosis. Phosphatase and tensin homologue (PTEN) has also been demonstrated to be a direct target of miR-21 in cardiac fibroblasts [77]. Previous reports characterize PTEN as a suppressor of matrix metalloproteinase-2 (MMP-2) expression [78,79]. I/R in the heart induced miR-21 in cardiac fibroblasts in the infarcted region. Thus, I/R-induced miR-21 limits PTEN function and causes activation of the Akt pathway and increased MMP-2 expression in cardiac fibroblasts.

Connective tissue growth factor (CTGF), a key molecule involved in fibrosis, was shown to be regulated by two miRNAs; miR-133 and miR-30, which are both consistently downregulated in several models of pathological hypertrophy and heart failure. [80]. The authors indicated that miR-133 and miR-30 are downregulated during cardiac disease, which inversely correlates with the upregulation of CTGF. *In vitro* experiments designed to overexpress or inhibit these miRNAs can effectively repress CTGF expression by interacting directly with the 3'UTR region of CTGF mRNA.

Together, these data indicate that miRNAs are important regulators of cardiac fibrosis and are involved in structural heart disease.

Arrhythmia

The electrical activities of the heart, the rate and force of contraction of the heart, are orchestrated by multiple categories of ion channels, which are transmembrane proteins that control the movement of ions across the cytoplasmic membrane of cardiomyocytes. Each heartbeat is initiated by a pulse of electrical excitation that begins in a group of specialized pacemaker cells and subsequently spreads throughout the heart. At rest, the membrane is selectively permeable to potassium (K^+) ions, and the electrochemical potential inside the myocyte is negative with respect to the outside. During electrical excitation, the membrane becomes permeable to Na^+ and the electrochemical potential reverses or depolarizes. Thus, sodium (Na^+) channels determine the rate of membrane depolarization. Connexin43 (Cx43) is critical for the ventricular gap junction communication, being responsible for inter-cell conduction of excitatory signals. L-type Ca^{2+} channels are mediators of Ca^{2+} influx and account for excitation-contraction coupling. L-type Ca^{2+} channels are located in sarcolemma, including the T-tubes facing the sarcoplasmic reticulum junction, and are activated by membrane depolarization. I_{CaL} is important in heart function because it modulates action potential shape and contributes to pacemaker activities in the sinoatrial and atrioventricular nodal cells. When K^+ channels open during repolarization, K^+ exits from the cell because the channels allow the passive movement of ions down their respective concentration gradients. Thus, K^+ channels govern the membrane potential and the rate of

membrane repolarization. Pacemaker channels, which carry the non-selective cation currents, are critical in generating sinus rhythm and ectopic heart beats as well. Because the heart beat is so dependent on the proper movement of ions across the surface membrane, disorders of ion channels, or channelopathies, which may result from genetic alterations in ion channel genes or aberrant expression of these genes, can render electrical disturbances predisposing to cardiac arrhythmias [81].

Recently, it was established with luciferase reporter activity and western blot analysis that gap junction protein $\alpha 1$ (GJA1) (encoding connexin43 (Cx43)) and potassium inwardly-rectifying channel, subfamily J, member 2 (KCNJ2) (encoding the K⁺ channel subunit Kir2.1) are target genes for miR-1 [82]. Cx43 is critical for inter-cell conductance of excitation [83–85], and Kir2.1 governs the cardiac membrane potential [86,87], both of which are important determinants for cardiac excitability. It was shown that miR-1 levels increased in individuals with coronary artery disease, and that when miR-1 is overexpressed in normal and infarcted rat hearts, it resulted in slowed conduction velocity and prolonged repolarization excessively and induced PVCs and arrhythmias. On the other hand, blocking miR-1 function with antisense oligoribonucleotides normalized the expression of Cx43 and Kir2.1, prevented QRS and QT prolongation, and reduced arrhythmias after MI.

Zhao *et al.* demonstrated that one of the miR-1-2 targets is the cardiac transcription factor Irx5, which represses potassium voltage-gated channel, Shal-related subfamily, member 2 (KCND2), a potassium channel subunit (Kv4.2) responsible for transient outward K⁺ current (*I_{to}*) by use of a targeted deletion technique [26]. The increase in iroquois homeobox 5 (Irx5) and Irx4 protein levels in miR-1-2 mutants corresponded well with a decrease in KCND2 expression. Their study suggests that the combined loss of Irx5 and Irx4 disrupts mouse ventricular repolarization with a predisposition to arrhythmias when miR-1 levels are enhanced.

To date, the cardiac ion channel genes that have been confirmed experimentally to be targets of miR-1 or miR-133 include GJA1/Cx43/*I_J* [82], KCNJ2/Kir2.1/*I_{K1}* [82], potassium voltage-gated channel, subfamily H (eag-related), member 2 (KCNH2)/human ether-à-go-go-related gene (HERG)/*I_{Kr}* [88], potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1)/KvLQT1/*I_{Ks}* [89], and potassium voltage-gated channel, Isk-related family, member 1 (KCNE1)/mink/*I_{Ks}* [89]. The fact that altered expression of miRNAs can deregulate expression of cardiac ion channels provided novel insight into the molecular understanding of cardiac excitability.

However, considering the inherent capacity of miRNAs to target a broad range of proteins, the link between miR-1 and arrhythmia is far from clear, and more miR-1 targets may be involved. Terentyev *et al.* investigated the effects of increased expression of miR-1 on excitation contraction coupling and Ca²⁺ cycling in rat ventricular myocytes using cellular electrophysiology and Ca²⁺ imaging [90]. They indicated that the protein phosphatase PP2A regulating subunit B56 α is potentially an important target for miR-1 in the heart, and through translational inhibition of this mRNA, miR-1 causes Ca/Calmodulin Kinase II (CaMKII)-dependent hyperphosphorylation of the ryanodine receptor (RyR2), enhances RyR2 activity, and promotes arrhythmogenic sarco(endo)plasmic reticulum (SR) Ca²⁺ release.

Thus, miR-1 may have important pathophysiological functions in the heart, and may be a potential antiarrhythmic target.

Angiogenesis and vascular diseases

Recently, a few specific miRNAs that regulate endothelial cell functions and angiogenesis have been described. Pro-angiogenic miRNAs include let7f and miR-27b [91], miR-17-92 cluster [92], miR-126 [93,94], miR-130a [95], miR-210, and miR-378, [96,97]. MiRNAs that exert anti-angiogenic effects include miR-15/16 [98,99], miR-20a/b[98], miR-92a [100], and miR-221/222 [101,102].

Inflammation is an important part of host defenses against infection and injury, but also contributes to the initiation and progression of atherosclerosis [103], [104]. The response-to-injury hypothesis describes that endothelial dysfunction caused by, for example, elevated low density lipoproteins (LDL), free radicals, hypertension, diabetes mellitus, and/or other factors is an early step in atherosclerosis [103].

Adhesion molecules expressed by activated endothelial cells play a key role in regulating leukocyte trafficking to sites of inflammation. Resting endothelial cells normally do not express adhesion molecules; however, cytokines activate endothelial cells to express adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), which mediate leukocyte adherence to endothelial cells. Harris *et al.* showed that endothelial cells predominantly express miR-126, which inhibits VCAM-1 expression [105]. On the other hand, transfection of endothelial cells with an oligonucleotide that decreases miR-126 permitted an increase in tumor necrosis factor (TNF)- α stimulated VCAM-1 expression and increased leukocyte adherence to endothelial cells.

Recently, Ji *et al.* revealed miRNAs that are aberrantly expressed in the vascular walls after balloon injury [106]. Modulating an aberrantly overexpressed miR-21, via antisense-mediated depletion, had a significant negative effect on neointimal lesion formation. They also demonstrated that PTEN and Bcl-2 were involved in miR-21-mediated cellular effects. The same group also revealed that miR-221 and miR-222 expression levels were elevated in rat carotid arteries after angioplasty [107]. Moreover, they found that the p27 (Kip1) and p57 (Kip2) were 2 target genes that were involved in miR-221- and miR-222-mediated effects on vascular smooth muscle cells (VSMC) growth. Knockdown of miR-221 and miR-222 resulted in decreased VSMC proliferation both *in vitro* and *in vivo*.

Another study demonstrated that the angiotensin II type 1 receptor (AT1R) and miR-155 are coexpressed in endothelial cells and VSMCs, and that miR-155 translationally represses the expression of AT1R [108]. The AT1R gene has been found to be highly polymorphic. In particular, a single nucleotide polymorphism (SNP) has been described in which there is an A/C transversion at position 1166 in the 3'-UTR of this gene. The increased frequency of the +1166 allele has been associated with essential hypertension, cardiac hypertrophy and MI [109-111], probably mediated by enhanced AT1R activity. Interestingly, the presence of the +1166 C-allele interrupts base-pairing complementarity within the 3'-UTR of AT1R, and thereby, decreases translational repression of human AT1R by miR-155 [108].

Thus, miR-21, miR-155, miR-126, miR-221, and miR-222 might be important modulators of vascular disease and vessel remodeling.

Heart failure

Since cardiac hypertrophy, fibrosis, arrhythmia, and coronary artery disease can cause heart failure, all of the miRNAs discussed so far are associated with this disease entity.

It is well known that heart failure is characterized by left ventricular remodeling and dilatation associated with activation of a fetal gene program triggering pathological changes in the myocardium associated with progressive dysfunction. Consistent with the reactivation

of the fetal gene program during heart failure, an impressive similarity has been found between the miRNA expression pattern occurring in human failing hearts and that seen in the hearts of 12-14-week-old fetuses [42]. Indeed, more than about 80% of the induced and repressed miRNAs were regulated in the same direction in fetal and failing heart tissue compared with the healthy adult control left ventricle tissue. The most consistent changes were upregulation of miR-21, miR-29b, miR-129, miR-210, miR-211, miR-212, and miR-423, with downregulation of miR-30, miR-182, and miR-526. Interestingly, gene expression analysis revealed that most of the upregulated genes were characterized by the presence of a significant number of the predicted binding sites for downregulated miRNAs and *vice versa*.

Recently, many profiling studies have been conducted and revealed a large number of miRNAs that are differentially expressed in heart failure, pointing to the new mode of regulation of cardiovascular diseases [2,38,40,41,49,80]. Horie *et al.* indicated that miR-133 may fine-tune glucose transporter 4 (GLUT4) via targeting kruppel-like factor-15 (KLF15) in heart failure [112] and there may be many other miRNA functions in specific disease settings. Nishi *et al.* suggested that four different miRNAs, which have the same seed sequence, regulate mitochondrial membrane potential during the transition from cardiac hypertrophy to failure [113].

miRNA exerts its role in the treatment with chemotherapeutic agent. It is suggested that the up-regulation of miR-146a after Dox treatment is involved in acute Dox-induced cardiotoxicity by targeting ErbB4 [114]. Inhibition of both ErbB2 and ErbB4 signalling may be one of the reasons why those patients who receive concurrent therapy with Dox and trastuzumab suffer from congestive heart failure (CHF).

Metabolic syndrome and cholesterol regulation

Recent reports have indicated that miR-33 controls cholesterol homeostasis based on knockdown experiments using antisense technology [115-117]. Mir-33 deficient mice were generated and the critical role for miR-33 in the regulation of ATP-binding cassette transporter A1 (ABCA1) expression and high density lipoprotein (HDL) biosynthesis was confirmed *in vivo* [118].

In humans, sterol regulatory element binding protein 1 (*SREBP1*) and *SREBP2* encode miR-33b and miR-33a, respectively [117]. It is well known that hypertriglyceremia in metabolic syndrome is caused by the insulin-induced increase in *SREBP1c* mRNA and protein levels [119,120]. Low HDL often accompanies this situation and it is possible that the reduction in HDL is caused by a decrease in ABCA1, because of the increased production of miR-33b from the insulin-induced induction of *SREBP1c*. Although it is impossible to prove this in animal models that lack miR-33b, antagonizing miR-33 could be a promising way to raise HDL levels when the transcription of both *SREBPs* is upregulated. Thus, a combination of silencing of endogenous miR-33 and statins may be a useful therapeutic strategy for raising HDL and lowering LDL levels especially for metabolic syndrome subjects.

The potential binding sites of miRNAs, which are shown in TargetScan (<http://www.targetscan.org/>), associated with cardiovascular diseases are summarized in the Table 1. Single nucleotide polymorphism (SNP) information is derived from the Single Nucleotide Polymorphism Database (dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP/>). All abbreviations are explained in Table 2.

Summary

Recent studies provide clear evidence that miRNAs modulate a diverse spectrum of cardiac functions with developmental, pathophysiological, and clinical implications. The biology of miRNAs in cardiovascular disease is a young research area and an emerging field.

Identifying the gene targets and signaling pathways responsible for their cardiovascular effects is critical for future studies.

Taken together, these recent pieces of evidence show that miRNAs play powerful roles in cardiovascular systems and are sure to open the door to previously unappreciated medical therapies.

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Table 1

Potential targets and binding sites of miRNAs associated with cardiovascular disease

MicroRNA	Targets	Gene symbol	Function	Binding site in mouse	Conservation in mouse	Binding sites in human	Conservation in human	SNPs (dbSNP)	Reference
Cardiac development									
miR-1	Hand2	HAND2	Decrease in cardiomyocyte proliferation.	Position 221-227 of Hand2 3' UTR	7mer-1A	Position 226-232 of HAND2 3' UTR	7mer-1A		[23]
miR-133a	CyclinD	CCND1	Inhibition of cell cycle progression.	ND		Position 1000-1006 of CCND1 3' UTR	7mer-1A		[28]
Cardiac hypertrophy									
miR-21	SPRY2		Promotion of cellular outgrowth.	Position 297-303 of Spry2 3' UTR	8mer	Position 235-241 of SPRY2 3' UTR	8mer		[44]
miR-23a	MuRF1	TRIM63	Induction of hypertrophy. Downstream of NFATc.	Position 257-263 of Trim63 3' UTR	7mer-m8	Position 279-285 of TRIM63 3' UTR	7mer-m8		[48]
miR-133	Nelf-A/WHSC2	WHSC2	Inhibition of cardiac hypertrophy.	Position 369-375 of Whsc2 3' UTR	8mer	Position 385-391 of WHSC2 3' UTR	7mer-m8		[33]
miR-208	THRAP1	MED13	Encoded by an intron of the α -MHC. Modulation of activity of the thyroid hormone receptor.	Position 546-552 of Med13 3' UTR	8mer	Position 564-570 of MED13 3' UTR	8mer		[50,51]
Myocardial Infarction and Cell Death									
miR-1	HSP60	HSPD1	Promotion of apoptosis, induced by H ₂ O ₂ in H9c2 cells.	Position 230-236 of Hspd1 3' UTR	7mer-m8	Position 234-240 of HSPD1 3' UTR	7mer-m8	rs12392, rs1804104	[56]
miR-1/206	IGF-1	IGF1	Increase in mitochondrial depolarization in H9c2 cells.	Position 155-161 of Igf1 3' UTR	8mer	Position 150-156 of IGF1 3' UTR	8mer	rs5031032	[57]
miR-21	PDCD4	PDCD4	Decrease of myocardial infarct size.	Position 289-295 of Pcd4 3' UTR	8mer	Position 242-248 of PDCD4 3' UTR	8mer		[55]
miR-199a	Hif-1a	HIF1A	Stabilization of p53 and inhibit apoptosis.	Position 91-97 of Hif1a 3' UTR	7mer-m8	Position 31-37 of HIF1A 3' UTR	7mer-m8		[60]
	Sirt1	SIRT1	Stabilization of p53 and inhibit apoptosis.	Position 450-456 of Sirt1 3' UTR	7mer-m8	Position 507-513 of SIRT1 3' UTR	7mer-m8		[60]
Cardiac Fibrosis									
miR-21	Spry1	SPRY1	Enhancement of ERK-MAP kinase pathway and fibroblast proliferation	Position 322-328 of Spry1 3' UTR	8mer	Position 415-421 of SPRY1 3' UTR	8mer		[76]
miR-29	collagens (Col4a5)	COL4A5	Inhibition of fibrosis in border zone of the infarcted area following coronary	Position 129-135 of Col4a5 3' UTR/Position	8mer	Position 106-112 of COL4A5 3' UTR/Position	8mer, 7mer-1A		[75]

MicroRNA	Targets	Gene symbol	Function	Binding site in mouse	Conservation in mouse	Binding sites in human	Conservation in human	SNPs (dbSNP)	Reference
			artery ligation.	410-416 of Col4a5 3'UTR		388-394 of COL4A5 3' UTR			
	fibrillin1	FBN1	Inhibition of fibrosis in border zone of the infarcted area following coronary artery ligation.	Position 405-411 of Fbn1 3' UTR. Position 655-661 of Fbn1 3'UTR	8mer, 7mer-m8	Position 415-421 of FBN1 3' UTR/Position 670-676 of FBN1 3' UTR	8mer, 7mer-m8		[75]
	elastin	ELN	Inhibition of fibrosis in border zone of the infarcted area following coronary artery ligation.	Position 37-43 of Eln 3' UTR. Position 284-290 of Eln 3'UTR	8mer	Position 38-44 of ELN 3' UTR/Position 297-303 of ELN 3' UTR/Position 310-316 of ELN 3' UTR	8mer, 8mer, 7mer-m8		[75]
miR-133	CTGF	CTGF	Inhibition of fibrosis in left ventricle (after thoracic aorta constriction)	Position 1026-1032 of CTGF 3' UTR	7mer-1A	Position 1026-1032 of CTGF 3' UTR	7mer-1A		[80]
Arrhythmia									
miR-1	GJA1	GJA1	QRS and QT prolongation	Position 477-483 of Gja1 3' UTR	8mer	Position 478-484 of GJA1 3' UTR	8mer		[82]
	KCNJ2	KCNJ2	QRS and QT prolongation	ND		Position 1076-1082 of KCNJ2 3' UTR	8mer		[82]
miR-133	ERG	ERG	Depression of Ikr and QT prolongation.	Position 274-280 of Erg 3' UTR	7mer-1A	ND			[88]
	KCNQ1	KCNQ1	QT prolongation	Position 709-715 of Kcnq1 3' UTR	7mer-m8	ND			[89]
Angiogenesis									
miR-92a	ITGA5	ITGA5	Inhibition of sprout formation and neovascularization.	ND		Position 988-994 of ITGA5 3' UTR	8mer		[100]
miR-130	HOXA5	HOXA5	Promotion of angiogenesis caused by endothelial cell tube formation in vitro.	Position 371-377 of Hoxa5 3' UTR	7mer-m8	Position 355-361 of HOXA5 3' UTR	7mer-m8		[95]
miR-210	Ephrin-A3	EFNA3	Increase of endothelial cell migration and tubulogenesis.	ND		Position 798-804 of EFNA3 3' UTR	7mer-m8		[96]
Vascular Disease									
miR-21	Bcl-2	BCL2	Activation of cell proliferation and decreased cell apoptosis	Position 703-709 of Bcl2 3' UTR	7mer-1A	Position 712-718 of BCL2 3' UTR	7mer-1A		[106]
miR-155	AT1R	AGTR1	The human AT1R polymorphism attenuates miR-155 binding.	ND		Position 83-89 of AGTR1 3' UTR	7mer-m8	rs5186	[108]

MicroRNA	Targets	Gene symbol	Function	Binding site in mouse	Conservation in mouse	Binding sites in human	Conservation in human	SNPs (dbSNP)	Reference
miR-221/222	p27(Kip1)	CDKN1B	Inhibition of cellular proliferation.	Position 202-208 of CDKN1B 3' UTR/Position 275-281 of CDKN1B 3' UTR	8mer/8mer	Position 202-208 of CDKN1B 3' UTR/Position 275-281 of CDKN1B 3' UTR	8mer/8mer		[107]
Cholesterol regulation									
miR-33	p57(Kip2)	CDKN1C	Inhibition of cellular proliferation.	Position 86-92 of CDKN1C 3' UTR	8mer	Position 86-92 of CDKN1C 3' UTR	8mer		[107]
	Abca1	ABCA1	Inhibition of HDL formation.	Position 134-140 of Abca1 3' UTR/Position 139-145 of Abca1 3' UTR/Position 149-155 of Abca1 3' UTR	8mer/7mer-m8/8mer	Position 134-140 of ABCA1 3' UTR/Position 139-145 of ABCA1 3' UTR/Position 149-155 of ABCA1 3' UTR	8mer/8mer/8mer		[115-118]

ND; not detected.

8mer: An exact match to positions 2-8 of the mature miRNA (the seed + position 8) followed by an 'A'

7mer-m8: An exact match to positions 2-8 of the mature miRNA (the seed + position 8)

7mer-1A: An exact match to positions 2-7 of the mature miRNA (the seed) followed by an 'A'

Table 2

A list of abbreviations

3'UTR	3' untranslated region
DGCR8	DiGeorge syndrome critical region gene 8
MCK	muscle creatine kinase
SRF	serum response factor
AVC	atrioventricular canal
MEF2	myocyte enhancer factor 2
NFATc3	nuclear factor of activated T cells isoform 3
MuRF1	muscle specific ring finger protein 1
THRAP	thyroid hormone receptor-associated protein
MHC	myosin heavy chain
MI	myocardial infarction
I/R	ischemia and reperfusion
HSP	heart shock protein
PDCD4	programmed cell death 4
AP1	activator protein 1
IGF-1	insulin-like growth factor-1
HIF	hypoxia inducible factor
ECM	extracellular matrix
SPRY1	sprouty homolog 1
ERK-MAPK	extracellular signal-regulated kinase/mitogen-activated protein kinase
PTEN	phosphatase and tensin homologue
MMP	matrix metalloprotease
CTGF	connective tissue growth factor
GJA1	gap junction protein a1
KCNJ2	potassium inwardly-rectifying channel, subfamily J, member 2
KCND2	potassium voltage-gated channel, Shal-related subfamily, member 2
Irx5	iroquois homeobox 5
KCNH2	potassium voltage-gated channel, subfamily H (eag-related), member 2
HERG	human ether-à-go-go-related gene
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1
KCNE1	potassium voltage-gated channel, Isk-related family, member 1
CaMK	Ca/Calmodulin Kinase
RyR2	ryanodine receptor 2
SR	sarco(endo)plasmic reticulum
LDL	low density lipoprotein
VCAM-1	vascular cell adhesion molecule 1
TNF	tumor necrosis factor
VSMC	vascular smooth muscle cell
AT1R	angiotensin II type 1 receptor
SNP	single nucleotide polymorphism

GLUT4	glucose transporter 4
KLF15	Kruppel-like factor-15
CHF	congestive heart failure
ABCA1	ATP-binding cassette transporter A1
SREBP	sterol regulatory element binding protein
HDL	high density lipoprotein