The Emerging Role of MicroRNAs in Cardiac Remodeling and Heart Failure

Vijay Divakaran, Douglas L. Mann

Abstract—Recent studies have suggested a potentially important role for a family of tiny regulatory RNAs, known as microRNAs (miRNAs or miRs), in the control of diverse aspects of cardiac function in health and disease. Although the field of miRNA biology is relatively new, there is emerging evidence that miRNAs may play an important role in the pathogenesis of heart failure through their ability to regulate the expression levels of genes that govern the process of adaptive and maladaptive cardiac remodeling. Here, we review the biology of miRNAs in relation to their role in modulating various aspects of the process of cardiac remodeling, as well as discuss the potential application of miRNA biology to the field of heart failure. (Circ Res. 2008;103:1072-1083.)

Key Words: heart failure ■ cardiac remodeling ■ microRNAs ■ neurohormonal activation ■ arrhythmia

The Emerging Role of MicroRNAs in Cardiac Remodeling and Heart Failure

Role of MicroRNAs in Cardiac Development

MicroRNAs and Angiogenesis

Eric Olson, Guest Editor

Biology of MicroRNAs

As reviewed elsewhere,1,2 miRNAs are noncoding RNAs that pair with specific “target” mRNAs and negatively regulate their expression through translational repression or mRNA degradation (gene silencing). The binding specificity of miRNAs depends on complementary base pairing of ≈7-nt region at the 5’ end of the microRNA with the 3’ untranslated region (UTR) of the corresponding mRNA target. However, because miRNAs are conserved more than the whole length of the mature miRNA, it is likely that the remainder of the miRNA (ie, outside of the 7 nucleotide region that pairs with the 3’ UTR of the mRNA) may also play a role in determining the target mRNA. As shown in Figure 1, binding of miRNAs to their cognate target mRNAs commonly leads to decreased expression of target genes through translation repression or mRNA degradation. Increased expression levels of miRNAs can also result in the “paradoxical” upregulation of previously suppressed target genes either directly, by decreasing the expression of inhibitory proteins and/or transcription factors, or indirectly, by inhibiting the expression levels of inhibitory miRNAs. Alternatively, decreased expression levels of inhibitory miRNAs can lead directly to increased target gene expression.4

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According to miRBase (http://microrna.sanger.ac.uk), the online reference repository of information on miRs, there are currently more than 6200 published mature miRNA sequences, including more than 700 miRNAs in humans. Moreover, additional miRNAs are continually being identified. The most common approach that has been used to study miRNAs thus far takes into account 2 characteristic features of miRs, that is, their tissue specificity and their disease specificity. With respect to tissue specificity, profiling miRNAs across various tissues reveals that miR-1, let-7, miR-26a, miR-30c, miR-126-3p, and miR-133 are highly expressed in the murine heart (18.5 weeks). However, at present we have very little information with respect to the temporal and spatial expression profiles of miRNAs in the heart. Disease-specific changes in miRNA expression have been studied using microarrays to determine which miRNAs are up- or downregulated in response to various forms of tissue injury. By identifying the signature miRNAs that are regulated in the heart, investigators have been able to use sophisticated computational approaches to predict the potential array of mRNA targets to which miRNAs can bind, which has led to the identification of previously unrecognized targets within disease pathways of interest. With that said, target prediction with the existing prediction algorithms remains challenging and requires careful experimental validation.

Expression Profiles of MicroRNAs in Experimental Models and Human Heart Failure

At the time of this writing, our understanding of the role that miRNAs play in heart failure is limited and is based largely on studies that have examined the miRNA expression profiles obtained from explanted hearts from patients with heart failure or studies that have examined miRNA microarrays in experimental models that lead to pathological remodeling of the heart, such as transaortic banding (TAB) of the aorta, cardiac-restricted overexpression of calcineurin, or cardiac-restricted overexpression of constitutively active mutant of Akt kinase. Another approach that has been used is to perform miRNA microarrays on cultured cardiac myocytes that have been stimulated with peptides that mimic neurohormonal activation and/or that have been shown to provoke myocyte hypertrophy in vitro, such as angiotensin (Ang) II, endothelin-1, and phenylephrine (PE).

Human studies of miRNA expression profiles in heart failure are limited by the lack of standardized protocols, the small numbers of patients in the studies, and the high degree of variability in expression levels between patients. Two independent groups have performed microarrays on RNA isolated from nonfailing and failing hearts. Ikeda et al have obtained miRNA expression profiles in human heart failure using left ventricular (LV) samples from nonfailing hearts or hearts from patients with ischemic or dilated cardiomyopathy or aortic stenosis. Interestingly, these authors were able to use a miRNA-based classifier to correctly classify the miRNA profiles into 1 of the above four disease-specific categories in 60% of the samples, suggesting that miRNA expression profiles are sufficiently distinct to predict clinical etiology with modest accuracy. Thum et al compared microarray data of miRNA expression profiles from nonfailing, failing, and fetal human hearts and observed that when compared to nonfailing hearts, 87% of the miRs that were upregulated and 84% of the miRs that were downregulated in the failing hearts were regulated concordantly in fetal heart tissue.

Figure 1. Mechanism for miRNA regulation of target mRNA levels. Stress signals (such as hemodynamic overload) activate signal transduction pathways that lead to either the upregulation or downregulation of specific miRNAs. Stress signals that increase the expression levels of miRNAs can result in the down regulation of several target miRNAs through gene silencing or, more commonly, translational blockade of the target miRNA. Alternatively, a stress-induced decrease in the expression levels of inhibitory miRNAs can lead to upregulation of previously suppressed target genes. Ultimately, it is the miRNA-induced pattern of change in gene expression that contributes to the resultant disease phenotype. Reproduced from Mann by permission of the Massachusetts Medical Society. Copyright © 2007.
suggesting that alterations in miRNAs levels may contribute to activation of the “fetal gene” program, which is among the signatures of hypertrophied and failing myocardium.

To better compare and contrast the altered expression profiles of miRNAs in various animal models of heart disease and in human heart failure, we have generated a 2D graphical map (Figure 2) that displays the relevant human and experimental studies in columns with the accompanying changes in miRNA expression levels depicted in rows. Analogous to conventional heat maps that have been used for gene and miRNA arrays, we have used red to indicate changes in miRNAs that are reported to be significantly upregulated and green to indicate miRNAs that were reported to be significantly downregulated. miRNA expression that was reported as unchanged is depicted in black, whereas miRNAs that

Figure 2. miRNA expression profiles in experimental models and human heart failure. A PubMed search (May to June 2008) was conducted using the MeSH titles: “microRNA,” “heart disease,” and/or “heart failure.” A total of 2314 articles were identified, of which 614 review articles were excluded. The content of the 1696 original articles were reviewed for relevance with respect to the role of miRNAs in cardiac remodeling. Six studies reported global miRNA expression data (miRNA profiling) using miRNA microarrays, of which 3 evaluated miR expression in 2 or more experimental model systems.8,9,11–14 Differentially expressed miR candidates that were observed in at least 2 studies are graphically displayed with the relevant studies in columns and the accompanying changes in miRNA expression illustrated in rows. Red indicates miRs that were significantly upregulated; green, miRs that were significantly downregulated; black, no change in miR expression levels; white, miRs that were either unreported, equally expressed or not significantly different \(P>0.05\) between disease phenotype and controls.
were either not reported or whose change in expression was not statistically interpretable were left uncolored. However, unlike a conventional heat map, the color intensity of the miRNAs depicted in Figure 2 has not been adjusted to reflect the magnitude of change in miRNA expression. Using this approach, we obtained miRNA expression profiles on 172 miRs from a total of 10 different experimental and clinical models, of which 5 were in human tissue, 4 were in vivo studies in animals, and 1 was an in vitro study in cultured cardiac myocytes. For the sake of brevity and clarity, we did not graphically display the 101 miRNAs whose expression levels were changed in only a single study (see the online data supplement for the entire analysis, available at http://circres.ahajournals.org). The remaining 71 candidate miRNAs whose expression levels changed significantly in 2 or more studies are illustrated in Figure 2. Inspection of this map reveals that for miRNAs with increased levels of expression, there was good concordance between human heart failure and experimental models of pathological remodeling. Indeed, 25 miRNAs (7b, 7c, 10b, 15b, 21, 23a, 23b, 24, 27a, 27b, 29a, 103, 125b, 140*, 195, 199a, 199a*, 199b, 208, 210, 211, 214, 330, 341, 424) were upregulated in one or more myocardial samples from failing human hearts and experimental models, suggesting that changes in miRNA expression patterns in experimental models may provide further insight into our understanding LV remodeling in human heart failure. Interestingly, the expression profile of miRNAs with decreased expression levels were far less concordant in experimental models and human heart failure samples. Indeed, there were only 10 miRNA species (1, 10a, 26b, 30a_5p, 30b, 30c_150, 218, 451, 499) that were downregulated in one or more myocardial samples from failing human hearts and experimental models. It should be emphasized that different microarray platforms were used in the above studies, which may account for some of the observed differences in miRNA regulation.

To better understand how miRNAs may play a role in cardiac remodeling and heart failure, it is useful to recognize that cardiac remodeling represents the anatomic summation of a series of changes in the biology of the cardiac myocyte and the myocardium, including the volume of cardiac myocytes and the volume/composition of the extracellular matrix (ECM) (see the Table). As discussed below, there is emerging evidence that miRNAs play an important role both in the changes in the biology of the cardiac myocyte, as well as changes in the myocardium.

**MicroRNAs Involved in Cardiac Myocyte Hypertrophy**

In both animal models and the human heart, it is generally held that changes in the biology of the cardiac myocyte (see the Table) are the primary initiating events that lead to cardiac remodeling, although it should be noted that cardiac remodeling can occur in the absence of myocyte dysfunction in some experimental models. One of the principal changes that occurs in the biology of the failing cardiac myocyte is an increase in cell size (hypertrophy). Based on the extant literature, there is evidence that various microRNAs control and/or modulate key components of the hypertrophic process in cardiac myocytes, including reactivation of the so-called fetal gene program. Indeed, the extant experimental literature suggests that miR-1, miR-18b, miR-21, miR-133, miR-195, and miR-208 play important roles in modulating cardiac hypertrophic growth.

### Table. Overview of LV Remodeling

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**miR-1 and miR-133**

miR-1 and miR-133 are preferentially expressed in cardiac and skeletal muscle and have been shown to regulate differentiation and proliferation of these cells. Although miR-1 and miR-133 form part of the same bicistronic unit, they are expressed as separate transcripts. Both miR-1 and miR-133 appear to play an important role in the remodeling of the heart that occurs during cardiogenesis. During cardiac development miR-1 levels are low, whereas its cognate target, Hand2 (a transcription factor that controls cardiac myocyte proliferation), is expressed at high levels, thereby promoting cardiac myocyte proliferation. As the heart develops, miR-1 levels increase and Hand2 protein levels decrease as cardiac myocytes exit the cell cycle and differentiate in mature myocytes. In relation to the present discussion, levels of miR-1 and miR-133 are decreased in heart failure models (Figure 2). Studies by Sayed et al demonstrated that levels of miR-1 were suppressed as early as 1 day after aortic banding and remained depressed 7 days after TAB. Independently, Care et al demonstrated reduced expression of miR-1 and miR-133 in 3 models of cardiac hypertrophic, namely, TAB, mice with cardiac-restricted overexpression of constitutively active Akt kinase and rats subjected to adaptive cardiac hypertrophy following endurance training. These same investigators confirmed that the expression level of miR-1 and miR-133 were decreased in the hearts of patients with hypertrophic cardiomyopathy or atrial dilation. Unfortunately, this study did not
examine miR-1 or miR-133 expression levels in myocardial samples from human heart failure.

The functional significance of the findings with respect to miR-1 has been demonstrated in gain-of-function studies in vitro and in vivo. For example, overexpression of miR-1 in cultured neonatal myocytes partially inhibited phosphorylation of ribosomal S6 protein and inhibited the computationally predicted in silico targets of miR-1 that were related to cardiac growth, including Ras GTPase-activating protein, cyclin-dependent kinase 9, fibronectin, and Ras homolog expressed in brain. miR-1 overexpression also suppressed myocyte spreading induced by serum and endothelin-1 and expression of atrial natriuretic factor (ANF).

Overexpression of miR-133 resulted in suppression of protein synthesis and inhibition of hypertrophic growth in PE- or endothelin-1-treated neonatal mouse cardiac myocytes and upregulation of fetal genes, including those encoded by ANF, skeletal and cardiac α-actin, and α-myosin heavy chain (αMHC) and βMHC. Loss-of-function studies showed that sequestering endogenous miR-133 using a targeted 3′ UTR decoy sequence resulted in marked cell hypertrophy and increased protein synthesis, increased fetal gene expression, and perinuclear localization of ANF, consistent with a potential role for miR-133 in suppressing hypertrophic growth. Furthermore, treatment of mice using an antisense RNA oligonucleotide (termed an antagonir) targeted to miR-133 resulted in cardiac hypertrophy and reinduction of the fetal gene program. Taken together, these studies suggest that downregulation of miR-1 and miR-133 allows for the increased expression (release) of growth-related genes that are responsible for cardiac hypertrophy. However, it is not clear whether these miRNAs contribute to the adverse cardiac remodeling that occurs in heart failure, insofar as the majority of studies in human tissue, save for 1 study, have not shown that miR-1 and miR-133 are downregulated. Of note, the 1 study that did show that miR-1 and miR-133 were downregulated in human tissue examined dilated atria and myomectomy samples from patients with hypertrophic cardiomyopathy, rather than LV myocardial specimens from patients with heart failure.

**miR-21**

The role of miR-21 in cardiac myocyte hypertrophy is controversial at present. Two studies have shown that miR-21 is highly upregulated in mouse models of pressure-overload hypertrophy post-TAB. Moreover, Ang II- and PE-induced cardiac myocyte hypertrophy is accompanied by a 4- to 5-fold increase in miR-21 expression in isolated cardiac myocytes. Antisense mediated knockdown of miR-21 partially inhibited PE- or Ang II–induced cell growth and protein synthesis in neonatal rat ventricular myocytes. In contrast, a different group of investigators has shown that antisense knockdown of miR-21 provokes hypertrophy and increased expression of fetal genes in cultured neonatal cardiac myocytes. The reason(s) for the discrepancy between these studies is unclear but may be that modulation of hypertrophic growth by miR-21 in myocytes is through an indirect mechanism, rather than a direct targeting effect of miR-21 on hypertrophy-related genes. Indeed, miR-21 has no validated gene targets that are related to cardiac myocyte hypertrophy. Unfortunately, further investigations into the role of miR-21 in myocyte hypertrophy have been hampered, insofar as exogenously administered premiR-21 fails to be processed in cardiac myocytes, resulting in the inability to overexpress mature miR-21 in these cells.

**Other Candidate miRNAs Involved in Cardiac Myocyte Hypertrophy**

Based on microarray data from aortic banding studies, calcineurin transgenic mouse models and human heart failure samples, van Rooij and colleagues identified 7 miRNAs that were upregulated and 4 miRNAs that were coordinately downregulated. Five of the miRNAs that were upregulated, miR-23a, -23b, -24, -195, and -214, provoked cardiac myocyte hypertrophy when transfected into neonatal cardiac myocytes (Figure 3), whereas transfection of miR-199a resulted in elongated spindle shapes myocytes that were reminiscent of the elongated cardiac myocytes observed in dilated cardiomyopathy. Of the miRNAs that were downregulated, miR-150 and-181b caused a reduction in cardiac myocyte size when transfected into cells. Cardiovascular-
Figure 4. Cardiac-specific overexpression of miR-195 is sufficient to drive cardiomyopathy. Hematoxylin/eosin-stained sections show that overexpression of miR-195 induces cardiac hypertrophic growth at 2 weeks of age that progresses to a dilated phenotype within 6 weeks. Moderate and high expression indicate transgenic mouse lines with 26- and 29-fold expression of miR-195, resulting in cardiac hypertrophy and dilated cardiomyopathy, respectively. The bottom 2 images show a high magnification of the hematoxylin/eosin section, indicating severe myocyte disorganization in the miR-195 transgenic (TG) animals compared with wild-type (WT). Adapted from van Rooij et al by permission of the National Academy of Sciences. Copyright © 2006.

overexpression of miR-195 resulted in a dilated cardiac hypertrophic phenotype at 6 weeks of age that was accompanied by exaggerated myocyte hypertrophy and activation of the fetal gene ensemble (Figure 4). In contrast, cardiac-restricted overexpression of miR-214 had no effect on cardiac phenotype. Of interest to the present discussion, miR-195 was significantly upregulated in 5 of the 10 studies illustrated in Figure 2, including human heart failure. Viewed together, these studies suggest that miR-195 may play an important role in adverse cardiac remodeling. Thum et al expressed 3 miRNAs that were upregulated in human heart failure (miR-21, miR-129, and miR-212) and showed that transfection of a single miRNA had little effect on cell morphology, the simultaneous overexpression of all 3 miRNAs resulted in myocyte hypertrophy and reinduction of the fetal gene program.

MicroRNAs Involved in Regulating Excitation–Contraction Coupling

Excitation–contraction coupling refers to the cascade of biological events that begins with cardiac action potential and ends with myocyte contraction and relaxation. Classic studies from explanted failing hearts have shown that patients with end-stage heart failure exhibit decreased contractility and impaired relaxation, which is believed to be secondary to changes in the abundance and/or phosphorylation state of critical calcium (Ca\(^{2+}\)) regulatory proteins that are thought to play an important role in cross-bridge activation and relaxation. An additional defect in myocyte function in hypertrophy and heart failure is thought to occur secondary to changes in the actin and myosin myofibrillar cross-bridges. Indeed, early studies showed that myofibrillar ATPase was reduced in the hearts of patients who died of heart failure and that these abnormalities in ATPase activity could be explained by an isoform switch from αMHC, which hydrolyzes ATP rapidly and is expressed in the adult heart, to βMHC, which hydrolyzes more slowly and is expressed in the fetal heart. Whereas αMHC accounts for approximately 33% of MHC mRNA in normal human myocardium, the abundance of αMHC mRNA decreases to ≈2% in the failing heart. Given the aforementioned link between expression levels of miRNAs and the development of cardiac myocyte hypertrophy, it is logical to consider that miRNAs might also regulate the Ca\(^{2+}\) regulatory proteins involved in excitation–contraction coupling that are downregulated in cardiac hypertrophy. However, at the time of this writing, there is no information on how, or whether, miRNAs target genes that are related to Ca\(^{2+}\) handling in the heart. This statement notwithstanding, there are interesting data that suggest that miRNAs affect cross-bridge cycling.

miR-208

Studies have shown that miR-208 is upregulated in response to a hemodynamic pressure overload, as well as in human heart failure. Olson and colleagues showed that miR-208, a cardiac specific microRNA that is encoded within an intron of the αMHC gene, is coordinately regulated with αMHC. Mice deficient for (miR-208\(^{-/-}\)) appeared phenotypically normal at baseline; however, these mice had a blunted hypertrophic response following TAB, as well as decreased myocardial fibrosis (Figure 5). Of note, miR-208\(^{-/-}\) mice did not express βMHC in response to pressure overload. Although mice with cardiac-restricted overexpression of miR-208 had increased expression of βMHC, they did not develop pathological remodeling. Taken together, these observations suggest that miR-208 is required for the development of cardiac hypertrophy and myocardial fibrosis and that miR-208 is a positive regulator of βMHC gene expression.

miR-21

Alterations in the expression or activity, or both, of myofilament regulatory proteins has also been proposed as a potential mechanism for the decrease in cardiac contractile function in heart failure, including changes in myosin light chains and the troponin–tropomyosin complex. As noted above, some, but not all, studies have implicated a role for miR-21 in regulating hypertrophic growth in cardiac myocytes. Although not yet studied in human heart failure, miR-21 targets the 3’ UTR of tropomyosin and inhibits its translation in tumor cells. Further studies will be necessary to delineate the role of miR-21 in cross-bridge cycling in heart failure.

MicroRNAs Involved in Regulating the Cytoskeleton

The cytoskeleton of cardiac myocytes consists of actin, the intermediate filament desmin, the sarcomeric protein titin, and α- and β-tubulin, which form the microtubules by polymerization. Vinculin, talin, dystrophin, and spectrin represent a separate group of membrane-associated cytoskeletal
proteins. Disruption of cytoskeletal and/or membrane-associated proteins has been implicated in the pathogenesis of heart failure in numerous studies. Indeed, the loss of integrity of the cytoskeleton, with a resultant loss of linkage of the sarcomere to the sarcolemma and ECM, would be expected to lead to contractile dysfunction at the myocyte level, as well as at the myocardial level. At present, there is limited evidence that miRNAs are involved in regulating the cytoskeleton; insofar as relatively few studies have examined these targets.

miR-1 and miR-133
As noted above, the decreased expression of miR-1 and miR-133 allows for the increased expression of growth-related genes that are responsible for cardiac hypertrophy. Adenoviral-mediated overexpression of miR-1 suppressed sarcomeric α-actin organization that is normally observed in serum-deprived neonatal cardiac myocytes. The effects of miR-1 on cytoskeletal reorganization were also thought to be responsible for the inhibition of endothelin-induced cell spreading in neonatal myocytes. Similarly, adenoviral transfection with miR-133 inhibited the reorganization of the actin myofilaments observed in hypertrophic growth of cardiac myocytes (Figure 6). Two validated gene targets of miR-133 are RhoA and Cdc42, a fact that is of interest in that members of the Rho family of GTP binding proteins are involved in myofibrillar rearrangement and are, therefore, important for cell motility and contractility. miR-1 also targets a number of cytoskeletal-related proteins, including microtubule-related proteins, kinectins, actin binding proteins, and cadherins. Insofar as miR-1 and miR-133 do not appear to be downregulated in human heart failure, the significance of the aforementioned miR-induced changes in the cytoskeleton vis-a-vis the biology of the failing myocyte remains to be determined.

MicroRNAs Involved in Myocardial Alterations in the Failing Heart
The alterations that occur in failing myocardium may be categorized broadly into those that affect the number of cardiac myocytes, as well as those changes that occur in the volume and composition of the ECM (Table). With respect to the changes that occur in cardiac myocyte component of the myocardium, there is increasing evidence that suggests that progressive myocyte loss, through necrotic, apoptotic, or autophagic cell death pathways, may contribute to progressive cardiac dysfunction and LV remodeling.

MicroRNAs Involved in Regulating Cell Fate
Progressive myocyte loss secondary to apoptosis occurs in failing hearts and contributes to progressive cardiac dysfunction and LV remodeling. Apoptosis requires activation of a...
phylogenetically conserved ensemble of proteins belonging to the extrinsic (death receptor–mediated) or intrinsic (mitochondrial) pathways that ultimately lead to activation of executioner caspases and myocyte loss. The extensive synergistic regulation of pro- and antiapoptotic proteins in response to stress signals raises the possibility that miRs may be involved in regulating programmed cell death.

**miR-21**

miR-21 is upregulated in various cancers (malignant glioblastoma, colorectal carcinoma, cervical adenocarcinoma) and, based on its in silico–predicted proapoptotic gene targets, has been proposed as a potential antiapoptotic miRNA. Knockdown of miR-21 in glioblastoma cells leads to caspase activation and apoptotic cell death,7 and depletion of miR-21 in vascular smooth muscle cells leads to a dose-dependent increase in apoptosis and decrease in cell proliferation.23 As noted above, several independent groups have shown that the expression levels of miR-21 are increased 2- to 4-fold in the heart post-TAB.8,11 Although miR-21 levels were not examined in relation to myocyte apoptosis in these studies, the upregulation of miR-21 may represent a prosurvival response in response to hemodynamic pressure overload. In this regard, it is interesting to note that the antiapoptotic protein Bcl-2 is an indirect target of miR-21.23

**miR-1 and miR-133**

Although miR-1 and miR-133 act synergistically in controlling myocyte proliferation and differentiation (see above), the extant literature suggests that these miRNAs have opposite roles with respect to regulating cell fate. Xu et al24 demonstrated that overexpression of miR-1 in H9c2 rat myoblasts provoked apoptotic cell death, which was partially rescued by treatment with miR-133. Similar effects on oxidative stress–induced apoptosis were observed in H2O2-treated H9c2 cells, wherein cotransfection with miR-1 led to 60% reduction in the IC50 value necessary for oxidative stress–induced DNA fragmentation, and cotransfection with miR-133 resulted in a 40% increase in the IC50 value required for oxidative stress–induced DNA fragmentation. Qualitatively similar results were obtained when rat neonatal ventricular myocytes were exposed to H2O2.24 Subsequent computational analyses predicted that heat shock protein (HSP)60 and HSP70 were targets for miR-1 and that caspase-9 was a target for miR-133. Indeed, experimentally miR-1 suppressed HSP60 and HSP70 protein levels by ≈70% and ≈60%, respectively, whereas miR-133 decreased protein levels of caspase-9 and caspase-9 activity levels in rat H9c2 cells. The authors postulated that the relative levels of miR-1 and miR-133 may determine cell fate.24 In this regard, it is noteworthy that miR-1 levels are overexpressed in coronary disease and are highly upregulated in ischemic myocardium,25 wherein apoptosis is an important mode of cell death.

**MicroRNAs Involved in Regulating Angiogenesis**

Adequate myocardial perfusion is essential for maintaining cardiac myocyte viability, particularly in the failing heart, wherein LV filling pressures are elevated and subendocardial perfusion is compromised. Prior studies have suggested that fewer capillaries are present in end-stage dilated cardiomyopathy26,27 and postpartum cardiomyopathy.28 The failure to appropriately regulate angiogenesis and vasculogenesis in the failing heart can result in myocardial ischemia with subsequent loss of cardiac myocytes and/or interstitial fibrosis and may thus contribute to progressive myocardial dysfunction in heart failure. There is increasing evidence that miRNAs may regulate vascular integrity, angiogenesis, and wound repair. Indeed, a number of pro- and antiangiogenic microRNAs have been identified that target key proteins involved in endothelial tube formation, as well as endothelial cell proliferation, migration, and apoptosis.29 However, it should be emphasized that many of the miRNAs that have been studied thus far have been identified in neoplastic tissue and may therefore not be relevant to cardiovascular physiology. Nonetheless, 2 recent studies have implicated miR-126 in angiogenesis and regulation of vascular integrity.30,31

**miR-126**

Srivastava and colleagues found that miR-126 was enriched in endothelial cells derived from mouse embryonic stem cells and in developing mouse embryos. Subsequent studies in zebrafish showed that miR-126 knockdown resulted in loss of vascular integrity, as well as hemorrhage during embryonic development. These authors found that miR-126 directly repressed 2 negative regulators of the vascular endothelial growth factor (VEGF) pathway, namely Sprouty-related protein (Spred1) and the phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2/p85-β). Increased expression of Spred1 or inhibition of VEGF signaling in zebrafish resulted in defects similar to miR-126 knockdown.30 Analogous studies in mice revealed that endothelial cell–restricted expression of miR-126–mediated developmental angiogenesis, whereas targeted deletion of miR-126 resulted in leaky vessels, hemorrhaging, and partial embryonic lethality caused by a loss of vascular integrity and defects in endothelial cell proliferation, migration, and angiogenesis. Relevant to the present discussion, the subset of mutant mice that were not embryonic lethal had impaired myocardial neovascularization following myocardial infarction. It was further shown that miR-126 enhanced the proangiogenic actions of VEGF and fibroblast growth factor and promoted formation of new blood vessels by repressing the expression of Spred1.31 Taken together, these findings illustrate that a miR-126 is sufficient to regulate vascular integrity and angiogenesis. However, the relevance of these experimental findings with respect to human heart failure is unclear, insofar as miR-126 is upregulated in failing human hearts.

**MicroRNAs Involved in Regulating the ECM**

Changes within the ECM constitute the second important myocardial adaptation that occurs during cardiac remodeling. The myocardial ECM consists of a basement membrane, a fibrillar collagen network that surrounds myocytes, proteoglycans, and glycosaminoglycans, and biologically active signaling molecules. Important changes occur in the ECM during cardiac remodeling, including changes in fibrillar collagen synthesis and degradation, as well as changes in the degree of collagen cross-linking. One of the histological...
signatures of advancing heart failure is the progressive increase in collagen content of the heart (myocardial fibrosis). The increased fibrous tissue would be expected to lead to increased myocardial stiffness, which would theoretically result in decreased myocardial shortening for a given degree of afterload. In addition, myocardial fibrosis may provide the structural substrate for atrial and ventricular arrhythmias, thus potentially contributing to sudden death.

miR-208
As noted above, Olson and colleagues have shown that miR-208, a cardiac-specific miRNA encoded within an intron of the αMHC gene, is required for cardiac fibrosis in response to hemodynamic pressure overload. Indeed, miR-208–deficient mice (mir-208+−) were resistant to developing fibrosis following TAB, as well as cardiac-restricted overexpression of calcineurin, a calcium/calmodulin-dependent phosphatase that provokes pathological remodeling of the heart. Thus, miR-208 appears to be required for the development of cardiac fibrosis.

miR-29
The miR-29 family is comprised of 3 members, mirR-29a to -c, that are expressed from 2 bicistronic miRNA clusters: miR-29b-1 is coexpressed along with miR-29a, whereas the second copy of miR-29b (miR-29b-2) is coexpressed with miR-29c. In an effort to identify miRNAs that were dysregulated in acute myocardial infarction, van Rooij and colleagues observed that miR-29 was downregulated in the left anterior descending artery and in the border zone of hearts border zone mouse hearts following acute occlusion of the left coronary artery. The increased fibrous tissue would be expected to lead to increased collagen content of the heart (myocardial fibrosis).

MicroRNAs Involved in Neurohormonal Activation

Extensive clinical and experimental data support the point of view that heart failure progresses as a result of the overexpression of biologically active molecules (collectively referred to as neurohormones) that are capable of exerting deleterious effects on the heart and the peripheral circulation. The portfolio of biologically active molecules that have been described thus far include those that belong to the adrenergic nervous system (norepinephrine) and renin–angiotensin system (RAS) (Ang II and aldosterone), which are responsible for maintaining cardiac output through increased retention of salt and water, peripheral arterial vasoconstriction, and contractility, as well as inflammatory mediators (tumor necrosis factor, interleukin-1, and interleukin-6) that are responsible for cardiac repair and remodeling. Although the role of miRNAs in regulating the components of RAS and the adrenergic system is not well understood, several recent observations are worth noting. As discussed above, treating isolated neonatal cardiac myocytes with ligands that mimic neurohormonal activation in vitro, such as PE, endothelin-1, or Ang II is sufficient to upregulate miRs that are linked to hypertrophic growth, including miR-21, -23a, and -133. As discussed below, miRNAs may also contribute to “neurohormonal activation” through enhanced RAS signaling.

miR-155
miR-155 is sufficient to suppress the levels of the Ang II type 1 receptor (AT1R) in primary human lung fibroblasts by binding directly to the 3’ UTR of AT1R mRNA. Functional studies demonstrated that transfection of miR-155 into human primary lung fibroblasts resulted in a significant reduction in Ang II-induced activation of the extracellular signal-related kinase (ERK)1/2. Furthermore, when fibroblasts were transfected with an antisense inhibitor to miR-155, endogenous AT1R expression and Ang II–induced ERK1/2 activation were increased significantly. Interestingly, a silent polymorphism (+1166 A/C) in the human AT1R gene that is associated with cardiovascular disease (possibly because of enhanced AT1R activity) is a target of miR-155. When the +1166 C allele is present, base-pairing complementarity to the 3’ UTR of the AT1R is interrupted, thereby decreasing the ability of miR-155 to interact with the cis-regulatory site. The result is that miR-155 is no longer able efficiently attenuate the translation of the AT1R gene, leading to increased endogenous levels of AT1R and increased Ang II–induced activation of ERK1/2. Whether these observations have any relevance in the setting of heart failure is unknown and will require further study.

mir-21
The role of mir-21 in cardiac hypertrophy and cell apoptosis has been discussed above. Of note, Ang II has been shown to upregulate miR-21 expression in human adrenal cells. Moreover, overexpression of miR-21 resulted in increased aldosterone secretion and cell proliferation in these cells. Although performed in a cell type that resides outside the heart, this study raises the interesting possibility that increased miRNA-21 expression levels outside of the heart may contribute to adverse cardiac remodeling through dysregulation of Ang II–mediated signaling and enhanced aldosterone secretion.

MicroRNAs Involved in Electrophysiological Alterations

In addition to cardiac remodeling and neurohormonal activation, there are important changes in ion channel function and expression in the failing heart that lead to alterations in the electric phenotype of both atrial and ventricular myocytes. This “electrophysiological remodeling” renders the heart more vulnerable to ventricular arrhythmias that underlie sudden cardiac death. Although the exact role of microRNAs in the development of atrial and ventricular arrhythmias in
the setting of clinical heart failure is unknown, the experimental literature suggests that cardiac specific microRNAs are sufficient to provoke cardiac arrhythmias.

**miR-1 and miR-133**
The pacemaker function of sinoatrial and atrioventricular cells and cells in the ventricular conducting system depends on their ability to undergo diastolic depolarizations. The ionic mechanism underlying pacemaker depolarizations is governed by activation of the hyperpolarization-activated current ($I_{f}$). The HCN ion channel subunit gene family encodes hyperpolarization-activated cation channels that are permeable to Na\(^+\) and K\(^+\). It has been suggested that upregulation of HCN channels may contribute to enhanced automaticity and arrhythmias in heart failure. Relevant to the present discussion, downregulation of miR-1 and miR-133 has been associated with increased protein levels of HCN2/HCN4 in hypertrophic hearts. Given than miR-1 and miR-133 (Figure 2) are downregulated in heart failure models, these findings may have relevance to the development of cardiac arrhythmias in heart failure. Changes in the inward rectifier K\(^+\) current ($I_{K1}$) have been suggested to play an important role in the genesis of both atrial and ventricular arrhythmias. In cardiac myocytes, $I_{K1}$ establishes the resting membrane potential and modulates the terminal phase of repolarization. Transfection of miR-1 into healthy or infarcted rat hearts resulted in significant widening of the QRS complex, prolonged the QT interval, and exacerbated arrhythmias, whereas knockdown of miR-1 in infarcted rat hearts using an antisense resulted in decreased arrhythmias. Importantly, miR-1 targets genes that encode for connexin 43 (GJA1) and the Kir2.1 potassium channel subunit (KCNJ2), which may partially explain the proarrhythmogenic potential of miR-1. miR-1 and miR-133 have been shown experimentally to target KCNE1 and KCNQ1, which are the genes that comprise the channels that regulates the slow delayed rectifier current ($I_{Ks}$). However, given that miR-1 and miR-133 are downregulated in heart failure models, the significance of these studies with respect to $I_{K1}$ and $I_{Ks}$ and the development of cardiac arrhythmia in heart failure is uncertain.

**Conclusion**
In the preceding discussion, we have reviewed the emerging importance of miRNAs in the failing heart. Although our understanding of miRNA biology in heart failure is embryonic at present, the experimental literature reviewed herein suggests that miRNAs contribute to adverse/pathological remodeling and hence are sufficient to contribute to disease progression in heart failure. As shown in Figure 7, miRNAs regulate key components of the remodeling process, including cardiac myocyte biology, cell fate, ECM remodeling, and neurohormonal activation. Given that miRNAs are coordinately regulated in response to stress signals, and given that miRNAs regulate the expression levels of gene networks that determine the so-called “heart failure phenotype,” it is tempting to speculate that miRNAs, acting singly or in combination, may be responsible for modulating the transition from adaptive to pathological cardiac remodeling. Although predicting the success of future therapeutic strategies in the field of heart failure is fraught with difficulty, one can envision that miRNA biology may contribute to our understanding of heart failure in at least 3 important areas. First, as modern computational methodologies continue to improve, it is likely that studying the expression patterns of miRNAs will lead to the identification of interesting new disease targets that are not obvious with classic “pathway-driven” (eg, neurohor-
monal) approaches. Moreover, this type of strategy would, for the first time, engender therapeutic strategies that directly address the fundamental biology of heart failure, insofar as they would be directed at the targets that are responsible for cardiac remodeling. Second, expression profiling of miRNAs in circulating leukocytes and/or myocardial tissue may one day be used to help to identify personalized strategies for heart failure patients, as is currently being done in the field of oncology. 42,43 For example, expression profiling of miRNAs might also be used to help in the earlier implantation and/or removal of circulatory assist devices if investigators are able to reliably determine miRNA signatures for worsening heart failure and/or myocardial recovery. Third, it is possible that certain miRNAs may themselves become therapeutic targets in heart failure. As discussed in depth elsewhere,57–59 certain miRNAs may themselves become therapeutic targets in heart failure and/or myocardial recovery. It is possible that they would be directed at the targets that are responsible for cardiac remodeling. Second, expression profiling of miRNAs in circulating leukocytes and/or myocardial tissue may one day be used to help to identify personalized strategies for heart failure patients, as is currently being done in the field of oncology. 42,43 For example, expression profiling of miRNAs might also be used to help in the earlier implantation and/or removal of circulatory assist devices if investigators are able to reliably determine miRNA signatures for worsening heart failure and/or myocardial recovery. Third, it is possible that certain miRNAs may themselves become therapeutic targets in heart failure. As discussed in depth elsewhere,57–59 certain miRNAs may themselves become therapeutic targets. As with all things in heart failure, progress in this field will require the collaborative efforts between basic scientists and clinical scientists to perform the requisite target validation and the careful clinical phenotyping that are required to move from the bench to the bedside. Given how little we know about miRNAs in heart failure at present, and given that there are seemingly an infinite number of interactions between miRNAs and their cognate target genes, some of which have yet to be characterized, it is almost certain that our learning curve will be extremely steep now and for the foreseeable future.

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**Disclosures**

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**References**


The Emerging Role of MicroRNAs in Cardiac Remodeling and Heart Failure
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Data Supplement for "The Emerging Role of MicroRNAs in Cardiac Remodeling and Heart Failure"

To compare and contrast the altered expression profiles of miRNAs in various animal models of heart disease and in human heart failure we have generated a two-dimensional graphical map (see Online Figure I) that displays the relevant human and experimental studies in columns with the accompanying changes in miRNA expression levels depicted in rows. Analogous to conventional heat maps that have been used for gene and miRNA arrays, we have used red to indicate changes in miRNAs that are reported to be up-regulated, and green to indicate miRNAs that were reported to be down-regulated. miRNA expression that was reported as unchanged is depicted in black, whereas miRNAs that were either not reported, or whose change in expression was not statistically interpretable were left uncolored. The definitions for changes that were considered to either up- or down-regulated in the various reports are given in the table below:

**Online Table I**

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental model</th>
<th>Criterion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Human HF vs control</td>
<td>&gt; 1.5 fold change</td>
<td>Thum et al.¹</td>
</tr>
<tr>
<td>B.</td>
<td>Human HF vs control</td>
<td>&gt; 1.3 fold change</td>
<td>van Rooij et al.²</td>
</tr>
<tr>
<td>C.</td>
<td>Human DCM vs control</td>
<td>p &lt; 0.05 and q &lt; 5%</td>
<td>Ikeda et al.³</td>
</tr>
<tr>
<td>D.</td>
<td>Human ICM vs control</td>
<td>p &lt; 0.05 and q &lt; 5%</td>
<td>Ikeda et al.³</td>
</tr>
<tr>
<td>E.</td>
<td>Human Aortic stenosis vs control</td>
<td>p &lt; 0.05 and q &lt; 5%</td>
<td>Ikeda et al.³</td>
</tr>
<tr>
<td>F.</td>
<td>Mouse TAC vs sham</td>
<td>p &lt; 0.05</td>
<td>Sayed et al.⁴</td>
</tr>
<tr>
<td>G.</td>
<td>Mouse TAC vs sham</td>
<td>30 % change</td>
<td>Cheng et. al.⁵</td>
</tr>
<tr>
<td>H.</td>
<td>Mouse TAC (or CnA Tg) vs sham</td>
<td>&gt; 1.5 or &lt; 0.5 fold change</td>
<td>van Rooij et al.⁵</td>
</tr>
<tr>
<td>I.</td>
<td>Mouse TAC vs sham at 14 days</td>
<td>None</td>
<td>Tatsuguchi et al.⁶</td>
</tr>
<tr>
<td>J.</td>
<td>Cardiomyocytes treated with PE</td>
<td>&gt; 1.5 fold or &lt; 0.75 fold</td>
<td>Tatsuguchi et al.⁶</td>
</tr>
</tbody>
</table>

Key: TAC: trans-aortic constriction, HF: heart failure, DCM: dilated cardiomyopathy, ICM: ischemic cardiomyopathy, CnA Tg: calcineurin transgenic mouse, PE: phenylephrine, q: false discovery rate. Reference article numbers refer to the references in the online supplement.
However, unlike a conventional heat-map, the color intensity of the miRNAs depicted in Online Figure I has not been adjusted to reflect the magnitude of change in miRNA expression. Using this approach, we obtained miRNA expression profiles on 172 miRs from a total of ten different experimental and clinical models, of which 5 were in human tissue, 4 were in-vivo studies in animals, and 1 was an in vitro study in cultured cardiac myocytes. An abridged version of this map that illustrates microRNAs that were changed in at least two studies is shown in Figure 2 of the main body of the manuscript.

References


5. 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*
Online Figure I: miRNA expression profiles in experimental models and human heart failure. A Pubmed search (May-June 2008) was conducted using the MeSH titles: ‘microRNA’, ‘heart disease’ and/or ‘heart failure.’ A total of 2314 articles were identified of which 614 review articles were excluded. The content of the 1696 original articles were reviewed for relevance with respect to the role of miRNAs in cardiac remodeling. Six studies reported global miRNA expression data (miRNA profiling) using micro-RNA micro-arrays, of which 3 evaluated miR expression in two or more experimental model systems. Red indicates miRs that were significantly up-regulated; green indicates miRs that were significantly down-regulated; black indicated no change in miR expression levels; white indicates miRs that were either unreported, equally expressed or not significantly different (p>0.05) between disease phenotype and controls (Reference article numbers in Online Figure I refer to the references in the Data Supplement).