Abstract: Disturbances in gene expression as a result of perturbed transcription or posttranscriptional regulation is one of the main causes of cellular dysfunction that underlies different disease states. Approximately a decade ago, the discovery of microRNAs in mammalian cells has renewed our focus on posttranscriptional regulatory mechanisms during pathogenesis. These tiny posttranscriptional regulators are differentially expressed in almost every disease that has been studied to date and can modulate expression of a gene via specifically binding to its messenger RNA. Because of their capacity to simultaneously target multiple functionally related genes, they are proving to be potentially powerful therapeutic agents/targets. In this review, we focus on the microRNAs that are differentially regulated in the more common cardiovascular pathologies, their targets, and potential function. (Circ Res. 2012;110:638-650.)

Key Words: angiogenesis ■ failure ■ hypertrophy ■ ischemia ■ microRNA
the mature form; the argonaute protein, consisting of four isoforms, of which argonaute 2 or slicer can cleave the target mRNA; the P-body protein PW1827; the human immunodeficiency virus transactivating response RNA-binding protein, known to recruit argonaute 2 to miRNA RNA-inducing silencing complex; and fragile X mental retardation protein, which associates with polyribosomes; and others whose miRNA RNA-inducing silencing complex-related functions have not been fully elucidated yet. This complex binds a target gene via partial sequence complementarity between the miRNA and, preferentially, a conserved site within the 3' untranslated region of the gene. Through this interaction, it can inhibit translation by any of three different mechanisms: (1) inhibiting translation at a postinitiation step without reducing mRNA abundance, polyadenylation, or polyribosomal content; (2) inhibiting translation initiation via a cap-dependent and poly(A)-dependent mechanism; and (3) inducing deadenylation of mRNAs independently of inhibition of translation.

Perturbation in gene expression is one of the main underlying mechanisms that contribute to disease development. Whereas transcriptional regulation of some genes has been recorded, others are regulated by posttranscriptional means. The discovery of miRNAs recently has advanced our understanding regarding the latter mode of gene regulation in development and disease, and is detailed for cardiovascular diseases, in particular, in this article.

miRNAs in Cardiac Hypertrophy and Failure: Cardiac-Enriched miR-1 and miR-133 in Cardiac Hypertrophy and Failure

miRNAs are differentially regulated during cardiac hypertrophy and failure in both rodents and humans. Reprogramming of gene expression in the heart to recapitulate the pattern of the fetal/neonatal heart is an established mechanism that contributes to the development of cardiac hypertrophy. Therefore, an analogous change in the expression pattern of miRNAs supports their role in the underlying pathogenesis. MiR-1 is one of the earliest miRNAs that are downregulated in the heart after the induction of pressure overload, which precedes the increase in cardiac mass, suggesting that it may potentially be involved in initiating the process. In accordance, miR-1 levels are also lower in genetic models of hypertrophy that include the calcineurin- and AKT-overexpressing transgenic mouse hearts. Furthermore, miR-1 is reduced in the hypertrophied left ventricle of acromegalic patients and in those who have aortic stenosis with normal ejection fraction. Experiments in cultured myocytes and those involving inhibition of miR-1 in vivo suggest that a decrease in miR-1 is necessary for an increase in cell size and mass. Some of its targets that were validated in these studies include insulin-like growth factor, calmodulin, and myocyte enhancer factor 2A, all of which are known to contribute to the development of cardiac hypertrophy. In contrast, the fluctuations in miR-1 levels were less consistent in the failing heart. For example, Matkovich et al. and Thum et al. recorded an increase in miR-1, Ikeda et al., Sucharov et al., and Naga Prasad et al. detected a reduction in miR-1 in ischemic and nonischemic dilated cardiomyopathies that were associated with a significant reduction in percent ejection fraction. Therefore, it would appear that an increase in cardiac mass is accompanied by downregulation of miR-1; however, as hypertrophy progresses into dilatation and contractile dysfunction, miR-1 concentrations return to normal or above normal levels.

miR-133, which is also a muscle-enriched miRNA that shares the same primary transcript as miR-1, is likewise downregulated during cardiac hypertrophy. Moreover, like miR-1, its knockdown via antagomirs was sufficient for inducing cardiac hypertrophy and the characteristic increase in some of the fetal genes. In contrast to these results, genomic knockout of miR-133a-1 or miR-133a-2, each of which contribute to approximately 50% reduction in miR-133 levels, which is equivalent to the decrease observed in cardiac hypertrophy, exhibited normal cardiac growth and function under quiescent or pressure overload conditions. This discrepancy in outcomes is not readily explainable. However, a transgenic mouse model overexpressing miR-133 that compensates for its downregulation during hypertrophy failed to inhibit cardiac enlargement, suggesting that downregulation of miR-133 in the heart is not required for the induction of cardiac hypertrophy.

The Regulation of Contractile Fibers by myomiRs During Cardiac Hypertrophy and Failure

While miR-1 and miR-133 are enriched in both skeletal and cardiac muscle, miR-208 is cardiac-restricted. It has two isoforms, miR-208a and miR-208b, that are contained within the introns of Myh6 (α-myosin heavy chain) and Myh7 (β-myosin heavy chain) genes, respectively. Homozygous miR-208a mice (miR-208a−/−) are developmentally normal, with the expected normal levels of α and β myosin heavy chain (Myh) at birth, although the adult mice fail to upregulate βMyh in response to thyroid hormone inhibition. Likewise, pressure overload in the heart did not induce the
expected increase in βMyh. In addition, there was absence of hypertrophic growth and fibrosis after an increase in workload, accompanied by reduced levels of miR-499. 38 Interestingly, transgenic overexpression of miR-499 in the miR-208a−/− background restored the normal response to thyroid hormone inhibition, suggesting that it is a downstream effector of miR-208 and is sufficient for mediating its effect. 39 The miR-499 was shown to target Sox6, 39 a negative regulator of αMyh, 40 and accordingly the transgene reduced its levels in the miR-208a−/− mice. Paradoxically, though, ablation of miR-499 did not recapitulate the miR-208a−/− phenotype.

Similar to miR-208a−/−, miR-208b−/− mice have no overt developmental defects. 39 Mirroring the expression pattern of their host genes, miR-208a is predicted to be the main source of mature miR-208 in the adult mouse heart, whereas miR-208b is mainly expressed during fetal and early postnatal cardiac development. Accordingly, the lack of a phenotype after targeted deletion of the latter offers a more conclusive result regarding the role of miR-208 in cardiac development. Thus, we can conclude that miR-208 is not required for the expression of βMyh during cardiac development, but it is necessary for the increase in its expression that occurs during cardiac hypertrophy and hypothyroidism. In contrast, in vivo delivery of antimiR-208 or antimiR-499 results in almost complete elimination of endogenous βMyh in the adult mouse, where it appeared uncharacteristically abundant in this study. 41 In accordance, antimiR-208a increased the survival rate of hypertensive rats and reduced the isovolumic relaxation time, which could be attributable to the reduction in βMyh. In addition, myocyte hypertrophy and perivascular fibrosis were also reduced.

In contrast to the absence of any spontaneous defects associated with the miR-208a/b knockout mice, cardiac-specific transgenic expression of miR-208a induced cardiac hypertrophy within 4 months of age. 42 The phenotype, though, did not exhibit downregulation of αMyh, upregulation of atrial natriuretic factor, or changes in any of the hypertrophy-related miRNAs. It was, however, associated with a decrease in myostatin, a negative regulator of hypertrophy, 43 and a validated target of miR-208a, which could potentially explain its antihypertrophic effect. 42 Additionally, these mice exhibited defects in conduction in the form of first-degree or second-degree atrioventricular bundle block compared to the miR-208a−/− mice, which experienced atrial fibrillation (AF). The latter could be attributed to a reduction in connexin 40 and its transcriptional regulator Hop; however, these proteins were unchanged in the miR-208a trans-
genic mice. Meanwhile, GATA4, a validated target of miR-208a and a positive regulator of connexin 40,44 is more abundant in the miR-208a−/− mice, although this obviously does not reconcile with the observed downregulation of connexin 40 in this model. Similar to the miR-208 transgenic, a cardiac-specific transgenic mouse model of the downstream effector of miR-208, miR-499 is sufficient for inducing cardiac hypertrophy.45 However, under these conditions, its validated target, Sox6, was not altered in the transgenic hearts.

miRNAs That Regulate Cardiac Fibrosis
One of the most highly and consistently increased miRNAs during cardiac hypertrophy is miR-21.19,23,25,27 Its function in this context has been elusive, because cardiac-specific transgenic mice appeared structurally and functionally normal and did not differ from wild-type mice in its response to pressure overload.46–47 Whereas miR-21 increases in the whole heart during pathological hypertrophy, it was suggested that its increase in myofibroblasts is more pronounced than it is in the myocytes.47 In the former cell type, it was shown to directly target and suppress sprouty-1, resulting in enhanced extracellular signal-regulated kinase 1/2 phosphorylation and myofibroblast survival, which, in turn, indirectly contributes to the increase in fibrosis during cardiac hypertrophy.47 This was confirmed by treating mice with a miR-21 antagonist, which resulted in the reduction of both fibrosis and cardiac hypertrophy.54 Moreover, miR-21 knockdown with LNA-modified antisense oligonucleotides, as it shows that global genomic ablation of miR-21, or gene/mechanism compensated for the genomic ablation of miR-21 during hypertrophy.56 Thus, the jury is still out regarding the role of miR-21 during hypertrophy.

Although cardiac hypertrophy is not inhibited as a result of normalizing miR-133 levels; apoptosis and fibrosis were restored to normal levels.36 Connective tissue growth factor is one of the main factors that mediate fibrosis in the heart.49,50 However, there is no evidence yet as to whether this contributes to the upregulation of βMyh and contractile dysfunction during cardiac hypertrophy in vivo. More recently, da Costa Martins et al.62 demonstrated that miR-199b, which is upregulated during cardiac hypertrophy, is also a direct transcriptional target of NFATc. In this case, miR-199b targets and suppresses the NFATc kinase dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1a, which is a negative regulator of NFAT. Accordingly, mutant mice overexpressing miR-199b in the heart, or those hemizygous for dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1a, exhibited exaggerated hypertrophy in response to pressure overload. Conversely, antisense “antagonist” treatment normalized miR-199a levels and not only prevented hypertrophy and failure but also almost completely reversed them.

The transcription factor GATA4 plays an essential role in the development of cardiac hypertrophy63,64 as it regulates the expression of some of the fetal genes that are re-expressed in this condition.65 In accordance, its DNA-binding activity and abundance increase during hypertrophy.56,67 Recent data utilizing state-of-the-art RNA polymerase II immunoprecipitation followed by deep sequencing established that the increase in GATA4 expression in cardiac myocytes is not attributable to an increase in the transcription of its gene.68 This prompted the search for miRNAs, which were downregulated during cardiac hypertrophy that targeted GATA4 3’ untranslated region. The miR-26a/b, which is highly expressed in the heart and other organs, inversely correlated with the expression of GATA4 levels in the developing and hypertrophied hearts.
It was proven to directly target GATA4 and, subsequently, reduce cardiac growth. However, although knockdown of miR-26 was sufficient for inducing GATA4 in the heart in vivo, it was insufficient for inducing cardiac hypertrophy. Another validated target of miR-26b is phospholipase C beta-1, which proved to be a major negative regulator of miR-26b, creating a double-negative feedback loop characteristic of many miRNAs and their targets. This is the first proven example of a gene that is primarily regulated by a posttranscriptional miRNA-dependent mechanism during disease, underscoring the functional relevance of miRNAs.

miRNAs in Ischemic Heart Disease

miRNAs That Regulate Apoptosis During Ischemia

Myocardial ischemia results from insufficient blood flow to the myocardium, which leads to apoptotic, autophagic, and necrotic cell death, with subsequent inflammatory cell infiltration, fibrosis and contractile dysfunction. MiRNAs’ expression is rapidly perturbed during myocardial ischemia or cellular hypoxia (Fig. 2). Some of the miRNAs that are downregulated that affect cell viability during ischemia include miR-320, miR-21, miR-494, and miR-24. MiR-320 is downregulated during ischemia/reperfusion (I/R) and is responsible for upregulation of its target HSP20. Further suppression of this miRNA with an antagonist successfully reduces infarct size after I/R. On the other hand, miR-21, which is ubiquitously expressed, has emerged as a major inhibitor of cell apoptosis in myocytes as well as a plethora of other cell types. It is acutely downregulated during myocardial ischemia, specifically within the ischemia zone, where replenishing it reduces the infarct size and retards the development of heart failure. These results were corroborated by experiments conducted in isolated cardiac myocytes in which miR-21 was proven to inhibit hypoxia-induced apoptosis by targeting Fas ligand and programmed cell death. Furthermore, miR-21 is induced by the AKT pathway and partly mediates its antiapoptotic effect via suppression of Fas ligand. Conversely, miR-21 activates AKT through suppression of phosphatase and tensin homolog (PTEN) via a double-negative feedback loop. In addition to myocytes, miR-21 is enriched in myofibroblasts and infiltrating cells within the infarct zone. In these cells, it was shown to inhibit PTEN and induce upregulation of metalloprotease-2, thereby promoting fibrosis. As discussed, miR-21 also has a profibrotic effect during cardiac hypertrophy by promoting the survival of myofibroblasts; however, this particular mechanism was not examined in the ischemic heart.

Similar to miR-21, miR-494 is also reduced in the infarct zone, where normalizing its levels by transgenic overexpression reduces infarct size and improves contractility. Intriguingly, miR-494 targets both proapoptotic genes that include PTEN, Rho-associated coiled-coil containing protein kinase-1, and calcium/calmodulin-dependent protein kinase II delta, as well as antiapoptotic genes such as fibroblast growth factor receptor-2 and leukemia inhibitory factor. However, as
mentioned, the net result is a cardioprotective effect that is associated with enhanced AKT phosphorylation, possibly attributable to inhibition of PTEN. Likewise, miR-24 is downregulated in the peri-infarct zone, where replenishing it reduces myocyte apoptosis, infarct size, and cardiac dysfunction after myocardial ischemia by targeting Bim. Additionally, miR-24, along with miR-21 and miR-221, inhibits Bim in cardiac progenitor cells and improves their survival and contribution to functional recovery after coronary artery ligation.

Other miRNAs are upregulated within the infarct zone. One example includes miR-15b, which is upregulated in the infarct zone of a porcine model after ischemia (60 minutes) and reperfusion (24 hours). When neutralized in cardiac myocytes, it enhances their viability during hypoxia in association with an increase of two of its validated targets, ADP-ribosylation factor-like 3 and Bcl2. In vivo, suppressing miR-15b with locked nucleic acid-modified "tiny" anti-miRs resulted in reducing the infarct size after ischemia (75 minutes) and reperfusion (24 hours) and improved percent ejection fraction and left ventricular end diastolic volume measured 2 weeks after ischemia/reperfusion. The mechanism underlying this protection is presumed to be improved myocyte survival, possibly because of an increase in the expression of suppressors of cell death.

The response of a cell to hypoxia or ischemia is biphasic; initially, ischemia induces an adaptive response, which then transitions into a damaging effect on the persistence of ischemia. Accordingly, exposing cells to brief periods of hypoxia or ischemia elicits a protective response, which involves activation of the AKT pathway. This process is known as ischemia preconditioning and has been proven to protect organs against subsequent damaging periods of ischemia. The miRNAs are involved in early preconditioning, which involves posttranscriptional versus transcriptional events, including the upregulation of hypoxia-inducible factor 1 alpha (Hif-1α). Specifically, miR-199a-5p is downregulated within minutes of applying ischemia preconditioning to the porcine or mouse hearts or hypoxia preconditioning to cardiac myocytes. At least in myocytes, this reduction proved to be both required and sufficient for upregulation of its target Hif-1α to the same extent as that observed after hypoxia preconditioning.

Sirt1 was also validated as a direct conserved target of miR-199a-5p, which is required for downregulation of prolyl hydroxylase 2 and, thus, stabilization of Hif-1α. These effects are mediated via an AKT-dependent pathway, which is sufficient for inducing downregulation of miR-199a-5p and upregulation of Hif-1α and Sirt1.

miRNAs That Regulate Angiogenesis During Ischemia

In addition to directly regulating cell viability during myocardial ischemia, miRNAs could also indirectly influence cell survival and the size of infarct zone or scar formation via regulating angiogenesis. The miR-126 is one that is highly expressed in murine lung and heart and, to a lesser extent, in the brain, liver, and kidney, where it is localized to endothelial and epithelial cells. Deletion of both its alleles results in approximately 50% embryonic lethality attributable to systemic edema and widespread hemorrhage from ruptured vessels. Those embryos that escaped the lethal phenotype developed normally with no apparent abnormalities until they were challenged with ischemia, which elicits reparative angiogenesis. In particular, when the left coronary artery was occluded, angiogenesis was compromised in the ischemic hearts, which resulted in precipitous cardiac failure. Similar results were observed when miR-126 antagonir was injected in mice. One of the relevant and validated targets of miR-126 include Spred-1, for which gain-of-function and loss-of-function experiments in an aortic ring sprouting assay proved that it is sufficient for inhibiting vascular outgrowths. In an opposing function, however, miR-126 was found to target stromal cell-derived factor-1. This study shows that silencing miR-126 during ischemia results in an increase in this protein, which mobilizes Sca-1(+/+)Lin(−) progenitor cells and enhances angiogenesis in a hind limb ischemia model. It is possible that the extent of silencing of miR-126 in different studies determines which of its functions predominates.

In contrast to miR-126, forced expression of miR-92a inhibits tube formation, cell migration, and adhesion of endothelial cells. Conversely, inhibition of miR-92a with antagonirs enhances angiogenesis during hind limb or myocardial ischemia in mice and improves blood flow and tissue viability in the region. The miR-92a targets alpha-5 integrin, which plausibly plays a role in mediating its function, although most likely other targets are also involved. The miR-92 is a member of the miR-17~92 cluster of miRNA. Recently, it was shown that other members of this cluster, including miR-17, miR-18a, and miR-20, also exhibited an antiangiogenic effect both in vitro and in vivo, where Janus kinase-1 was identified as a miR-17 target. In contrast, another study reported that myc induces upregulation of miR-17~92 and that overexpression of the cluster with Ras enhanced tumor growth by increasing neovascularization. In this case, it was suggested that the targeting of thrombospondin by miR-19 mediates the effects of the cluster. This does agree with the fact that c-myc induces angiogenesis by posttranscriptionally inhibiting the expression of thrombospondin-1 and provides an explanatory mechanism for it. The discrepancy between these studies may reflect differences in the models that were investigated.

Whereas miR-24 is downregulated in the peri-infarct zone and contributes to myocyte apoptosis and cardiac dysfunction during myocardial ischemia through an increase in its target suppressing proapoptotic genes such as PTEN, Fas ligand, and programmed cell death 4.
miRNAs That Regulate Arrhythmogenesis

Abnormalities in electric conduction or repolarization is an underlying cause of arrhythmias that are manifest during coronary/ischemic heart disease. Moreover, miRNAs do not only regulate cell viability during ischemia but have also been found to regulate the expression of molecules involved in mounting an action potential and in conductivity. miR-1, for example, is upregulated during coronary artery disease in humans and ischemic rat hearts, which are accompanied by an increase in the incidence of arrhythmias. As proof of the role of miR-1 in arrhythmogenesis, overexpressing or inhibiting miR-1 in the ischemic rat heart resulted in an equivalent effect on the frequency of arrhythmias. Validated targets of miR-1 include the gap junction protein Cx43 and Kir2.1 subunit of the K+ channel that mediates I_{K1}, both of which could potentially be mediating its effects. Deletion or nonsense mutation of Cx43 or I_{K1}, respectively, were shown to increase the incidence of arrhythmias. Moreover, their ablation resulted in the reversal of the knockdown arrhythmogenic effect of miR-1. However, the downregulation of miR-133 during cardiac hypertrophy is responsible for a reduction in the I_{CaL} accessory subunit, Kcnn2, through an indirect effect, which could be a cause of prolonged QT interval.

An increase in miR-1 in adult myocytes has been shown to regulate inward calcium current (I_{CaL}) and calcium release from the sarcoplasmic reticulum (SR). Overexpression of miR-1 increases I_{CaL}; however, this does not elicit an increase in maximum SR Ca2+ release, because it is possibly counteracted by a 25% lower SR Ca2+ content. Increased spark frequency and SR Ca2+ leak in the miR-1–overexpressing myocytes might be contributing factors to the lower SR Ca2+ content. These effects of miR-1 could be attributed to its targeting and suppression of the protein phosphatase 2A regulatory subunit B56 and, consequently, an increase in the phosphorylation of the ryanodine and dihydropyridine receptors. Phosphorylation of ryanoide by CAMKII has been linked to SR diastolic Ca2+ leakiness and lower SR Ca2+ content and to ventricular tachycardia. In concordance, overexpression of miR-1 dramatically increased the frequency of extrasystolic Ca2+ transients and after depolarizations in isoproterenol-stimulated paced rat adult myocytes, which was reversed by a CAMKII inhibitor. miR-133, which is coexpressed with miR-1, collaborates in this function by targeting both the alpha and beta subunits of the catalytic subunit of protein phosphatase 2A and has a similar capacity to increase arrhythmogenesis in isolated rat myocytes. This mechanism has not been validated in vivo; however, supportive evidence for that includes an increase in miR-1 and miR-133 and a decrease in protein phosphatase 2A catalytic and regulatory subunits in a canine heart failure model. These results would suggest that higher levels of both miR-1 and miR-133 in the failing or ischemic heart contribute to arrhythmogenesis via perturbation of the expression of genes involved in conduction, depolarization, and calcium handling.

However, miR-328, but not miR-1 or miR-133, was reported to be upregulated in a canine model of AF and in human AF patients with rheumatic heart disease.
increased susceptibility to AF. Similarly, transgenic overexpression of the miRNA in the mouse heart induced spontaneous AF 28 days after birth and, vice versa, knockdown of miR-328 reduced the incidence of pacing-induced AF. In this case, miR-328 targeted and suppressed both the α1c and β1 subunits of the L-type calcium channel, which accordingly explained the dramatic reduction in the L-type Ca^{2+} current observed in the transgenic model. Thus, it would appear that the miRNA-regulated mechanisms underlying arrhythmogenesis in the failing or ischemic heart differ from those involved with AF.

miRNAs in Vascular Disease and Remodeling

miRNAs in Atherosclerogenesis

Perturbation of cholesterol homeostasis is a main underlying cause of atherosclerosis. One of the regulatory factors in cholesterol metabolism include sterol regulatory element-binding protein transcription factors, which regulate the expression of cholestereogenetic genes such as low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase. The human sterol regulatory element-binding protein-1 and sterol regulatory element-binding protein-2 genes harbor miR-33b and miR-33a, respectively, which appear to be coexpressed with their host genes. Interestingly, miR-33 was found to target adenosine triphosphate-binding cassette A1 cholesterol transporter, which mediates intracellular cholesterol efflux from cells to form high-density lipoprotein that is protective against atherosclerosis. Thus, as expected, when antisense miR-33 is injected in western diet-fed mice, serum high-density lipoprotein is significantly elevated. The clinical relevance of this was tested in low-density lipoprotein receptor-deficient mice with atherosclerosis. Treatment of these mice with anti-miR-33 for 4 weeks resulted in upregulation of adenosine triphosphate-binding cassette A1 in the liver and macrophages, an increase in high-density lipoprotein levels, and ultimately reduced plaques size and inflammatory gene expression. Similar results were observed in African green monkeys, in which anti-miR-33 resulted in elevated high-density lipoprotein, in addition to reduced levels of very-low-density lipoprotein-associated triglycerides.

Other miRNAs, such as miR-145/143 cluster, miR-133, and miR-221, have a more direct role in atherosclerosis. The miR-145/miR-143 cluster is enriched in visceral and vascular smooth muscle cells (VSMC) as early as embryonic day 11.5 and throughout adulthood. Genomic deletion of this cluster resulted in viable mice with a mild vascular phenotype but no cardiac abnormalities. In particular, the smooth muscle cells were smaller and exhibited an increase in rough endoplasmic reticulum and a decrease in actin stress fibers, with a resultant thinner tunica media. In addition, there were small neointimal lesions and a megacolon phenotype, which suggested that knockout of miR-145/143 may inhibit differentiation and, subsequently, enhance proliferation and migration of smooth muscle cells. However, the failure to detect a consistent increase in VSMC proliferation or apoptosis argued that the miRNA may not be directly required for differentiation but rather for the ability of VSMC to differentiate in response to the physiological contractile demand.

miR-145 is downregulated in response to vascular injury. Although the knockout models dispute the fact that downregulation of miR-145 is sufficient for reducing α-actin and myosin heavy chain, they do show that abrogation of miR-145 induces limited neointimal lesion formation. In particular, one study has shown that these lesions are only observed in the femoral artery of older knockout mice (18 months), which suggested that although downregulation of miR-145/143 does not result in fulminant neointimal formation, it does promote it. In consensus, restoring miR-145 levels during vascular injury inhibits neointimal formation, with promising therapeutic prospects. The effect of miR-145 in this context could be, at least partially, explained by the suppression of its target, Kruppel-like factor-5. Kruppel-like factor-5, first identified as a transcription factor that induces embryonic smooth muscle myosin heavy chain, is involved in enhancing smooth muscle proliferation by upregulating cyclin D1.

miR-133 is mainly known to be enriched in the heart and skeletal muscle, but a recent report shows that it is also expressed in smooth muscle cells, albeit at relatively low levels. After balloon injury, miR-133 is downregulated in the tunica media and becomes undetectable in the neointimal layer. Overexpression of miR-133 in the carotid artery halts VSMC proliferation and prevents neointimal hyperplasia. The levels of miR-133 in this tissue inversely correlate with its targets, Sp1 and moesin. The prediction is that by inhibiting Sp1, Kruppel-like factor-5 is deactivated, which in turn prevents downregulation of Myh11, and VSMC phenotype switching and proliferation.

In contrast to miR-145 and miR-133, miR-221, but not the coclustered miR-222, positively regulates smooth muscle proliferation. It is induced by platelet-derived growth factor, which is known to stimulate VSMC switching and proliferation during angiogenesis and neointimal formation. Gain-of-function and loss-of-function experiments proved that it is required for mediating the effects of platelet-derived growth factor via targeting and suppressing the cell-cycle inhibitors p27^Kip1, p57^Kip2, and p53 as well as c-kit. In concordance, knockdown of miR-221 during vascular injury reduced neointimal thickness by 40%. The effects of miR-221 are likely to be reinforced by the concurrent upregulation of miR-21 in neointimal lesions. miR-21, which is a ubiquitously expressed prosurvival miRNA, was shown to inhibit PTEN expression under these conditions. Accordingly, its abrogation increased apoptotic VSMC death and reduced neointimal thickness. Thus, neointimal formation is the result of a combinatorial effect of changes in the expression of several miRNAs and their targets that regulate VSMC differentiation, proliferation, and survival.

miRNAs in Aneurysm Formation

Patients with thoracic aortic aneurysm have reduced levels of miR-1, miR-133, miR-21, and miR-29a, all of which significantly inversely correlated with the aortic diameter.
confirmed that miR-29a targeted metalloproteinase-2, but not metalloproteinase-9, which showed a positive correlation with aneurysm size in these patients. In contrast, Boon et al found no change in miR-29a, but found an increase in miR-29b in thoracic aortic aneurysm specimens obtained from a similar patient demographic (30 patients undergoing tricuspid valve replacement vs control samples from patients undergoing coronary artery bypass). The one difference, though, was that the control group of the former study included some samples from the aorta of heart donors, which may account for the difference, considering that coronary artery disease in the control group might have been associated with changes in miRNA expression in the aorta. In rodents, miR-29b was also upregulated in the dilated aorta of angiotensin II-treated aged mice and in fibulin-4 knockdown mice. Treatment of the former mice with anti-miR-29 induced an increase in the targets elastin, collagen 1A1 and 3A1, and reversed the phenotype of miR-29. In this model, metalloproteinase-9 was decreased but the levels of metalloproteinase-2 were undetermined. Marfan syndrome, which is characterized by the development of aortic aneurysms because of a defect in connective tissue, is also characterized by the development of aortic aneurysms which is accompanied by an increase in miR-29b and a decrease in elastin in the aorta of the Fbn1^{-/} mouse model. In contrast, though, metalloproteinase-2, which is also a miR-29 target, was increased, suggesting that miR-29b may not be the main regulator of its expression under these conditions. Similar to the angiotensin II-treated aged model, prenatal treatment of these mice with anti-miR-29 completely inhibited aortic dilation at 4 weeks of age. Overall, miR-29 emerges as a major regulator of extracellular matrix protein expression with potential therapeutic utility.

**Conclusion**

The discovery of miRNAs has introduced a new dimension to our understanding of gene regulation in cardiac disease. They are characterized by targeting multiple functionally related versus single genes, which renders them a potentially powerful therapeutic target/tool. However, to fully exploit these molecules in diagnostics and therapeutics, more studies are necessary. Specifically, we need to discover the full spectrum of genes that a single miRNA may target in a given context, how these targets functionally interact, and the extent to which each contributes to the observed functionality of the miRNA. In addition, because a single miRNA could be simultaneously regulated by multiple miRNAs, the collaborative effects of these miRNAs, in a given function, also need to be explored. These details will further explain the functional significance of miRNAs and aid in utilizing them for therapeutic purposes.

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Differential Expression of MicroRNAs in Different Disease States
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