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Review Series

The management of cardiovascular risk through lifestyle modification and pharmacotherapy is paramount to the prevention of cardiovascular disease. Epidemiological studies have identified obesity, dyslipidemia, diabetes, and hypertension as interrelated factors that negatively affect cardiovascular health. Recently, genetic and pharmacological evidence in model systems has implicated microRNAs as dynamic modifiers of disease pathogenesis. An expanded understanding of the function of microRNAs in gene regulatory networks associated with cardiovascular risk will enable identification of novel genetic mechanisms of disease and inform the development of innovative therapeutic strategies.

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Introduction

In the early 20th century, cardiovascular disease surpassed infectious agents as the leading cause of mortality in the developed world (1). Ensuing epidemiological studies to uncover the etiology of heart disease significantly reduced patient mortality rates through the identification of diabetes, obesity, hypertension, and dyslipidemia as major, modifiable cardiovascular risk factors (2). In spite of these successes, cardiovascular diseases remain the predominant cause of morbidity and mortality worldwide (3). Today, knowledge of cardiovascular risk factors provides the means to proactively reduce patient death, but unfortunately also predicts an expanding pandemic of heart disease. This current and impending burden of disease underscores the need for further insight into the molecular mechanisms that contribute to the pathogenesis of cardiovascular disease and an expanded arsenal of therapeutic agents for prevention and treatment.

Disease phenotypes frequently involve the modulation of gene expression through non-coding RNAs (4). MicroRNAs (miRNAs) are short, non-coding nucleic acid regulators of mRNA stability and translation that play diverse roles in development and disease (5–7), and can be antagonized pharmacologically (8–10). In this Review, we focus on the in vivo role of miRNAs in the pathogenesis of several major risk factors for the development of cardiovascular disease, and their sequelae. We also highlight the potential for targeting miRNAs as preventative and reparative therapeutics for the treatment of cardiac disease.

The biology of miRNAs

miRNAs are highly conserved RNAs, 18–25 nucleotides in length, that regulate gene expression. miRNAs are encoded within the genome as intronic miRNAs, which are located in and processed from introns of protein-coding gene transcripts, or as intergenic miRNAs, which are transcribed under the control of their own promoters. Processing of the primary miRNA-encoding transcript in the nucleus by the RNase Drosha produces a pre-miRNA stem loop 80–110 nucleotides in length. Exportin 5 facilitates nuclear export of the pre-miRNA to the cytoplasm, where it is then processed into a mature miRNA/miRNA* duplex by Dicer (11). The mature miRNA is then loaded into the RNA-induced silencing complex (RISC) and dictates targeting of RISC to the 3′-untranslated region (UTR) of mRNA transcripts. Recent data suggest that miRNAs primarily affect gene expression via subtle changes in mRNA transcript stability (12), thus resulting in small changes in protein levels (13). While the effects of any individual miRNA on a single target might be subtle, the combinatorial effects of a miRNA on multiple mRNA targets within a regulatory network can profoundly change the output of a pathway.

Risk factors

Hypertension. Arterial hypertension is defined by a consistent measurement of systolic blood pressure exceeding 140 mmHg or diastolic blood pressure greater than 90 mmHg, with approximately 95% of clinical cases stemming from essential (primary) hypertension (14). Patients with hypertension have an increased lifetime risk of cardiovascular disease and heart failure (15, 16), and those with high-normal blood pressure similarly demonstrate a heightened risk (17). Though essential hypertension is idiopathic in nature, two hallmarks of the pathogenesis of this condition, and foci of current therapeutic strategies, are an increase in vascular tone and hyperactivation of the renin-angiotensin-aldosterone system (RAAS).

Vascular smooth muscle constitutes the medial layer of arteries and veins. The phenotype of a differentiated VSMC is plastic and can exhibit either proliferative or contractile properties that affect vascular function in normal and pathological settings (Figure 1 and ref. 18). miRNAs have been demonstrated to play an integral role in the transcriptional regulation of VSMC development, phenotype, and function during vascular pathology. Loss of miRNA processing via conditional deletion of Dicer specifically in embryonic or adult VSMCs results in embryonic lethality or loss of VSMC function, respectively, suggesting that miRNA expression is necessary for the development and maintenance of VSMCs (19, 20).

The smooth muscle–enriched miRNAs miR-143 and miR-145 are co-expressed, resulting from the transcriptional regulation of their bicistronic mRNA transcript by serum response factor and myocardin (21, 22). Genetic deletion of the miR-143/145 cluster in vivo demonstrates that these miRNAs are dispensable for smooth muscle specification; however, they are required for the transition between proliferative and contractile VSMC phenotypes. Consequently, mice lacking miR-143/145 display reduced arterial medial

Conflict of interest: Eric N. Olson is a cofounder of miRagen Therapeutics, a biotechnology company developing microRNA-based therapeutics.

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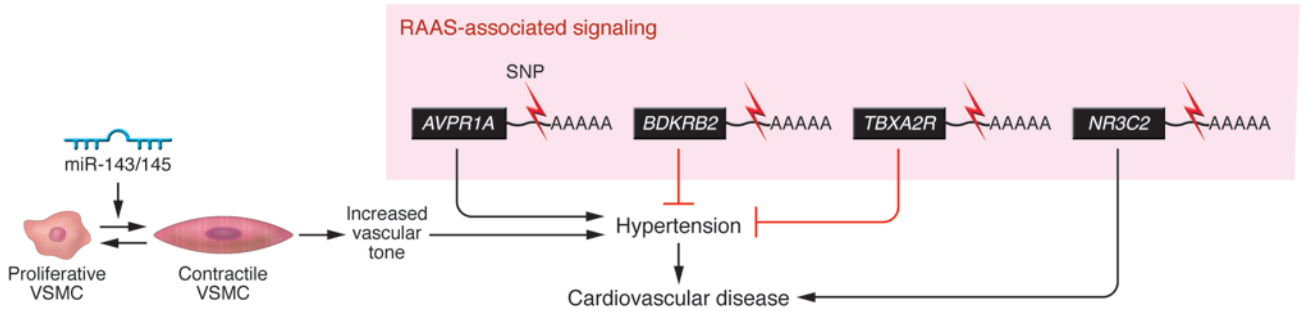

Figure 1
miRNA regulation of blood pressure. Pathological changes in vascular tone and RAAS signaling underlie primary arterial hypertension. miR-143 and miR-145 promote a contractile VSMC phenotype and are required for maintenance of normal vascular tone and RAAS-induced vasoconstriction. Human genome-wide association studies have identified SNPs in the miRNA binding sites of several RAAS-associated genes that correlate with a dysregulation of blood pressure.

thickness, decreased vascular tone, and reduced systemic blood pressure during homeostasis and following hypertensive challenge (21, 23). These in vivo effects of miR-143/145 are mediated by their destabilizing effect on transcripts encoding known repressors of the contractile VSMC phenotype, including *Klf4*, *Klf5*, and *Ace*.

Under normal physiological conditions, the RAAS hormonal axis functions to preserve fluid volume and arterial pressure through antinatriuretic and vasopressive effects. Pathological activation of RAAS is believed to be an underlying cause of essential hypertension. A recent linkage study demonstrated that SNPs in the miRNA binding sites of several RAAS-related genes are associated with alterations in blood pressure in humans (24). Patients with SNPs in the 3′UTR of the vasopressin receptor (*AVPR1A*) that lead to a loss of miR-526b and miR-578 binding exhibit increased blood pressure. In addition, several miRNA loss-of-function SNPs in potential miRNA binding sites of transcripts encoding the bradykinin 2 (*BDKRB2*) and thromboxane A2 (*TBXA2R*) receptors are associated with reduced blood pressure. Intriguingly, an additional SNP in the 3′UTR of the aldosterone receptor (*NR3C2*) is not linked with alterations in blood pressure but is associated with an increased risk of myocardial infarction in young males (24). While the effect of these SNPs on the expression of their associated genes remains to be elucidated in vivo, their linkage to blood pressure phenotypes implicates miRNAs as possible modulators of RAAS signaling.

Hyperlipidemia. Elevated levels of serum cholesterol and other lipid molecules are broadly defined as hyperlipidemia. Clinically, total cholesterol measurements greater than 240 mg/dl, with LDL fraction over 160 mg/dl and HDL under 40 mg/dl, designate a state of dyslipidemia (25). The LDL/HDL ratio is a powerful indicator of cardiovascular risk (26, 27). Patients with elevated total or LDL cholesterol are at greater risk for coronary heart disease (28), while those patients with serum HDL levels in the 80th percentile have a significantly reduced risk of cardiovascular disease (29).

Regulation of serum lipid levels by the liver is influenced by hepatic miRNA content (Figure 2). Liver-specific genetic ablation of Dicer-mediated miRNA processing results in aberrant gene expression and metabolism (30). These changes are associated with increased levels of free serum cholesterol and fatty acids, as well as an increase in the concentration of cholesterol, fatty acids, and triglycerides in the liver. Together, these data suggest that hepatic miRNA expression is required for normal lipid homeostasis.

The transcriptional activator SREBP is a master regulator of endogenous sterol biosynthesis (31). In humans, the primary mRNA transcripts encoding SREBP1 and SREBP2 proteins also encode miR-33b and miR-33a, respectively. miR-33a/b negatively regulate the membrane transporter ABCA1 to reduce cholesterol efflux (32, 33) and target the transcripts *CROT*, *CPT1a*, *HADHB*, and *AMPKa* to dampen the β-oxidation of fatty acids (34). Thus, intronically encoded miR-33a/b collaborate with their protein coding host transcript to promote the cholesterologenic state (Figure 2). Antisense inhibition of the singular miR-33 species in mice increases total and HDL serum cholesterol concentrations, consistent with increased cellular cholesterol export via the upregulation of ABCA1 in macrophages and hepatocytes (32, 33). Subsequent investigation of anti–miR-33a/b therapy in primates demonstrated a lowering of serum VLDL triglyceride levels and an increase in HDL cholesterol, suggesting that antagonism of miR-33 might beneficially alter a patient's serum lipid profile (35).

miR-122 is one of the most abundantly expressed miRNAs in the liver (36, 37) and is specifically enriched in hepatocytes (38). This hepatic enrichment makes miR-122 highly amenable to pharmacological inhibition by various anti-miR chemistries that are efficiently distributed to the liver. Two studies have demonstrated that antagonism of miR-122 in mice, with various anti-miR chemistries, reduces total serum cholesterol levels (8, 39). These studies reveal that inhibition of miR-122 leads to the de-repression of a multitude of transcripts with miR-122 seed matches, resulting in increased fatty acid oxidation and reduced lipid synthesis in the liver. Further development of locked nucleic acid–modified (LNA-modified) anti–miR-122 therapies, primarily as antiviral therapy against hepatitis C virus infection, have similarly revealed the beneficial effects of anti–miR-122 treatment on lipid profiles in primates and established proof of efficacy and minimal toxicity of LNA-modified anti-miR therapeutics in a preclinical model (40, 41).

Obesity and diabetes. Type II diabetes is characterized by a dysregulation of blood glucose levels and results from progressive peripheral insulin resistance in conjunction with pathologically altered pancreatic insulin secretion. Diabetes is diagnosed by repeated measurement of fasting glucose levels over 126 mg/dl, or over 200 mg/dl following oral glucose challenge, and type II diabetes often presents in obese, inactive individuals. Men and women with diabetes have an increased risk for cardiovascular

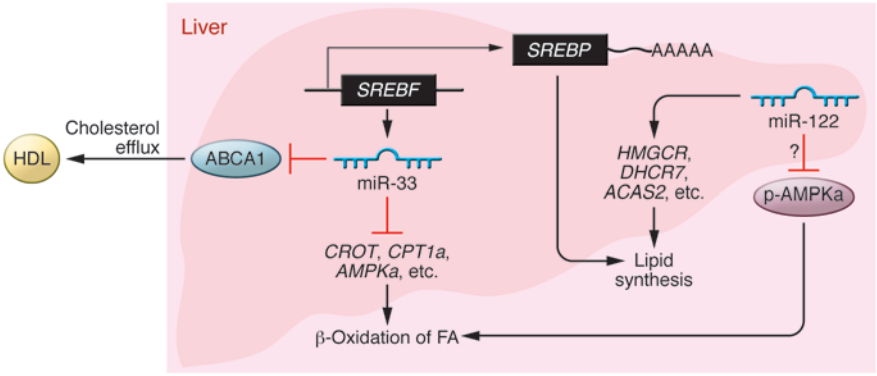

Figure 2
Role of miR-33 and miR-122 in hepatic lipid metabolism. The *SREBF* locus encodes the lipogenic transcription factor SREBP and miR-33, which represses mRNA transcripts of genes in the fatty acid (FA) β-oxidation and cholesterol efflux pathways. miR-122 promotes lipid synthesis by supporting the expression of lipid biosynthetic genes, and dampens fatty acid oxidation by inhibiting phosphorylation of AMPKa by an unknown mechanism.

disease (42), and the frequent comorbidity of type II diabetes with other cardiovascular risk factors, such as hypertension, dyslipidemia, and obesity, significantly increases the risk of heart failure in these patients (16).

miRNA processing is required for proper pancreatic endocrine function. Deletion of Dicer in the developing pancreas inhibits pancreatic islet formation and insulin-secreting β cell differentiation, indicating the importance of miRNAs in lineage specification of the endocrine pancreas (43). Removal of Dicer in mature β cells using an insulin promoter–driven Cre-recombinase does not affect β cell number, suggesting that miRNAs are not required for maintenance of differentiated β cells. However, this study did not interrogate the function of adult β cells lacking mature miRNAs. Additionally, miRNAs are integral to the function of fat-storing adipocytes, which are central to the pathology of obesity and type II diabetes. Conditional ablation of Dicer in the adipocyte lineage results in decreased fat mass and lipogenesis in white but not brown fat depots (44).

miR-375 is a β cell–enriched miRNA whose expression is elevated in the pancreatic islets of *ob/ob* diabetic mice and patients with type II diabetes (45–47). Pharmacological studies have revealed that miR-375 negatively regulates the exocytosis of insulin by targeting myotrophin, as treatment with anti–miR-375 antagomir in mice improves insulin secretion (45). Genetic interrogation of miR-375 function revealed that mice lacking miR-375 are viable, exhibit reduced β cell mass, develop hyperglycemia under normal conditions, and demonstrate exacerbated hyperglycemia in the context of a genetic (*ob/ob*) model of diabetes (47). This phenotype can be attributed to the de-repression of a constellation of growth inhibitory genes in the absence of miR-375. Together, these data suggest that dysregulation of miR-375 in the setting of diabetes is a compensatory mechanism to increase β cell mass in order to counteract increased peripheral insulin resistance.

The expression of miR-103 and miR-107 is elevated in the liver of rodent models of obesity and type II diabetes (48, 49). The miRNAs miR-103 and miR-107 differ by only 1 nucleotide 3′ of their seed region and constitute a miRNA family. Adenoviral-mediated overexpression of miR-107 in the liver leads to hyperglycemia, hyperinsulinemia, and an increase in hepatic gluconeogenesis. In contrast, pharmacological inhibition of miR-103/107 with anti–miR-103 antagomir improves glucose homeostasis in obese mice, reduces adipocyte size, and decreases overall fat mass (49). Pharmacological inhibition of miR-103 potentiates insulin signaling via the upregulation of the target gene caveolin-1 and its subsequent stabilization of the insulin receptor (49). This modulation of upstream insulin signaling components suggests that anti–miR-103 could be used to therapeutically sensitize peripheral tissue to insulin signaling in the diabetic state.

The miR-143/145 cluster is similarly upregulated in the liver of diabetic rodents (50). Overexpression of miR-143 in the liver is sufficient to drive glucose intolerance and insulin resistance, while knockout mice lacking miR-143/145 display reduced obesity-induced insulin resistance. These phenotypic effects are associated with dampened or heightened activation of the AKT pathway, respectively. The effect of miR-143 in glucose homeostasis is mediated by the target oxysterol binding protein-like 8 (ORP8), which has been shown to have a novel role in promoting AKT phosphorylation (50).

The let-7 family of miRNAs was first identified through forward genetics as a regulator of developmental timing in *C. elegans* (51), but has been shown to exert a powerful effect on growth and metabolism in mammals (Figure 3A). Global transgenic overexpression of Lin28a, a negative regulator of let-7 biogenesis, significantly increases the growth and organ size of mice and markedly reduces age-associated and high-fat diet–induced obesity (52). Conversely, ubiquitous overexpression of let-7 at modest levels results in reduced body size and aberrant glucose homeostasis, leading to hyperglycemia (53, 54). Conditional overexpression of let-7 in the pancreas is sufficient to reduce insulin secretion (54). The effects of let-7 on peripheral insulin sensitivity were identified by deletion of Lin28a and the concomitant elevation of let-7 specifically in skeletal muscle, which was sufficient to induce a hyperglycemic phenotype in mice (53). Additionally, knockdown of the let-7 family with a LNA-modified anti-miR reduces peripheral insulin resistance induced by high-fat diet (54). In these studies, the metabolic effects of the Lin28/let-7 regulatory cascade were attributed to let-7 targeting of the insulin receptor (*Insr*) and its downstream mediators, such as *Irs2* and *Igf1r*, in skeletal muscle and liver. Finally, Zhu et al. showed in large-scale genome wide association studies that multiple let-7 targets are associated with type II diabetes (53), suggesting a potential role for aberrant regulation of let-7 target genes in the diabetic patient. Together, these studies indicate that let-7 acts to decrease insulin secretion centrally and induce insulin resistance in peripheral metabolic tissues such as skeletal muscle. Of particular interest is the ability of short-term anti–let-7 administration to reduce blood glucose levels by decreasing peripheral insulin resistance, suggesting anti–let-7 therapy as a potential insulin-sensitizing agent (54).

The continuous contractile mechanics of the heart demand a high amount of substrate for energy production and are sensitive to metabolic derangements (55). mRNA transcripts coding for

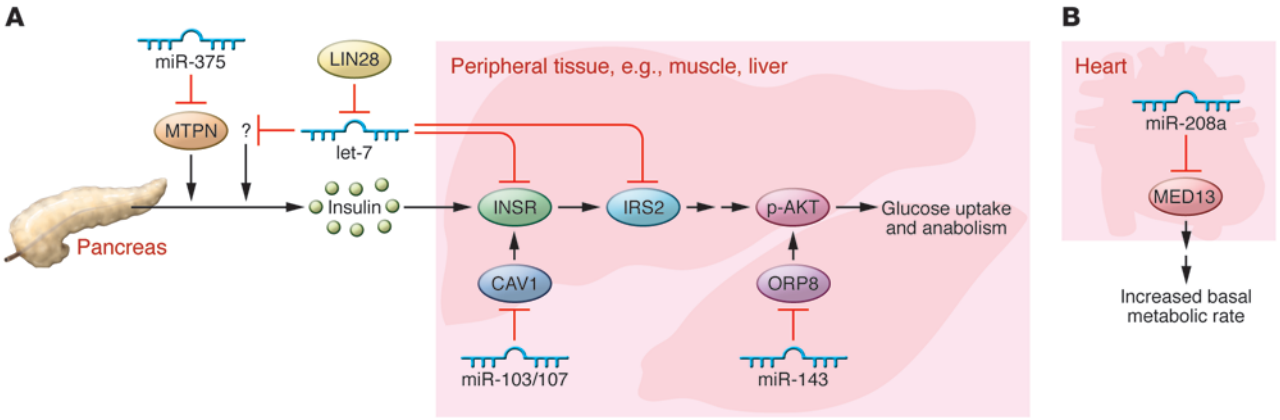

Figure 3
miRNA modulation of insulin signaling and metabolic homeostasis. (**A**) miRNAs regulate pancreatic insulin secretion and the insulin signaling cascade in peripheral tissues. Pathological changes in the expression of these miRNAs contribute to the type II diabetic phenotype. (**B**) Modulation of Med13 expression in the heart by the cardiac-specific miRNA, miR-208a, regulates whole body metabolism and adiposity.

three cardiac myosin heavy chain contractile proteins, *Myh6*, *Myh7*, and *Myh7b*, encode miRNAs miR-208a, miR-208b, and miR-499, respectively, within their introns (56). These miRNAs function redundantly to control cardiac contractility in response to pathological hypertrophic remodeling and thyroid hormone signaling (57, 58). This action is mediated in part by MED13, a component of the mediator transcriptional complex (58). Acute inhibition of miR-208a with LNA-modified anti-miR leads to the upregulation of *Med13* transcript in the heart and significantly reduces weight gain in mice that are fed a high-fat diet (Figure 3B and ref. 59). Transgenic overexpression of *Med13* specifically in the murine heart increases the basal metabolic rate, as measured by O_2 consumption and CO_2 production, and reduces overall fat mass on normal- and high-fat diets. In contrast, conditional deletion of *Med13* in the heart exacerbates weight gain in mice fed a high-fat diet. This study demonstrates the influence of a cardiac miRNA family on whole-body metabolism via the regulation of *Med13* and suggests a novel mechanism by which cardiac contractility and energy demand interface with global energy homeostasis. Given the multiplicity of miR-208 target transcripts, additional target genes likely contribute to this regulatory pathway.

Sequelae

Atherosclerosis. The prolonged pathogenesis of atherosclerosis is marked by endothelial cell dysfunction, followed by deposition of lipid-laden macrophages and VSMC proliferation, leading to occlusion of the arterial lumen. Hypertension and hyperlipidemia significantly contribute to the process of atherosclerotic plaque formation (60), as do the miRNAs that regulate these processes. The positive regulation of cholesterol efflux by the miR-33 family promotes atheroma formation. Treatment of hypercholesterolemic *Ldlr*-null mice with an anti–miR-33 compound causes a regression of atherosclerotic lesions, likely due to the regulation of ABCA1 in resident plaque macrophages (61).

Gain and loss of function of smooth muscle–enriched miR-143/145 in vivo results in reduced smooth muscle cell proliferation and consequently prevents neointima formation in surgical models of vascular injury, suggesting that closely titrated levels of miR-143/145 are required for the pathological proliferative response to injury (21, 62). Similarly, antisense oligonucleotide inhibition of the broadly expressed miR-21 reduces neointima formation following vascular balloon injury via increased levels of the pro-apoptotic proteins BCL2 and PTEN (63).

miRNAs also appear to mediate direct anti-atherogenic signals between healthy endothelial cells and vascular smooth muscle (64). Normal laminar blood flow induces transcriptional activation of miR-143/145 by KLF2 in endothelial cells, where these miRNAs are packaged into vesicles and secreted to neighboring vascular smooth muscle to support contractile gene expression (Figure 4A). miR-143/145–enriched vesicles from endothelial cells reduce atherosclerotic plaque size in *Apoe*$^{-/-}$ mice. In the setting of endothelial cell injury, this miRNA-mediated communication is disrupted, leading to a proliferative VSMC phenotype in the absence of endothelial-derived miR-143/145.

miR-126 is encoded within an intron of *Egfl7* and is enriched in endothelial cells (65, 66). Genetic deletion of miR-126 disrupts vascular integrity and results in partial embryonic lethality. miR-126 knockout mice that survive to adulthood demonstrate defective neo-angiogenesis following a surgical model of myocardial infarction. miR-126 is enriched in the apoptotic bodies of dying endothelial cells and signals in a paracrine manner to induce the anti-apoptotic, pro-repair, CXCL12-CXCR4 signaling cascade in neighboring vascular cells (67). Direct administration of pre–miR-126, or infusion of miR-126–containing apoptotic bodies, respectively decreases the size or promotes the stabilization of atherosclerotic lesions in *Apoe*$^{-/-}$ mice.

Cardiac ischemia. Atherosclerotic plaque formation in the coronary vasculature impedes blood flow, resulting in transient bouts of cardiomyocyte ischemia, and often presents clinically as angina. Cardiomyocytes possess inducible protective mechanisms to dampen injury associated with ischemia and infarction (68), and a thorough understanding of miRNA involvement in these intrinsic defense mechanisms may provide new therapeutic targets to reduce injury following myocardial infarction.

miRNAs are dynamically regulated in rodent models of ischemia/reperfusion injury and infarction, as well as in cardiac biopsies from human subjects following myocardial infarction (69, 70). miR-214 is significantly upregulated in the heart following ischemia

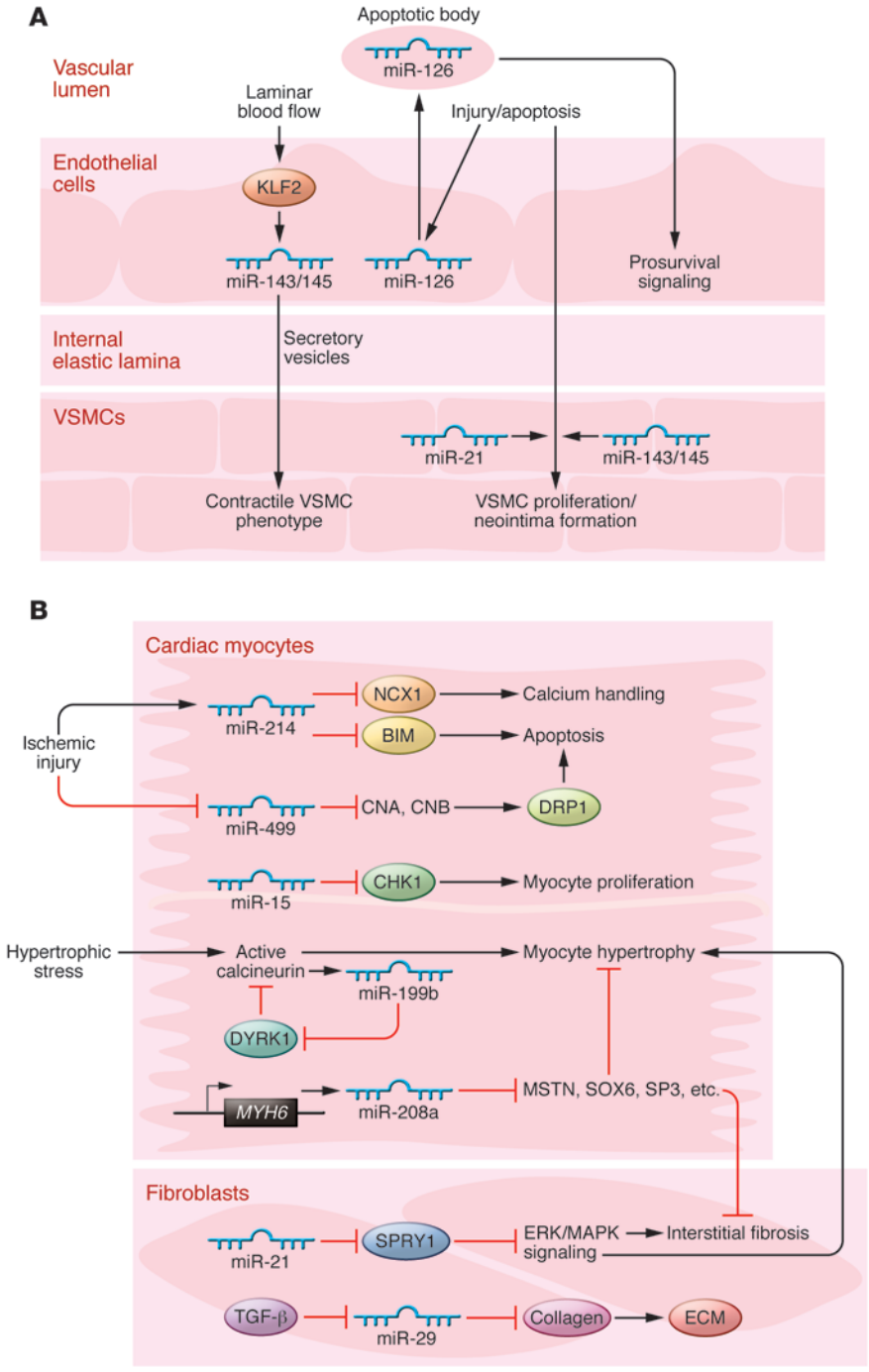

Figure 4
miRNAs in cardiovascular pathology. (**A**) miRNAs in endothelial cells act as paracrine signals to preserve vascular structure under normal conditions and to promote cell survival following injury. VSMC miRNAs modulate the smooth muscle response to injury. These mechanisms are dysregulated during atherosclerosis. (**B**) Cardiomyocyte and fibroblast miRNAs regulate cellular survival and pathological responses to multiple cardiac stressors.

(Figure 4B and refs. 69, 71). In mice, deletion of miR-214 exacerbates injury following myocardial ischemia/reperfusion and increases mortality following myocardial infarction (71). In the pathology of ischemia/reperfusion injury, miR-214 attenuates calcium overload by titrating the expression of the sodium/calcium exchanger NCX1 in cardiomyocytes and promotes cell survival by inhibiting expression of the pro-apoptotic protein BIM.

The expression of myosin heavy chain–encoded miR-499 is downregulated in response to hypoxic or ischemic stress in cardiomyocytes (72). Transgenic overexpression of miR-499 blunts cardiomyocyte apoptosis and infarct size following ischemia/reperfusion injury, while knockdown of miR-499 with a cholesterol-modified antagomir promotes myocardial apoptosis and broadens the infarct zone (72). This cardioprotective effect of miR-499 results, at least in part, from its targeting of the α- and β-isoforms of calcineurin, a central regulator of pathological cardiac remodeling (73), and their downstream effects on dynamin-related protein 1 induction of mitochondrial fission and apoptosis. Of note, elevated miR-499 levels prime the heart for dysfunction in response to hypertrophic stress signals (74, 75), suggesting that acute but not chronic elevation of miR-499 might be of therapeutic interest for cardioprotection from ischemia and infarction.

Cardiac remodeling. Cardiac diseases of various origin are linked by their common histological features of myocyte death, associated with compensatory pathological remodeling and minimal functional repair. miRNAs are intimately involved in cardiac repair and remodeling responses to injury, and their ablation in the adult heart, either by conditional or temporal deletion of Dicer or *Dgcr8*, is sufficient to drive heart failure and death (76, 77).

Increased contractile demand in the setting of systemic hypertension, or in response to loss of neighboring viable myocytes drives pathological cardiomyocyte hypertrophy and interstitial fibrosis. This compensatory hypertrophic response preserves cardiac output in the pathological setting and involves a signature pattern of miRNA dysregulation (78, 79).

miR-21 is widely expressed and is one of the most significantly upregulated miRNAs in rodent models of cardiac hypertrophy (78). Pharmacological antagonism of miR-21 with antagomir exhibits antihypertrophic and antifibrotic effects, resulting in functional improvement following hypertrophic stress (80). This therapeutic effect has been attributed to reduced activation of the ERK/MAPK signaling cascade in cardiac fibroblasts, resulting from the upregulation of the miR-21 target Sprouty1. Intriguingly, genetic ablation of miR-21 or inhibition with 8-mer LNAs are not sufficient to blunt the hypertrophic and fibrotic responses to a

variety of cardiac stress stimuli (81). These fundamental differences between the pathological response following genetic loss or pharmacological inhibition of miR-21 might reflect unique dosage effects of miR-21, differences between constitutive and temporal loss of miR-21, differential uptake of miR-21 inhibitors in cardiac fibroblasts versus cardiomyocytes, or off-target effects of antagomir-21. Additionally, alternate conclusions from these miR-21 loss-of-function studies might be explained in part by the use of different anti-miR chemistries that vary in nucleotide length and chemical composition.

The miR-199 family of miRNAs is similarly upregulated in genetic and surgical rodent models of cardiac hypertrophy (78, 79). miR-199b is induced by, and positively regulates, the pro-hypertrophic calcineurin/NFAT signaling cascade by inhibiting expression of DYRK1 kinase, thus forming a feed-forward mechanism that potentiates pathological growth (82). Inhibition of miR-199b with antagomir increases the expression of DYRK1 and blunts or reverses pathological remodeling.

miR-208a is encoded by the α–myosin heavy chain gene (*Myh6*) transcript and dominantly regulates the expression of the related miRNAs miR-208b and miR-499. Loss of miR-208a results in loss of miR-208b/499 expression in the heart, and prevents pathological hypertrophic remodeling and fibrosis (56–58). miR-208a regulation of myocyte hypertrophy and contractile protein expression occurs through the target genes myostatin, *Sox6*, *Purb*, *Sp3*, and *Hp1b*. Inhibition of miR-208a by injection of LNA–anti-miR prevents cardiac remodeling and improves cardiac function and survival in a Dahl rat hypertensive model of cardiac hypertrophy (83).

The cardiac response to infarction injury is characterized by coagulative necrosis of myocytes, followed by extracellular matrix deposition and scar formation by cardiac fibroblasts. Myocardial infarction is associated with a decrease in levels of miR-29 family members, allowing for the expression and deposition of collagen components of the fibrotic scar (84). Conversely, heightened miR-29 expression is associated with reduced vascular stromal integrity, and anti–miR-29 therapy stabilizes extracellular matrix, preventing aortic dilation and aneurysm formation (85, 86). These in vivo functions for miR-29 highlight the fragile balance of beneficial and pathological fibrotic deposition that should be considered when developing miR-29 therapies.

Cardiomyocyte regeneration. Though the heart has limited regenerative capacity, recent evidence suggests that replacement of cardiomyocytes can occur through the proliferation and migration of existing cardiomyocytes (87–89). The miR-15 family of miRNAs, composed of miR-15a/b, miR–16-1/2, miR-195, and miR-497, contain a homologous sequence at their seed regions and exhibit marked upregulation coinciding with the last wave of cardiomyocyte proliferation in the postnatal heart (90). The miR-15 family promotes mitotic cell cycle exit by diminishing the expression of checkpoint kinase 1 and other promitotic genes. Antagonism of all miR-15 family members with a single inhibitory chemistry increases myocyte proliferation in hearts of neonatal mice, prevents ischemic damage in the adult mouse heart, and improves cardiac function following myocardial infarction (90, 91).

Translating miRNA biology to therapeutics

The preceding studies convincingly implicate miRNAs in the pathogenesis of multiple conditions that lead to the development of cardiovascular disease and the subsequent cardiac response to the diseased state. The robust functions of miRNAs in these pathways present unique challenges to incorporating pharmacological targeting of miRNAs into patient treatment. The following examples highlight these obstacles.

Preventative miRNA therapies. The role of miRNAs in the pathogenesis of the aforementioned cardiovascular risk factors suggests that they might be interesting candidate drug targets. The administration of miRNA-targeted therapies for long-term risk modification warrants the consideration of several potential adverse effects.

Many of the miRNAs involved in cardiovascular pathology have also been implicated as tumor suppressors in vivo (92). For example, the let-7 family of miRNAs, which can be antagonized to induce peripheral insulin sensitivity, functions to promote differentiation and suppress tumor initiation, and is downregulated in a variety of malignancies (93). Thus, the potential use of anti–let-7 therapy to counteract the diabetic state should take into consideration the long-term risk of developing cancer. Notably, tumorigenic effects of let-7 inhibition have not been reported, though in vivo evidence has demonstrated the antitumor affect of let-7 in gain-of-function experiments (94). These concerns might warrant development of targeted let-7 antagonism specifically in skeletal myofibers. Furthermore, let-7 inhibition in vivo promotes β2-adrenergic receptor expression and may complicate the use of β-adrenergic antagonists in patients with cardiovascular disease (95).

Other undesirable side effects of chronically administered miRNA therapeutics could be mitigated by modulation of miRNA expression in a particular tissue of interest. This could be achieved by targeted delivery of miRNA therapeutics or specifically targeting miRNAs that display restricted expression in a tissue of interest and/or specific regulation of a biochemical process. Chronic inhibition of miR-122 and miR-33a/b, hepatic and cholesterol metabolism–specific miRNAs, respectively, might beneficially alter serum lipids and may be less likely to provoke undesirable side effects. Additionally, targeting of β cell–enriched miRNAs to increase insulin secretion could aid in glycemic control in diabetic patients. Finally, chronic antagonism of smooth muscle–enriched miR-143/145 to treat essential hypertension and confer metabolic improvement in diabetic patients would ideally result in minimal on-target effects in other tissues. This therapeutic strategy may minimize undesirable off-tissue effects but does not address the potential adverse on-target effects of chronic miRNA antagonism in the cell type of interest.

Reparative miRNA therapies. The biologically diverse functions of miRNAs underscore the need for targeted delivery and desirable pharmacokinetic profiles of inhibitory and mimic miRNA chemistries for reparative therapeutics. Acute miR-15 antagonism might confer benefits to cardiac repair and function following myocardial infarction by inducing myocyte proliferation, while prolonged anti–miR-15 therapy could potentially induce global cellular proliferation and promote neoplasia. Targeted delivery of anti–miR-15 might circumvent these adverse systemic effects, as might rapidly metabolized chemistries that transiently inhibit miR-15 following myocardial infarction.

The therapeutic manipulation of the miR-29 family presents a similar requirement for targeted therapy. In the context of post–myocardial infarction remodeling, overexpression of miR-29 might beneficially reduce the extent of scar formation. However, such treatment might disrupt the vascular integrity of the aorta. Again, local delivery of molecules that mimic miR-29, possibly via drug-eluting coronary stents, could prevent such adverse outcomes.

The outlook for the clinical application of miRNA discoveries is promising. miRNA diagnostics and therapeutics, such as the use of cardiovascular miRNA biomarkers (96) and ongoing phase II clinical trails of miRNA inhibitory molecules (97), exemplify this potential. Additionally, a breadth of intriguing in vitro data implicate miRNAs in many more cardiovascular disease processes and pathways, but the role of miRNAs remains to be tested in vivo in disease models. In conclusion, the involvement of miRNAs in cardiovascular risk factor pathology and subsequent cardiovascular disease warrant further genetic and pharmacological elucidation of their function in vivo in order to support the development of miRNA and gene regulatory therapies for the prevention and treatment of cardiovascular disease.

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