Review

Huaping Li, Jiabing Zhan, Chen Chen* and Daowen Wang*

MicroRNAs in cardiovascular diseases

https://doi.org/10.1515/mr-2021-0001
Received March 12, 2021; accepted December 29, 2021;
published online April 26, 2022

Abstract: Cardiovascular diseases (CVDs) are the leading causes of death and disability worldwide, despite the wide diversity of molecular targets identified and the development of therapeutic methods. MicroRNAs (miRNAs) are a class of small (about 22 nucleotides) non-coding RNAs (ncRNAs) that negatively regulate gene expression at the post-transcriptional level in the cytoplasm and play complicated roles in different CVDs. While miRNA overexpression in one type of cell protects against heart disease, it promotes cardiac dysfunction in another type of cardiac cell. Moreover, recent studies have shown that, apart from cytosolic miRNAs, subcellular miRNAs such as mitochondria- and nucleus-localized miRNAs are dysregulated in CVDs. However, the functional properties of cellular- and subcellular-localized miRNAs have not been well characterized. In this review article, by carefully revisiting animal-based miRNA studies in CVDs, we will address the regulation and functional properties of miRNAs in various CVDs. Specifically, the cell–cell crosstalk and subcellular perspective of miRNAs are highlighted. We will provide the background for attractive molecular targets that might be useful in preventing the progression of CVDs and heart failure (HF) as well as insights for future studies.

Keywords: apoptosis; cardiovascular diseases; fibrosis; heart failure; hypertrophy; miRNAs; subcellular organelle.

Introduction

Cardiovascular diseases (CVDs) are the leading causes of death and disability in developed as well as in developing countries, and their incidence is on the rise globally. The annual mortality due to CVD is expected to reach 23.6 million by 2030 [1]. CVDs are a group of disorders of the heart and blood vessels including hypertension, heart diseases (such as coronary heart disease [CHD], rheumatic heart disease, congenital heart disease, hypertrophic cardiomyopathy [HCM], dilated cardiomyopathy, and diabetic cardiomyopathy [DCM]), cerebrovascular disease (stroke), peripheral vascular disease, deep vein thrombosis, pulmonary embolism, and arrhythmias [2]. Heart failure (HF) is one of the most common and often unavoidable outcome of CVDs. HF is a complex syndrome in which the ability of the heart to maintain blood circulation is impaired owing to structural or functional impairment of ventricular filling or ejection. Despite the best medical therapy, 50% of all HF patients die within five years of diagnosis, thus stressing the importance of understanding the underlying mechanisms of CVDs and HF thereby calling for innovative treatments.

Non-coding RNAs (ncRNAs) are a class of functional RNA molecules that are transcribed from DNA but are not translated into proteins. Epigenetic-related ncRNAs include microRNAs (miRNAs), short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), and long non-coding RNAs (lncRNAs). miRNAs are a subclass of small (about 22 nucleotides) ncRNAs that negatively regulate gene expression at the post-transcriptional level. However, recent studies suggest that miRNAs may also regulate gene expression in a positive manner in subcellular organelles such as the mitochondria and nucleus. miRNAs are the most well-studied ncRNAs in CVDs. Emerging studies have linked altered miRNA expression to various CVDs such as hypertension, CHD, arrhythmia, DCM, and HF [3, 4]. Over the last two decades, molecular investigations have revealed numerous signaling pathways involved in the underlying mechanisms of heart diseases.

Huaping Li and Jiabing Zhan contributed equally to this work.

***Corresponding authors: Chen Chen**, MD, PhD and **Daowen Wang**, MD, PhD, Division of Cardiology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095# Jiefang Ave., Wuhan 430030, China; and Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Wuhan 430030, China, Phone: +86 27 6937 8422, E-mail: chenchen@tjh.tjmu.edu.cn (C.Chen) and dwwang@tjh.tjmu.edu.cn (D.W. Wang). https://orcid.org/0000-0002-9774-3980 (D. Wang)
Huaping Li and Jiabing Zhan, Division of Cardiology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; and Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Wuhan, China

miRNAs usually act as nodes of signaling networks that regulate the progression of CVDs. Although numerous studies have revealed the potential function of several miRNAs, most of these studies are preliminary. There is still a lack of high-quality data and in-depth mechanistic insights into CVD-related miRNAs.

In this review, we carefully describe the *in vivo* studies regarding miRNAs in CVDs, as well as the dynamic interplay between CVD-related pathways mediated by miRNAs. Specifically, the cell–cell crosstalk and subcellular perspective of miRNAs are highlighted. This overview will provide a background for attractive molecular targets that might be beneficial in preventing the progression of CVDs and HF.

miRNAs in CHD and myocardial ischemia

Coronary artery disease almost always occurs due to atheromatous narrowing and subsequent occlusion of the vessel [5]. Pathophysiological processes such as oxidative stress, inflammation, and apoptosis are involved in coronary atherosclerosis, myocardial ischemia injury, and post-ischemia cardiac remodeling. miRNAs may serve as fine-tuning tools and play regulatory roles in virtually all these signaling pathways (Figure 1).

During the early stage of CHD (atherosclerosis), damaged endothelial cells (ECs), macrophages, and vascular smooth muscle cells (VSMCs) are frequently observed. As the interface between the bloodstream and the vessel wall, the endothelium plays a critical role in sensing and transducing the stimulus that induces vascular remodeling. The endothelium maintains homeostasis within the vasculature by a balance between growth inhibitors and growth promoters [6, 7]. Dysfunction of the endothelial lining of lesion-prone areas is an important contributor to the pathophysiology of atherosclerosis. Among the available models, apolipoprotein E-deficient (ApoE (–/–)) mice are frequently used because of their propensity to spontaneously develop atherosclerotic lesions. miRNAs are potently involved in the initiation and progression of atherosclerosis. In particular, miR-1 prevented high-cholesterol diet-induced endothelial permeability and endothelial barrier dysfunction in ApoE-deficient mice. Detailed mechanistic studies

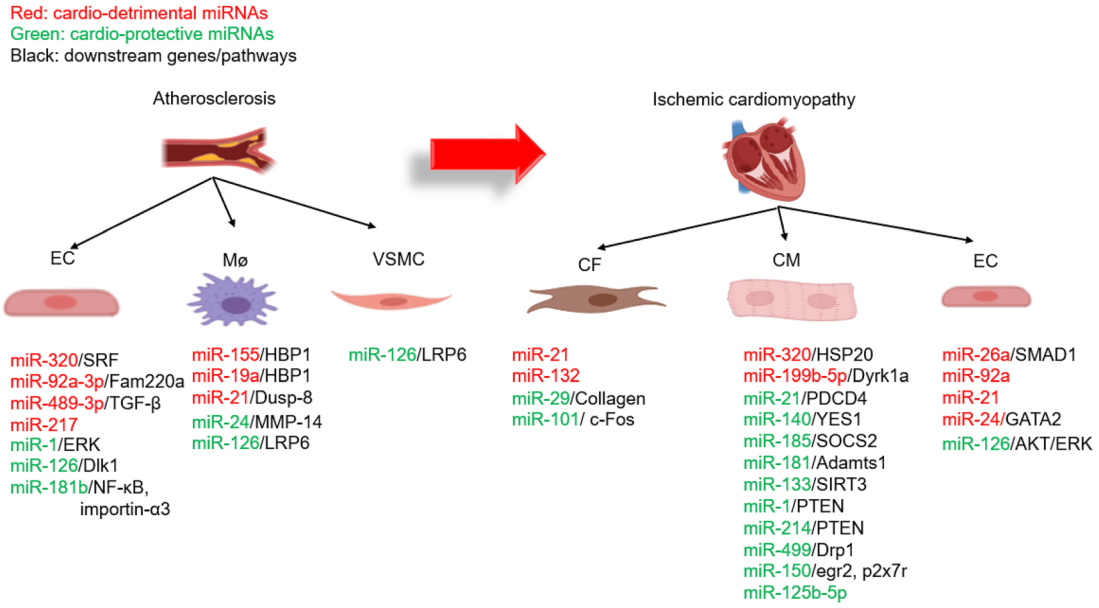

Figure 1: miRNAs with key roles in the process of atherosclerosis and ischemic cardiomyopathy. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. EC, endothelial cell; Mø, macrophage; VSMC, vascular smooth muscle cell; CF, cardiac fibroblast; CM, cardiomyocyte; SRF, serum response factor; TGF-β, transforming growth factor beta; ERK, extracellular signal regulated kinase; Dlk1, Notch1 inhibitor delta-like 1 homolog; NF-κB, nuclear factor kappa-B; HBP1, HMG box-transcription protein1; Dusp-8, dual specificity protein phosphatase 8; MMP-14, type-1 matrix metalloproteinase; LRP6, low density lipoprotein receptor-related protein 6; HSP20, heat-shock protein 20; Dyrk1a, specificity tyrosine-phosphorylation-regulated kinase 1A; PDCD4, programmed cell death 4; YES1, YES proto-oncogene 1; SOCS2, suppressor of cytokine signaling 2; Adamts1, ADAM metallopeptidase with thrombospondin type 1 motif 1; SIRT3, sirtuin 3; PTEN, phosphatase and tensin homolog; Drp1, dynamin-related protein-1; egr2, zinc-binding transcription factor induced by ischemia; p2x7r, pro-inflammatory ATP receptor; SMAD1, SMAD Family Member 1.

showed that high-cholesterol-induced extracellular signal regulated kinase (ERK) activation is enhanced by miR-1 antagomir and attenuated by miR-1 mimic [8]. Our group showed that miR-320a was markedly elevated in the peripheral blood vessels of patients with CHD. Overexpression of miR-320a in ApoE (−/−) mice attenuated endothelium cell function and promoted atherogenesis. Mechanistically, miR-320a post-transcriptionally downregulate the expression of serum response factor (SRF), a key regulator of EC function and essential for VGEF signaling [9]. Circulating levels of miR-181b were reduced in human subjects with CHD. However, systemic delivery of miR-181b inhibited the activation of nuclear factor kappa-B (NF-κB) and atherosclerosis through cell-specific mechanisms in the vascular endothelium. Specifically, miR-181b inhibited the expression of the target gene importin-α3 and thereby reduced NF-κB nuclear translocation specifically in the vascular endothelium of lesions [10]. A milestone study reported that miR-126-5p promoted endothelial proliferation and limited atherosclerosis by suppressing Notch1 inhibitor delta-like 1 homolog (Dlk1). The miR-126-5p mimic potently reduced atherosclerosis and increased EC proliferation in the aortic root of Mir126(+/+) ApoE(−/−) mice, suggesting that miR-126-5p mimics may have therapeutic value in human atherosclerosis [11]. Wiese and colleagues found that dual inhibition of endothelial miR-92a-3p and miR-489-3p reduce renal injury-associated atherosclerosis in ApoE (−/−) mice. Tgfβ2 and Fam220a were identified as targets of miR-489-3p and miR-92a-3p, respectively [12]. Yébenes et al. used an inducible endothelium-specific knock-in mouse model to investigate the role of miR-217 in vascular function and atherosclerosis. They found that increased endothelial miR-217 expression led to exacerbated atherosclerosis in proatherogenic ApoE (−/−) mice [13]. Conversely, inhibition of endogenous vascular miR-217 in ApoE (−/−) mice improved vascular contractility and diminished atherosclerosis [13]. In an angiotensin II (Ang II)-induced and aging-related atherogenesis mouse model, locked nucleic acid-modified antisense miR-92a attenuated inflammasome formation, improved vasodilation, and ameliorated atherogenesis. Mechanistically, sterol regulatory element binding protein 2 (SREBP2)-induced miR-92a targets key molecules involved in endothelial homeostasis, including sirtuin 1 (SIRT1), Krüppel-like factor 2, and Krüppel-like factor 4, leading to NOD-like receptor family pyrin domain-containing three inflammasome activation and endothelial nitric oxide synthase (eNOS) inhibition [14]. miR-29 inhibition by a locked nucleic acid-miR-29 (LNA-miR-29) in an atherosclerotic ApoE (−/−) mouse model increased fibrous cap thickness and SMA staining and reduced necrotic zones in lesions. Interestingly, LNA-miR-29 increased the secretion of ECM proteins from VSMCs that induced intra-plaque collagen gene expression but not systemic fibrosis [15].

Atherosclerosis also exhibits a continuous inflammatory response mediated by macrophages and other inflammatory cells. Macrophages actively participate in lipoprotein ingestion and accumulation giving rise to foam cells filled with lipid droplets. Accumulation of foam cells contributes to lipid storage and atherosclerosis [16, 17]. In macrophages, silencing miR-24 significantly increased type-1 matrix metalloproteinase (MMP-14) expression and enhanced their invasive capacity. In atherosclerotic ApoE (−/−) mice, miR-24 inhibition increased plaque size by increasing macrophage membrane MMP-14 expression [18]. miR-155 expression was also increased in both plasma and macrophages isolated from ApoE(−/−) mice with atherosclerosis. Inhibition of miR-155 systemically by antagomir-155 decreased lipid loading in macrophages and reduced atherosclerotic plaques in ApoE(−/−) mice. HMG box-transcription protein1 (HBP1), a repressor of macrophage migration inhibiting factor, is a novel target of miR-155 [19]. HBP-1 is also a target gene of miR-19a, and miR-19a antagonist delivery via the caudal vein decreased atherosclerotic plaques and lipid load in ApoE(−/−) mice fed with a high-fat diet [20]. ApoE(−/−) mice fed with a high-fat diet and treated with miR-126 overexpressed endothelial microparticles (EMPs), reduced macrophage infiltration, neointima formation and VSMC proliferation by targeting low-density lipoprotein receptor-related protein 6 (LRP6) [21]. In a recent study, reduced atherosclerotic plaque formation and inactivated macrophage adhesion were observed in miR-21(−/−) ApoE(−/−) (double knockout, DKO) mice. miR-21 increased macrophage infiltration mediated by the dual specificity protein phosphatase 8 (Dusp-8) gene, a potent negative regulator of the mitogen-activated protein kinase (MAPK) signaling pathway [22]. By regulating the inflammatory response in macrophages, these miRNAs determine the progression of atherosclerosis and could serve as potential therapeutic targets. Together, ECs, macrophages and VSMCs disorders collectively contributed to progression of atherosclerosis. Interestingly, we have observed that inhibiting of miR-3201 increased apolipoprotein A5 (APOA5) expression in hepatic cells [4]. Therefore, it's very likely that hepatic cell-derived miRNAs are also involved in atherosclerosis, which require further investigations.

Abrupt release of atherosclerotic thrombi is the most common cause of myocardial ischemia. Adverse cardiac remodeling post-myocardial infarction (MI) directly damage the recovery of cardiac function and survival rate of

patients with MI. Cardiac fibroblasts are the most prevalent cell type in the heart and play a pivotal role in cardiac remodeling. Many of the functional effects of fibroblasts are mediated through differentiation to a myofibroblast phenotype that exhibits increased migratory, proliferative, and secretory properties [23]. The function of fibroblasts are potently regulated by various miRNAs. The expression of miR-29 family members was downregulated in the region of MI as well as in the heart region adjacent to the infarct. The miR-29 family targets several genes including multiple collagens, fibrillin, and elastin. Downregulation of miR-29 induced the expression of collagens, whereas its overexpression in fibroblasts reduced collagen expression and post-ischemia cardiac remodeling [24]. In addition, miR-101a expression was decreased in the peri-infarct area. Adenovirus-mediated overexpression of miR-101a in rats with MI improved cardiac performance and decreased fibroblast proliferation and collagen production by impairing c-Fos and its downstream protein, transforming growth factor-β1 [25]. In a pig model of ischemia-reperfusion (IR) injury, anti-miR-21 and anti-miR-132 treatment protected against cardiac fibrosis, cardiac hypertrophy, and HF [26, 27].

Cardiomyocytes are another major cell type that play a critical role in cardiac remodeling. Immediately following an ischemic insult, irreversible injury and subsequent cell death occurs to the cardiomyocytes, leading to cardiac dysfunction [28, 29]. Ren and colleagues found that miR-320 expression level was significantly decreased in the hearts after IR. Overexpression of miR-320 enhanced cardiomyocyte apoptosis and death, whereas knockdown (KD) by antagomir-320 reduced infarct size. Heat-shock protein 20 (Hsp20), a known cardio-protective protein, is the downstream target of miR-320 in cardiomyocytes [30]. In a coronary microembolization (CME)-induced pig model, ultrasound microbubble-mediated miR-21 transfection effectively improved CME-induced cardiac dysfunction via inhibition of the PDCD4/NF-κB/TNF-α signal transduction pathway in cardiomyocytes [31]. Consistently, another study revealed that localized injection of miRNA-21-enriched extracellular vesicles effectively restored cardiac function after MI. miRNA-21-loaded EVs effectively delivered miRNA-21 into cardiomyocytes and ECs, thereby drastically inhibiting cell apoptosis and resulting in significant improvement of cardiac function [32]. Recently, Hinkel et al. found that miR-21 knockdown by intracoronary infusion of anti-miR-21 prevented inflammatory response and MAPK signaling and protected against myocardial dysfunction in a pig model of IR Injury [27]. These two completely opposite conclusions might be explained by the different targeting cells by miRNA-21-loaded EVs and antisense microribonucleic acid based antimir-21 treatment. Another apoptosis-related miRNA, miR-140, is downregulated after IR injury. Overexpression of miR-140 in myocytes reduced infarct size by inhibiting mitochondrial-mediated apoptosis by targeting YES proto-oncogene 1 (YES1) [33]. Li and colleagues noticed that bone marrow mesenchymal stem cell (BMSC)-derived exosomal miR-185 could repress apoptosis of cardiomyocytes and ventricular remodeling in mice with MI by inhibiting suppressor of cytokine signaling 2 (SOCS2) [34]. Genetic knockout of miR-181a in a murine model of MI deteriorated cardiac function, promoted cardiac remodeling, and activated the aldosterone-mineralocorticoid receptor (Aldo-MR) pathway. However, AAV9-mediated miR-181a overexpression improved cardiac function and deactivated the Aldo-MR pathway by targeting ADAM metallopeptidase with thrombospondin type 1 motif 1 (Adamts1) in cardiomyocytes [35]. miR-133 overexpression could reduce cardiomyocyte apoptosis, inflammation, and oxidative stress through the SIRT3/AMPK pathway, thereby improving the cardiac function in mice with MI [36]. Izarra et al. also reported that miR-133a cardiac progenitor cells clearly improved cardiac function in a rat model of MI by increasing cardiomyocyte proliferation and vascularization [37]. Transplantation of miR-1-transfected mesenchymal stem cells (MSCs) reportedly repaired infarct injury and improved heart function [38]. Delivery of miR-214 by adenovirus improved cardiac remodeling and decreased the apoptosis of myocardial cells through phosphatase and tensin homolog (PTEN) [39]. Glass and colleagues reported that miR-1-transfected embryonic stem cells enhanced cardiac myocyte differentiation and inhibited apoptosis by modulating the PTEN/Akt pathway in the infarcted heart [40]. Wang et al. found that miR-499 prevented MI and cardiac dysfunction induced by IR. Mechanistically, both α and β isoforms of the calcineurin catalytic subunit are direct targets of miR-499, which inhibits cardiomyocyte apoptosis by suppressing calcineurin-mediated dephosphorylation of dynamin-related protein-1 (Drp1), thereby decreasing its accumulation in the mitochondria and Drp1-mediated activation of the mitochondrial fission program [41]. Transgenic animals with cardiomyocyte-restricted overexpression of miR-199b-5p displayed enhanced cardiac hypertrophy and fibrosis after MI by specifically targeting the notch1 receptor tyrosine phosphorylation-regulated kinase 1A (DYRK1a) and its ligand jagged1 [42]. miR-150 is downregulated in patients with acute myocardial infarction (AMI). Genetic deletion of miR-150 in mice causes structural and functional abnormalities in cardiac remodeling after MI through the EGR2 (zinc-binding transcription factor induced by ischemia) and P2X7R (pro-inflammatory ATP receptor) pathways in cardiomyocytes [43]. Bayoumi et al. showed that miR-125b-5p, in response to ischemic stress, protects against

cardiomyocyte apoptosis by targeting the pro-apoptotic genes Bak1 and Klf13. Moreover, loss-of-function of miR-125b-5p in MI-induced mouse heart caused abnormalities in cardiac function [44]. Collectively, these apoptosis-related miRNAs play crucial roles in post-MI cardiac remodeling, and have considerable therapeutic potential.

The formation of new blood vessels through neoangiogenesis is also essential for cardiac repair following MI. miRNAs regulate the formation of new blood vessels by affecting the homeostasis of ECs. Transplantation of MSCs overexpressing miR-126 enhanced angiogenesis and cardiac function in the infarcted myocardium of mice by activating the AKT/ERK-related pathway [45]. The expression level of miR-26a is increased in mice with AMI and in human subjects with acute coronary syndrome. Systemic intravenous administration of an miR-26a inhibitor increased SMAD1 expression level in ECs and rapidly induced robust angiogenesis, thereby reducing myocardial infarct size and improving heart function [46]. Interestingly, in an adult pig model of reperfused AMI, a single intracoronary administration of antagomir-92a encapsulated in specific microspheres resulted in significant vessel growth, reduced regional wall-motion dysfunction, and prevented adverse remodeling in the infarct area [47]. While systemic inhibition of anti-miR-21 did not impede re-endothelialization, local miR-21 suppression using anti-miR-21-coated stents effectively reduced in-stent restenosis (ISR) [48]. Blocking of endothelial miR-24 limited myocardial infarct size in mice via prevention of endothelial apoptosis and enhanced vascularization, thereby maintaining cardiac function and survival through the endothelium-enriched transcription factor GATA2 and the p21-activated kinase PAK4 pathways [49].

However, it should be noted that although knockdown of a specific miRNA might reduce cardiac dysfunction, it does not imply that overexpression of this miRNA at any dosage would naturally protect against cardiac dysfunction. The effects of miRNAs vary at different dosages. Therefore, in this review, the cardio-protective miRNAs or cardio-detrimental miRNAs that are applicable in a specific pathophysiological stage of CVDs have been discussed with the particular miRNA concentration detailed in each study.

miRNAs in hypertension and hypertensive heart disease

Hypertensive heart disease comprises several changes including hypertrophy in the left ventricle and left atrium as a result of chronic blood pressure elevation [50], which can progress to HF [51]. Loss of nitric oxide (NO), increase in ROS, excessive Na^+ and H_2O reabsorption, and renin-angiotensin-aldosterone system activation have been reported to cause impaired endothelium-dependent relaxation and hypertension [51, 52]. miRNAs determine the progression of hypertension and cardiac complications by targeting various organs through different pathways (Figure 2).

It is generally accepted that hypertension is driven by chronic hyperactivity of the sympathetic nervous system (SNS) [53]. Friese et al. demonstrated that *in vivo* administration of miR-22 antagomir to spontaneous hypertensive rats (SHRs) caused substantial reduction in blood pressure. Interestingly, miR-22 appeared to regulate its target gene chromogranin A (CgA) in brainstem cardiovascular control nuclei to influence the pathogenesis of hypertension in SHRs [54].

Hypertension and kidney diseases are closely linked, and hypertension is both the cause and effect of kidney diseases. Modification of kidney-localized miRNAs enabled in determining the outcomes of hypertension and cardiac complications. Inhibition of miR-429 in the renal medulla increased the salt sensitivity of blood pressure in Sprague Dawley rats. Mechanistically, miR-429 is the upstream mediator in PHD2/HIF-1α-associated renal adaptation to high salt intake [55]. Kidney-specific inhibition of miR-214-3p significantly attenuated salt-induced hypertension in Dahl salt-sensitive (SS) rats [56]. miR-214-3p directly targeted eNOS and the effects of its inhibition in hypertension were abrogated by heterozygous loss of eNOS [56]. miR-192-5p targeted ATP1B1 mRNA (β1 subunit of Na^+/K^+-ATPase), which drives renal tubular reabsorption. Anti-miR-192-5p delivered through renal artery injection in uninephrectomized SS rats significantly increased hypertension [57]. Carr et al. found that miR-153 level was increased in renal and mesenteric arteries (MAs) of SHRs, whereas its direct target Kv7.4, a voltage-dependent potassium channel expressed throughout the vasculature that controls arterial contraction, was decreased [58]. Functional assays revealed that delivery of miR-153 to MAs increased vascular wall thickening and downregulated Kv7.4 expression/Kv7 channel function [58]. Inflammatory cells in the kidneys also regulate blood pressure. Notably, Ang II-induced hypertension resulted in increased expression level of miR-31 in the kidney leukocytes, aorta, and splenic CD4+ T cells. miR-31 deficiency markedly reduced blood pressure and attenuated vascular damage through protein phosphatase 6C (PPP6C)-mediated Treg cell differentiation [59]. miR-181a was decreased in BPH/2J mice, a genetic model of hypertension with overactivity of the

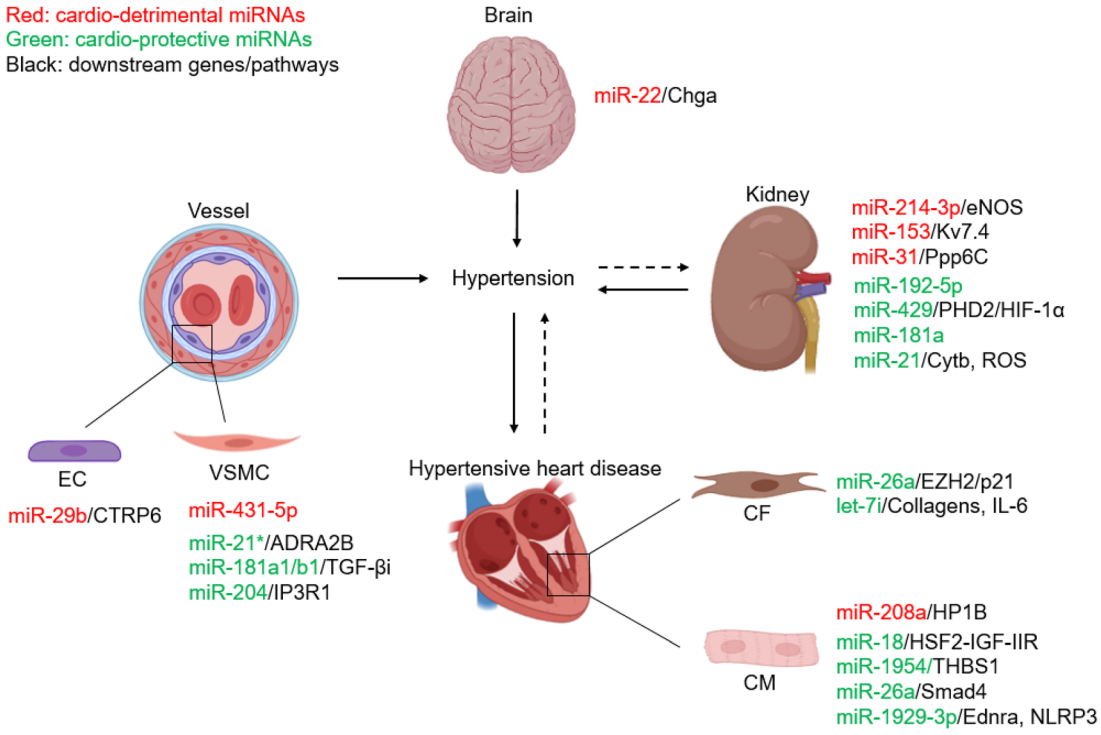

Figure 2: miRNAs with key roles in hypertension and hypertensive heart disease. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. Chga, Chromogranin A; CTRP6, C1q/tumor necrosis factor-related protein 6; ADRA2B, adrenal α2B-adrenergic receptor; TGF-βi, TGF-β induced; IP3R1, type 1 Inositol 1, 4, 5-trisphosphate receptor; eNOS, endothelial nitric oxide synthase; Ppp6C, protein phosphatase 6c; PHD2, prolyl hydroxylase domain-containing protein 2; HIF-1α, hypoxia-inducible factor -1α; Cytb, cytochrome *b*; EZH2: zeste homolog 2; IL-6, interleukin 6; HP1B, heterochromatin protein 1 homolog beta; HSF2-IGF-IIR, heat shock factor 2-insulin-like growth factor receptor II; THBS1, thrombospondin 1; Ednra, endothelin A receptor; NLRP3, NOD-like receptor pyrin domain containing 3; EC, endothelial cell; VSMC, vascular smooth muscle cell; CF, cardiac fibroblast; CM, cardiomyocyte.

SNS and renin-angiotensin system. Overexpression of miR-181a mimic reduced blood pressure in BPH/2J mice partially through an interaction between the renal sympathetic nerves and miR-181a-mediated regulation of RAS [60]. These studies linked kidney-localized miRNAs directly with hypertension. Our group has revealed that miR-21 delivered via recombinant adeno-associated virus (rAAV) is sufficient to lower blood pressure and prevent hypertensive hypertrophy as well as cardiac fibrosis in the SHR model. Interestingly, miR-21 directly targeted mitochondrial cytochrome-b (mt-cytb) to enhance its translation, thereby decreasing mitochondrial ROS production in cardiomyocytes. Moreover, rAAV-delivered miR-21 targeted the kidney and liver and may collectively contribute to the observed reduction in blood pressure [61]. Interestingly, genetic ablation of miR-21 increased Sprouty 2 (Spry2) expression level and promoted aldosterone/salt-mediated cardiac dysfunction. However, it did not affect aldosterone/salt-mediated hypertension [62], indicating that different approaches to modify miR-21 expression *in vivo* might exert conflicting effects.

In the peripheral blood vessels, phenotypic transformation of VSMCs is a major initiating factor for vascular remodeling in hypertension. We revealed the protective role of miR-21* (miR-21-3p) in hypertension by showing that intravenous delivery of rAAV-mediated miR-21-3p induced a persistent attenuation of hypertension, with marked amelioration of target organ damage, including cardiac hypertrophy, cardiac fibrosis, and kidney fibrosis in SHRs. Mechanistically, miR-21-3p-mediated hypotensive reduction effect was accomplished by regulating the phenotypic switch of VSMCs via suppression of the adrenal α2B-adrenergic receptor (ADRA2B) in arteries [63]. The miR-181 family has also been reported to be involved in blood pressure regulation. Hori et al. demonstrated a progressive increase in pulse wave velocity and elevated systolic blood pressure in miR-181a1/b1(−/−) mice compared to those in wild-type (WT) controls. Mechanistically, miR-181b targeted TGF-βi (TGF-β-induced) in aortic VSMCs to modify vascular stiffness, but endothelial function was unaltered [64]. In VSMCs, miR-431-5p targeted ETS homologous factor (Ehf) and *in vivo* miR-431-5p

knockdown by injecting miR-431-5p inhibitors delayed Ang II-induced blood pressure elevation and reduced vascular injury [65]. Gabani et al. reported a marked increase in blood pressure in Ang II-treated miR-204−/− mice compared to that in control animals. miR-204 protected against VSMC contractility and blood pressure through regulating sarcoplasmic reticulum (SR) calcium (Ca^{2+}) release by targeting type 1 inositol 1, 4, 5-trisphosphate receptor (IP3R1) [66].

ECs in the peripheral blood vessels also play an important role in vascular remodeling in hypertension. Sun and colleagues demonstrated that miR-29b was highly upregulated in SHRs. Intraventricular injection of miR-29b inhibitor decreased arterial systolic pressure, reduced serum Ang II and endothelin 1 (ET-1) concentrations, and enhanced serum NO content through the C1q/tumor necrosis factor-related protein 6 (CTRP6) pathway. In accordance, CTRP6 recombinant protein could antagonize the suppressive effect of miR-29b by activating the ERK/PPARγ axis and function of aortic ECs [67].

In the heart, miRNA treatment might directly determine the progression of hypertensive complications (cardiomyopathy) without affecting blood pressure. Huang et al. reported that transgenic overexpression of miR-18, specifically in cardiomyocytes, using the myosin light chain promoter was sufficient to protect against hypertrophy and fibrosis in SHRs [68]. Mechanistically, their study revealed that the p53-miR-18-HSF2-IGF-IIR axis is a critical regulatory pathway of cardiomyocyte hypertrophy, suggesting that miR-18 is a therapeutic target for alleviation of cardiomyopathy during hypertension-induced HF [68]. It has been suggested that cardiac-specific miRNA overexpression can reverse blood pressure levels, which might be due to the translocation of miRNAs from the heart to other tissues. For example, Chiasson et al. reported that cardiac-specific miR-1954 overexpression using the α-myosin heavy chain (α-MHC) promoter significantly reduced cardiac mass and blood pressure in Ang II-infused mice. By targeting thrombospondin 1 (THBS1), miR-1954 decreased fibrotic and inflammatory gene expression level and restored the expression of the calcium-regulated gene, ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2 (SERCA2), without affecting apoptotic genes [69]. Expression level of miR-26a was decreased in the myocardium of SHRs, and overexpression of miR-26a by rAAV9 significantly prevented the increase in blood pressure and inhibited myocardial fibrosis in Ang II-treated mice by directly targeting connective tissue growth factor (CTGF) and Smad4. In addition, miR-26a overexpression inhibited cardiac fibroblast proliferation by the enhancer of the zeste homolog 2 (EZH2)/p21 pathway [70]. Wang et al. reported that delivery of let-7i by an agomir significantly inhibited Ang II-induced cardiac inflammation and fibrosis without affecting blood pressure. Mechanistically, let-7i protected against Ang II-induced cardiac inflammation and fibrosis by suppressing the expression of interleukin-6 (IL-6) and multiple collagens [71]. Montgomery et al. demonstrated that, during hypertension-induced HF in Dahl hypertensive rats, therapeutic inhibition of cardiac-specific miR-208a by subcutaneous delivery of anti-miR-208a dose-dependently prevented pathological myosin switching, cardiac remodeling, and cardiac dysfunction. Anti-miR-208a treatment resulted in the derepression of the characterized miR-208a target, heterochromatin protein 1 homolog beta (HP1B) [72]. In C57BL/6 mice with hypertension induced by murine cytomegalovirus (MCMV), the expression level of miR-1929-3p was decreased. Overexpression of miR-1929-3p by rAAV vector in MCMV-infected mice protected against hypertension and myocardial remodeling through downregulating the expression of endothelin receptor type A (EDNRA) and NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome activation [73].

Collectively, these miRNAs are promising candidates for the treatment of hypertension and hypertensive cardiomyopathy.

miRNAs in diabetic cardiomyopathy

DCM is defined as structural and functional abnormalities of the myocardium in diabetic patients without coronary artery disease or hypertension [74]. Mechanistically, lipotoxicity, ROS, mitochondrial dysfunction, impaired Ca^{2+} handling, advanced glycation end-products, endothelial disorders, inflammation, autophagy, and apoptosis/necrosis are involved in the development and progression of DCM. miRNAs effectively determine the cardiac function in diabetic models by regulating the function of myocytes, ECs, and fibroblasts in the heart (Figure 3).

Hyperglycemia-induced endothelial injury is a key pathogenic factor in DCM. Feng et al. generated endothelial-specific miR-146a-overexpressing transgenic mice (Tg) using the tie-2 promoter and investigated the role of miR-146a in DCM. miR-146a overexpression protected against streptozotocin (STZ)-induced inflammatory markers and extracellular matrix proteins (IL6, TNFα, IL-1β, MCP-1, NF-κB, Col1α1, and Col4α1) and cardiac dysfunction [75]. Zheng et al. demonstrated the upregulation of miR-195 in STZ-treated and db/db mouse hearts. Systemic delivery of a nanoparticle-based anti-miR-195 construct reduced caspase-3 activity, decreased ROS, and improved myocardial function in STZ-induced mice

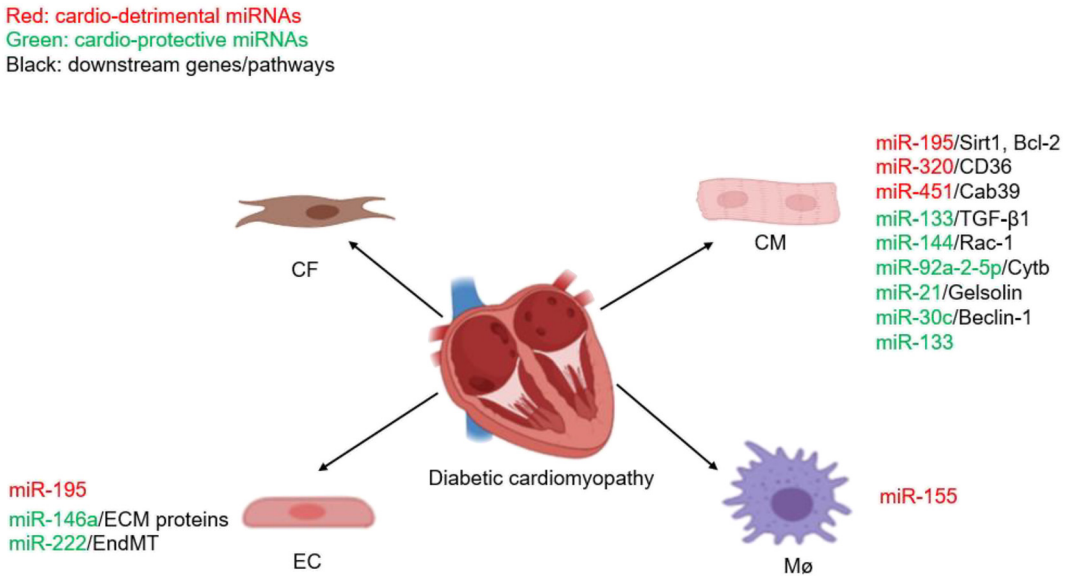

Figure 3: miRNAs with key roles in DCM. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. DCM, diabetic cardiomyopathy; EC, endothelial cell; Mø, macrophage; CF, cardiac fibroblast; CM, cardiomyocyte; ECM proteins, extracellular matrix proteins; EndMT, Wnt/β-catenin-mediated endothelium to mesenchymal transition; Sirt1, sirtuin 1; Bcl-2, B-cell lymphoma 2; TGF-β1, transforming growth factor beta 1; Rac-1, Rac Family Small GTPase 1; Cab39, calcium-binding protein 39; Cytb, cytochrome *b*;

by targeting Sirt1 and Bcl-2 mRNA in ECs and cardiomyocytes [76].

Cardiomyocyte hypertrophy, apoptosis, and fibrosis are critical pathogenic events that occur due to DCM. Chen et al. reported a marked decrease in miR-133a in the hearts of STZ-induced diabetic mice. Transgenic overexpression of miR-133a under the α-myosin heavy-chain promoter prevented cardiac fibrosis, at least partly through the TGF-β1 signaling pathway [77]. Xu et al. found that lentivirus-based inhibitors of miR-223 attenuated NLRP3 inflammasome activation, fibrosis, and apoptosis in STZ-induced DCM [78]. The expression level of cardiac miR-144 was decreased in the STZ-treated mouse heart tissues. Systemic overexpression of miR-144 using an agomir improved cardiac function and mitochondrial biogenesis by targeting the Rac family small GTPase 1 (Rac-1) pathway [79]. A study by Wang et al. revealed that intravenous injection of Ad-miR-222 inhibited cardiac fibrosis in STZ-treated mice by regulating Wnt/β-catenin-mediated endothelial-to-mesenchymal transition (EndMT) [80]. Kuwabara et al. found that miR-451 levels were significantly increased in the hearts of mice with type 2 diabetes mellitus (DM). Moreover, HFD-induced cardiac hypertrophy and contractile reserves were ameliorated in cardiomyocyte-specific miR-451 knockout mice compared to those in controls [81]. Mechanistically, miR-451 inhibits the expression of its target gene, the calcium-binding protein 39 (Cab39), which is a scaffold protein of liver kinase B1 (LKB1), an upstream kinase of AMP-activated protein kinase (AMPK) [81]. Kambis et al. demonstrated that in the Akita model, which exhibits insulin resistance and deficiency (double DM), miR-133a was decreased. Crossbreeding of cardiac-specific miR-133a transgenic mice with Akita mice prevented DM-induced cardiac fibrosis, hypertrophy, and impaired contractility without affecting glucose levels [82].

Our group has systematically investigated the role of miRNAs in DCM. Our recent study revealed that miR-320 is significantly upregulated in the hearts of mice and patients with DM. Interestingly, miR-320 translocates into the nucleus to directly enhance CD36 transcription [83]. CD36 is responsible for increased fatty acid uptake and cardiac lipid accumulation, thereby causing lipotoxicity in the heart. The miR-320/CD36 pathway links glucose toxicity to lipotoxicity in the heart. In another study, we found that miR-92a-2-5p and let-7b-5p were downregulated in the mitochondria of db/db hearts. Re-expression of miR-92a-2-5p and let-7b-5p in cardiomyocytes led to reduced ROS production via enhanced translation of the mitochondrial gene, cytochrome-b (mt-cytb). *In vivo*, rAAV-mediated delivery of miR-92a-2-5p, but not let-7b-5p, rescued cardiac diastolic dysfunction in db/db hearts. Mechanistically, let-7b-5p not only upregulated mt-cytb in the mitochondria, but also downregulated insulin receptor substrate 1 (IRS1)

in the cytosol, leading to unaltered cardiac function in db/db mice [84]. We also reported downregulation of miR-21 and miR-30c in db/db diabetic hearts. miR-30c or miR-21 overexpression using rAAV9 vector rescued functional and structural cardiac changes via targeting *beclin-1*-regulated autophagy and gelsolin-mediated bioavailable NO, respectively [85, 86].

miRNA-mediated regulation of inflammatory cells has been observed in DCM. Jia et al. reported that delivery of miR-155 antagonist using gold nanoparticles (AuNPs) preferentially released the nucleic acids into macrophages via phagocytosis, increased M2 ratio, reduced inflammation, and protected against cell apoptosis and cardiac dysfunction [87].

Taken together, targeting these miRNAs might protect the heart from diabetes-induced cardiomyopathy in the future.

miRNAs and cardiac arrhythmias

Cardiac arrhythmia is defined as abnormal heart rate or rhythm. miRNAs regulate cardiac rhythm through key ion channels, transporters, and cellular proteins in arrhythmogenic conditions [88].

In animal studies, arrhythmia is usually induced by MI. Yang et al. found that the muscle-specific miRNA miR-1 is overexpressed in individuals with CHD, and when overexpressed in normal or infarcted rat hearts, it exacerbates arrhythmogenesis. miR-1 knockdown by an antisense inhibitor relieved arrhythmogenesis in infarcted rat hearts. Mechanistically, miR-1 overexpression slowed conduction and depolarized the cytoplasmic membrane by post-transcriptionally repressing the K^+ channel subunits KCNJ2 and GJA1, which might be the possible reason for its arrhythmogenic potential [89]. Terentyev et al. found that adenoviral-mediated overexpression of miR-1 enhanced Ca2+ release and promoted cardiac arrhythmogenesis by targeting the PP2A regulatory subunit B56alpha, and causing CaMKII-dependent hyperphosphorylation of RyR2 in cardiomyocytes [90]. Cardiac-specific miR-1 transgenic (Tg) mice showed a higher incidence of atrioventricular (AV) block than controls [91]. In guinea pigs treated with the anti-promyelocytic leukemia drug arsenic trioxide (As_2O_3), QT interval and QRS complex were significantly prolonged. Forced expression of miR-133 and miR-1 widened the QT interval and QRS complex and increased the mortality rate by targeting the ether-a-go-go related gene (ERG) and K(+) channel subunits such as IKr, Kir2.1, and IK1. Knockdown of miR-1 and miR-133 abolished these electrical disorders induced by As_2O_3 [92].

miR-1231 is overexpressed in human and rat hearts after MI. Functionally, inhibition of miR-1231 *in vivo* ameliorates arrhythmias in rat hearts caused by MI by targeting the calcium channel gene CACNA2D2 [93]. The miR-206 inhibitor alleviated IR-induced arrhythmias by regulating connexin 43 (Cx43) [94]. Osbourne et al. reported that cardiac-specific miR-130a transgenic mice developed sustained ventricular tachycardia, six weeks after overexpression, by targeting Cx43 [95]. Constitutive expression of miR-17-92 in cardiac and smooth muscle tissues led to defective heart function and arrhythmogenic susceptibility, partly through direct repression of PTEN and Cx43 [96]. Upregulation of miR-223-3p in AMI repressed KCND2/Kv4.2 expression, leading to a reduction in transient outward K^+ current (Ito density) that could cause prolonged action potential duration and thereby promote ischemic arrhythmias [97]. miR-130a transgenic mice demonstrated right ventricular dilation and an arrhythmogenic phenotype (spontaneous premature ventricular complexes) by downregulating desmocollin2 (DSC2), an important protein required for cell adhesion [98].

In a rat atrial fibrillation (AF) model established by tail vein injection of acetylcholine- (ACh-)$CaCl_2$, miR-27b-3p level was decreased. miR-27b-3p overexpression by tail vein injection of rAAV reduced AF incidence and duration, alleviated atrial fibrosis, and increased atrial Cx43 expression by targeting the Wnt3a pathway [99]. miR-328 levels were elevated in dogs and patients with AF relative to those in non-AF subjects. Overexpression of miR-328 through adenovirus infection in canine atrium and a transgenic approach recapitulated the phenotypes of AF by targeting CACNA1C and CACNB1, which encode cardiac L-type Ca^{2+} channel α1c- and β1 subunits, respectively. Inhibition of miR-328 levels with an antagomir or genetic knockdown dampened AF vulnerability [100]. Luo et al. demonstrated that knockdown of miR-26 promoted AF, whereas adenovirus-mediated expression of miR-26 reduced AF vulnerability in mice through potassium inwardly rectifying channel subfamily J member 2 (KCNJ2) [101]. Chiang et al. reported that genetic knockdown of the miR-106b-25 cluster promoted AF via enhanced RyR2-mediated SR Ca^{2+} leak [102]. Reilly et al. showed that upregulation of miR-31 in human AF leads to arrhythmia by depleting dystrophin and neuronal nitric oxide synthase (nNOS) [103]. Atrial overexpression of miR-27b by adeno-associated virus attenuated Ang II-induced fibrillation and atrial fibrosis by targeting activin-like kinase 5 (ALK5) [104]. In zinc finger homeobox 3 (ZFHX3) knockdown (KD)-induced AF, miR-133a and miR-133b were significantly downregulated. Of note, miR-133a/b mimic treatment diminished ZFHX3 KD-induced atrial ectopy in mice, possibly by targeting adrenergic, Wnt/calcium, and fibroblast growth factor receptor 1

signaling [105]. Wang et al. found that both miR-17-92-and miR-106b-25-deficient mice exhibited sinoatrial node dysfunction and pacing-induced AF. Mechanistically, miR-17-92 and miR-106b-25 directly repressed the genes Shox2 and Tbx3, which are required for sinoatrial node development [106].

Callis et al. found significantly prolonged PR interval rates in miR-208a transgenic mice compared to those in controls. Interestingly, approximately 30% of miR-208a Tg mice also had second-degree AV blocks, causing failure of ventricular contraction. In contrast, the ECGs of approximately 80% of miR-208a(–/–) mice lacked P waves preceding QRS complexes, suggesting that these mice suffered from AF [107]. Mechanistically, miR-208a is required for the expression of connexin 40 (Cx40), a potent cardiac conduction regulator that is restricted to the atria and specialized cardiomyocytes constituting the His bundle and Purkinje fibers [107, 108].

Knockdown of miR-423-5p with anti-miR-423-5p reversed training-induced bradycardia via rescue of hyperpolarization-activated cyclic nucleotide gated channel 4 (HCN4), and the corresponding ionic current, I_f, in mice [109]. Yanni et al. observed sinus bradycardia in mice with transverse aortic constriction (TAC)-induced HF. Upregulation of miR-370-3p resulted in downregulation of the pacemaker ion channel HCN4, and the corresponding ionic current, I_f. Functionally, intraperitoneal injection of miR-370-3p anti-miR into mice with TAC restored HCN4 expression and I_f in the sinus node, thus diminishing sinus bradycardia [110]. Decrease in Cav1.2 and Cav1.3 Ca^{2+} channels have been linked to sick sinus syndrome. Moreover, miR-1976 levels in rabbit SAN tissues were negatively correlated with Cav1.2 and Cav1.3 expression but positively correlated with corrected sinus node recovery time. Functionally, miR-1976 transgenic mice displayed attenuated Cav1.2 and Cav1.3 protein expression, which led to sinus node dysfunction [111].

Most studies regarding cardiac arrhythmias have focused on the roles of myocyte-localized miRNAs (Figure 4). Reports regarding the role of ECs or fibroblast-enriched miRNAs in arrhythmias are scarce and require further investigation.

TAC-induced HF

In animal models, TAC and thoracic aortic banding (TAB) are commonly used to investigate the mechanism of pressure overload-induced hypertrophy, fibrosis, and cardiac dysfunction, which are clinically exhibited by many patients with CVDs. Therefore, examination of miRNAs using the TAC model might also have therapeutic potential in general (Figure 5).

Fibrosis is critical in pressure-overload-induced cardiac remodeling. miR-21 levels are increased selectively in

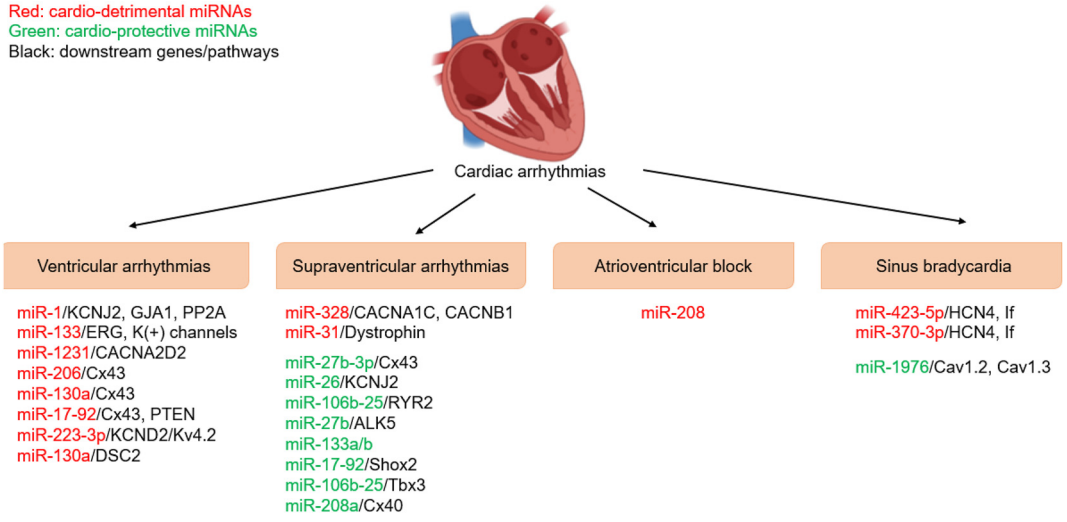

Figure 4: miRNAs with key roles in cardiac arrhythmias. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. KCNJ2, Potassium Inwardly Rectifying Channel Subfamily J Member 2 (which encodes the K^+ channel subunit Kir2); GJA1, gap junction protein alpha 1 (which encodes connexin 43); PP2A, protein phosphatase 2A regulatory subunit; ERG, ether-a-go-go related gene; CACNA2D2, calcium voltage-gated channel auxiliary subunit alpha 2 delta 2; Cx43, Connexin 43; PTEN, phosphatase and tensin homolog; KCND2, potassium voltage-gated channel subfamily D member 2 (which encodes voltage-gated channel Kv4.2); DSC2, desmocollin2; CACNA1C, calcium voltage-gated channel subunit alpha1 C; CACNB1, calcium voltage-gated channel auxiliary subunit beta 1; RYR2, ryanodine receptor type 2; ALK5, activin like kinase 5; Shox2, short stature homeobox; Tbx3, T-box transcription factor 3; Cx40, connexin 40; HCN4, hyperpolarization-activated cyclic nucleotide gated channel 4.

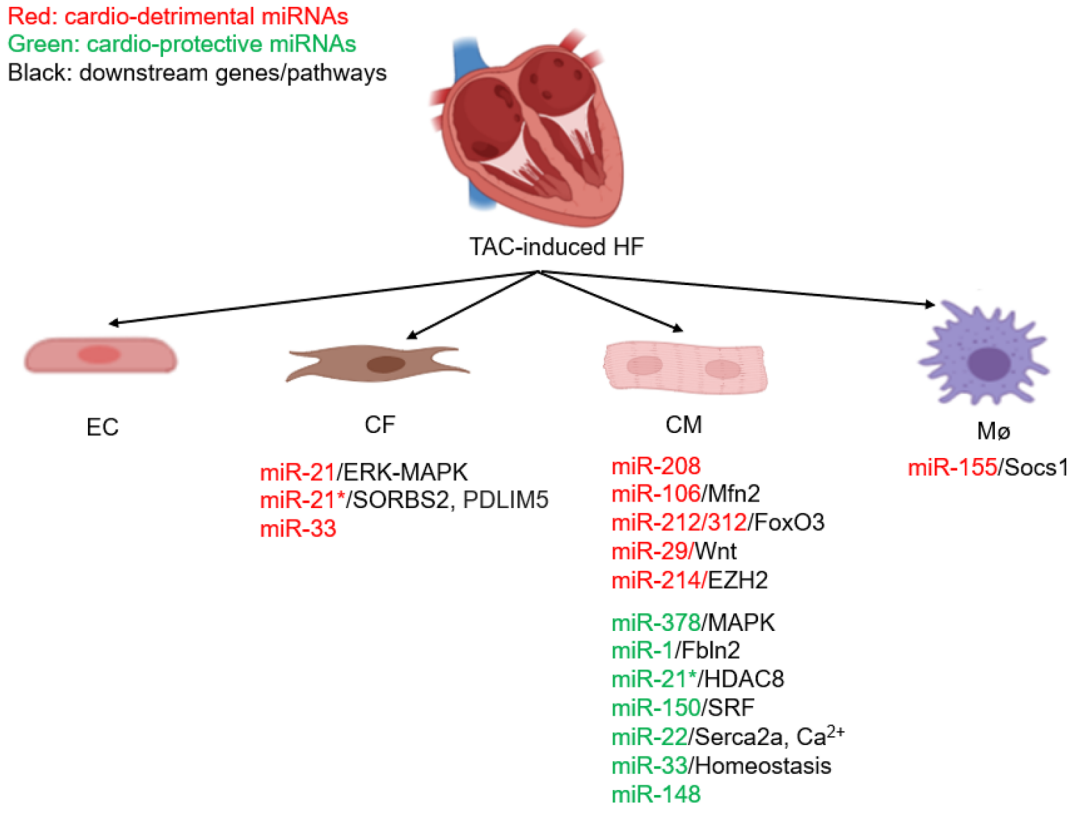

Figure 5: miRNAs with key roles in TAC-induced heart failure. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. Mø, macrophage; CF, cardiac fibroblast; CM, cardiomyocyte; ERK, extracellular signal regulated kinase; MAPK, mitogen-activated protein kinase; SORBS2, sorbin and SH3 domain-containing protein 2; PDLIM5, PDZ and LIM domain 5; Mfn2, mitofusin 2; FoxO3, forkhead transcription factors of the O class; EZH2, zeste homolog 2; Fbln2, fibulin 2; HDAC8, histone deacetylase-8; SRF, serum response factor; Socs1, suppressor of cytokine signaling 1.

fibroblasts of TAC-induced failing heart. *In vivo* silencing of miR-21 by antagomir in a TAC model reduced cardiac ERK-MAP kinase activity, inhibited interstitial fibrosis, and attenuated cardiac dysfunction [112]. Bang et al. reported that fibroblast exosomal-derived miR-21-3p induced cardiomyocyte hypertrophy. Proteome profiling identified sorbin and SH3 domain-containing protein 2 (SORBS2) and PDZ and LIM domain 5 (PDLIM5) as targets of miR-21-3p [113]. However, our group has observed a decrease in miR-21-3p levels in cardiac hypertrophy induced by TAC and Ang II infusion in mice. Overexpression of miR-21-3p by rAAV9 markedly suppressed TAC-induced cardiac hypertrophy and cardiac dysfunction. Mechanistically, the effects of miR-21-3p on myocyte hypertrophy are dependent on histone deacetylase-8 (HDAC8) [114]. Interestingly, Ramanujam et al. found that in the TAC model, cardiac function was maintained only in mice with miR-21 deficiency in nonmyocyte cardiac cells but not in mice with global or cardiac myocyte-specific ablation. These data demonstrated that miR-21 exerts its pathologic activity differently in CMs and non-CMs [115]. Nishiga et al. observed upregulation of miR-33a/b in human failing hearts and TAC-treated mouse hearts. miR-33 KO mice showed impaired systolic function despite amelioration of cardiac fibrosis. Mechanistically, miR-33 deficiency impaired cardiac fibroblast proliferation through altered lipid raft cholesterol content. Therefore, targeting cardiac fibroblast-localized miR-33 rather than global miR-33 might be promising for HF treatment in the future [116].

Myocyte apoptosis and hypertrophy are also closely regulated by miRNAs. Ucar et al. found that miR-212/132 knockout mice were protected from TAC-induced HF, whereas cardiomyocyte-specific overexpression of the miR-212/132 family led to pathological cardiac hypertrophy and HF. Both miR-212 and miR-132 directly target the anti-hypertrophic and pro-autophagic FoxO3 transcription factor and overexpression of these miRNAs leads to hyperactivation of pro-hypertrophic calcineurin/NFAT signaling [117]. Targeted deletion of miR-29 in cardiac myocytes *in vivo* prevented cardiac hypertrophy and

fibrosis through Wnt signaling in a TAC-induced model of HF [118]. A study by Liu et al. revealed that transgenic mice that overexpress miR-150 in the heart were resistant to cardiac hypertrophy and fibrosis through downregulation of SRF in the TAC model [119]. miR-208, a cardiac-specific miRNA encoded by an intron of the aMHC gene, is required for cardiomyocyte hypertrophy, fibrosis, and expression of bMHC in response to stress, TAB and hypothyroidism. Although genetic deletion of miR-208 in mice failed to induce an overt phenotype at baseline in response to cardiac stress, miR-208 knockout mice showed virtually no cardiomyocyte hypertrophy or fibrosis [120]. Ganesan et al. found a significant decrease in miR-378 in the failing hearts of mice. Functionally, cardiomyocyte-targeted expression of miR-378 by AAV9 partially prevented cardiac hypertrophy by regulating the MAPK pathway [121]. In Sprague Dawley rats subjected to ascending aortic stenosis, AAV9-mediated miR-1 overexpression reduced cardiac hypertrophy and prevented fibrosis by targeting fibulin-2 (Fbln2) [122]. Knockdown of miR-106a by an antagomir through tail vein injection nearly completely reversed the hypertrophic phenotypes induced by TAC and Ang II pretreatment by targeting mitofusin 2 (Mfn2) [123]. Gurha et al. reported the induction of miR-22 expression due to TAC stress during the early phase(s) of cardiac remodeling. Genetic ablation of miR-22 sensitized mice to cardiac decompensation and left ventricular dilation after long-term stimulation with TAC-induced pressure overload. Mechanistically, miR-22 knockdown led to a decrease in Serca2a and muscle-restricted genes, encoding proteins in the vicinity of the cardiac Z disk/titin cytoskeleton, which partly regulate the SRF pathway [124]. Yang et al. reported that cardiomyocyte-specific overexpression of miR-214 resulted in enlarged left ventricular internal diameter, wall thinning, and reduced ejection fraction by zeste homolog 2 (EZH2). *In vivo* silencing of miR-214 using an antagomir prevented cardiac hypertrophy and dysfunction in a TAC model [125]. Raso et al. showed that antagomir-mediated silencing of miR-148a caused wall thinning, chamber dilation, increased left ventricle volume, and reduced cardiac performance in mice with TAC-induced HF. Overexpression of miR-148a prevented the transition from TAC-induced concentric hypertrophic remodeling to eccentric hypertrophy [126]. Systemic delivery of miR15a/miR16-1 by nanoparticles prevented the hypertrophic phenotype and cardiac dysfunction induced by TAC through multiple targets, including INSR, IGF-1R, AKT3, and serum/glucocorticoid regulated kinase 1 (SGK1) [127].

Inflammatory cells are also involved in the progression of pressure overload-induced HF. Heymans et al. demonstrated that macrophage-derived miR-155, but not cardiomyocyte-specific miR-155, promoted cardiac inflammation, hypertrophic growth, and systolic dysfunction in the TAC model by inhibiting the suppressor of cytokine signaling 1 (SOCS1). SOCS1 knockdown in miRNA-155 knockout macrophages largely restored their hypertrophy-stimulating potency [128].

Kawasaki disease

Kawasaki disease is an acute febrile illness and systemic vasculitis of unknown etiology that predominantly afflicts young children, causing coronary artery aneurysms and long-term cardiovascular sequelae [129]. Mechanistically, it is characterized by infiltration of the coronary artery wall by a broad variety of innate and adaptive immune cells, vascular EC damage, VSMC proliferation, and endothelial-mesenchymal transition [129].

Patients with kawasaki disease with the most severe coronary pathology (giant coronary artery aneurysms) exhibited lack of miR-223 induction. In a mouse model of kawasaki disease (induced by *Lactobacillus casei* cell wall extract injection), Zhang et al. found that miR-223 knockout mice exhibited increased medial thickening, loss of contractile VSMCs in the media, and fragmentation of medial elastic fibers compared with WT mice. Mechanistically, reduced platelet-derived miR-223 induction in kawasaki disease led to persistent VSMC dedifferentiation via the miR-223/PDGFRβ-VSMC axis [130]. In another mouse model of kawasaki disease generated using *Candida albicans* water-soluble fraction, miR-223-3p levels were increased during the acute stage of kawasaki disease. Systemic miR-223-3p overexpression alleviated vascular endothelial damage and decreased inflammation in mice with kawasaki disease by targeting interleukin-6 receptor subunit beta (IL-6ST) [131]. Despite these findings, the role of miRNAs in kawasaki disease is still largely unknown which might be due to lack of appropriate animal models of kawasaki disease. Therefore, further *in vivo* studies are the need of the hour.

miRNAs and myocarditis

The pathogenesis of viral myocarditis (VMC) is based on an adverse immune response evoked by infection of the cardiac muscle by cardiotropic viruses, which results in viral elimination as well as cardiomyocyte destruction, cardiac fibrosis, and HF [132]. Recent studies have revealed that miRNAs regulate the progression of myocarditis by

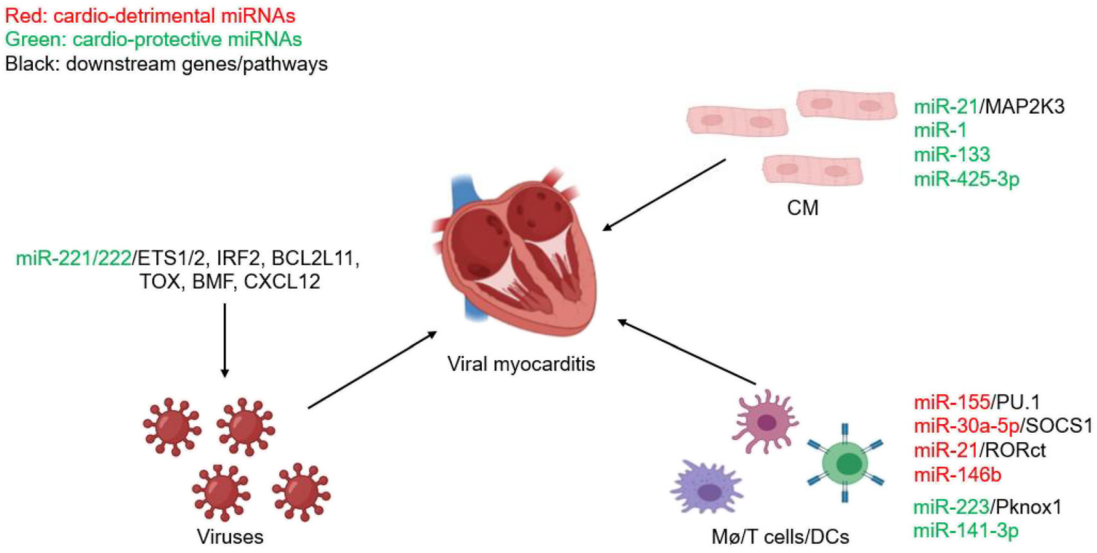

Figure 6: miRNAs with key roles in VMCs. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. VMCs, viral myocarditis; CM, cardiomyocyte; Mø, macrophage; DCs, dendritic cells; MAP2K3, mitogen-activated protein kinase 3; SOCS1, suppressor of cytokine signaling 1; RORct, retineic-acid-receptor-related orphan nuclear receptor gamma; Pknox1, PBX/Knotted 1 Homeobox 1; ETS1/2, ETS proto-oncogene 1/2; IRF2, interferon regulatory factor 2; BCL2L11, BCL2-like 11; TOX, thymocyte selection associated high mobility group box; BMF, Bcl2 modifying factor; CXCL12, C-X-C motif chemokine ligand 12.

affecting the function of inflammatory cells, viral replication, and myocyte apoptosis (Figure 6).

In mice with coxsackievirus B3 (CVB3)-induced VMC, miR-155 was consistently and strongly upregulated primarily in infiltrating macrophages and T lymphocytes. Inhibition of miR-155 by systemic delivery of an LNA-antimiR attenuated cardiac inflammatory cell infiltration and reduced myocardial damage during VMC through regulation of PU.1, an E26 transformation-specific family transcription factor and an inhibitor of dendritic cell (DC) antigen presentation to T cells [132]. Corsten et al. demonstrated that miR-221 and miR-222 levels were significantly elevated during acute VMC caused by CVB3. Systemic inhibition of miR-221/-222 by antagomirs in mice increased cardiac viral load and strongly aggravated cardiac injury and inflammation. Viral replication and inflammation-related targets of miR-221/-222 were identified including ETS1/2, IRF2, BCL2L11, TOX, BMF, and CXCL12 [133]. miR-223 expression was downregulated in heart tissues and heart-infiltrating macrophages of CVB3-infected mice. miR-223 overexpression using a lentiviral vector protected the mice against CVB3-induced myocardial injuries and cardiac dysfunction by directly targeting PBX/Knotted 1 Homeobox 1 (Pknox1), thereby suppressing the expression of M1 markers (iNOS, TNF-α, and CD 86), and promoting the expression of M2 markers (Arginase-1, Fizz-1, and CD 206) [134]. miR-30a-5p was found to be highly expressed in mice with VMC, and silencing its expression using antagomir polarized macrophages toward the M2 phenotype to alleviate cardiac dysfunction by targeting suppressor of cytokine signaling 1 (SOCS1) [135]. miR-21 and miR-146b were upregulated in a mouse model of CVB3-induced VMC. Inhibition of miR-21 and miR-146b ameliorated myocardial inflammation by decreasing the expression of Th17 through regulating retinoic-acid-receptor-related orphan nuclear receptor gamma-t (RORγt) expression [136]. However, He et al. demonstrated that mice injected with plasmid encoding miR-21 (pMDH-miR-21) showed reduced myocardial injury, lowered myocarditis score, and increased survival rate by repressing PDCD4-mediated myocyte apoptosis [137]. Another study showed that miR-21 overexpression by lentiviral vectors inactivated MAP2K3/P38/MAPK signaling in VMC, leading to prolonged survival time without affecting the viral load within host cells [138]. Therefore, miR-21 seems to play a dual role in VMC. Moreover, it might have conflicting roles in different target cells such as myocytes and inflammatory cells.

Cardiac-specific miR-1 and miR-133 decreased cardiomyocyte apoptosis by mediating the expression of apoptosis-related genes in the hearts of mice with CVB3-induced VMC [139]. Another apoptosis-related miRNA, miR-425-3p, was downregulated in VMC. Overexpression of

miR-425-3p agomir prevented cardiomyocyte apoptosis and improved cardiac function by targeting transforming growth factor β1 (TGF-β1) [140].

In experimental autoimmune myocarditis (EAM) induced by subcutaneous injection of porcine cardiac myosin, miR-590-3p was reduced, while the p50 subunit of NF-κB was increased. *In vivo*, miR-590-3p overexpression by adeno-associated virus decreased p50 expression, suppressed NF-κB activity, and blocked IL-6/TNF-α expression, leading to improved cardiac function in EAM [141]. miR-223-3p expression level was reduced in mice with EAM. *In vivo* transfer of miR-223-3p-overexpressing DCs attenuated autoimmune myocarditis and improved cardiac performance by inhibiting NLRP3 inflammasome expression, which promoted the polarization of DCs toward a tolerogenic DC phenotype [142]. Overexpression of miR-141-3p by an agomir alleviated the inflammatory response and improved cardiac function in mice with EAM by inhibiting the STAT4 pathway [143]. A study by Mirna et al. revealed that miR-21a-5p knockdown by antagomir-21a-5p resulted in a significant reduction in the expression of pro-inflammatory cytokines as well as attenuation of fibrosis and cardiac performance of EAM [144].

miRNAs and chemotherapy-induced HF

Doxorubicin (Dox) is one of the most powerful and widely used anticancer anthracycline agents. Cardinale et al. described a 9% incidence of cardiotoxicity among anthracycline-treated patients [145]. The mechanism of Dox-induced cardiotoxicity involves increased reactive oxidative stress, DNA damage, and cardiomyocyte apoptosis [146]. Cardiomyocyte-localized miRNAs and EC-localized miRNAs have been reported to participate in Dox-induced HF (Figure 7).

In a doxorubicin-treated cardiotoxicity mouse model, significant decline in cardiac expression of miR-132 and miR-212 was observed. Overexpression of AAV9-mediated miR-212/132 cluster limited cardiac atrophy by increasing left ventricular mass and wall thickness, decreasing

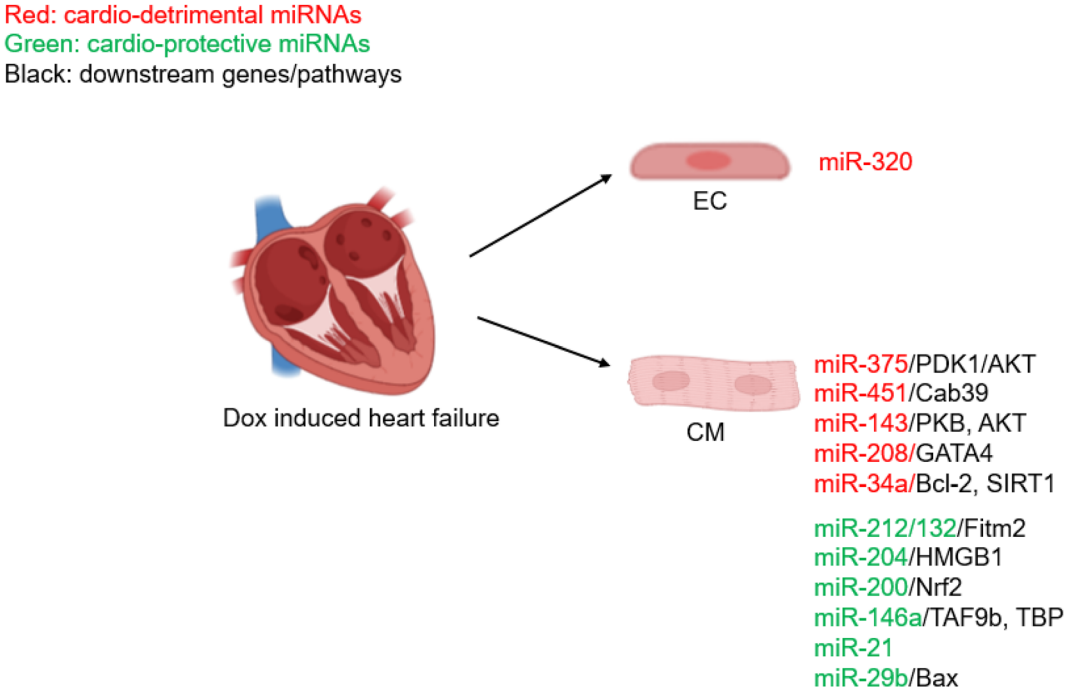

Figure 7: miRNAs with key roles in process of Dox induced heart failure. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. Dox, doxorubicin; CM, cardiomyocyte; EC, endothelial cell; PDK1, phosphoinositide-dependent kinase 1; AKT, AKT serine/threonine kinase; Cab39, calcium binding protein 39; PKB, protein kinase B; Bcl-2, B-cell lymphoma 2; SIRT1, sirtuin 1; Fitm2, fat storage-inducing transmembrane protein 2; HMGB1, high mobility group box 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; TAF9b, TBP associated factor 9b; TBP, TATA-binding protein; Bax, BCL2 associated X.

doxorubicin-mediated apoptosis, and preventing myofibril damage. Fat storage-inducing transmembrane protein 2 (FITM2) is a novel target and downstream effector molecule of miR-212/132 [147]. miR-204 expression was also decreased in doxorubicin-treated mice. miR-204 overexpression by rAAV prevented Dox-induced injury in cardiomyocytes by directly decreasing high mobility group box 1 (HMGB1) expression [148]. Hu et al. reported that rAAV-miR-200a reduced ROS and cardiac apoptosis by upregulating nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expression, and without affecting matrix metalloproteinase and inflammatory factors in mice with acute Dox injection [149]. miR-146a was reported to target TATA-binding protein (TBP)-associated factor 9b (TAF9b), a coactivator and stabilizer of p53, to attenuate apoptosis and regulate autophagy levels. Therefore, miR-146a knockout mice showed deteriorated cardiac dysfunction under Dox stress [150]. Dox treatment upregulated miR-31-5p expression in myocytes, and miR-31-5p antagomir increased quaking (QKI) expression, attenuated myocardial apoptosis, and improved cardiac function [151]. Inhibition of miR-375 prevented ROS damage in Dox-treated mice through the PDK1/AKT signaling pathway [152].

miR-451 was increased in the hearts of Dox-treated mice, while miR-451 inhibitor delivered via the retro-orbital plexus protected against ROS and myocyte apoptosis via activation of the calcium binding protein 39 (CAB39) and AMPK signaling pathway [153]. miR-143 knockdown by antagomir reduced doxorubicin-induced mortality through activation of the protein kinase B (PKB) and AKT signaling pathway [154]. Ultrasound-targeted microbubble destruction (UTMD)-assisted exosomal miR-21 delivery into the heart decreased apoptosis and restored cardiac function in a mouse model Dox-induced cardiotoxicity [155]. Jing et al. demonstrated that miR-29b was significantly downregulated in the myocardium of Dox-treated rats. Local delivery of miR-29b agomir to the myocardium prior to Dox treatment prevented myocyte apoptosis and improved cardiac function by decreasing BCL2-associated X (Bax) expression and caspase activation [156]. Tony et al. found that Dox upregulated miR-208a expression to promote cardiomyocyte apoptosis. Silencing miR-208a by an antagomir attenuated myocyte apoptosis and improved cardiac function through the GATA4 signaling pathway [157]. Piegari et al. reported improvement of cardiac function and alleviation of myocyte apoptosis in rats with Dox-induced HF by systemic antimiR-34a treatment. B-cell lymphoma 2 (Bcl-2) and sirtuin 1 (SIRT1) are the direct targets of miR-34a [158].

Our group has reported enhanced cardiac miR-320a expression and reduced cardiac microvessel density in ECs of mice with Dox-induced HF. While rAAV-mediated overexpression of miR-320a aggravated vessel abnormalities and cardiac dysfunction, miR-320a knockdown resulted in enhanced proliferation of ECs and attenuated cardiac abnormalities [159]. Mechanistically, the negative effects of miR-320a in vascular homeostasis and cardiac function were alleviated by VEGF-A, the direct target of miR-320a, re-expression in Dox treated mice [159].

Therefore, targeting these miRNAs might protect against Dox-induced HF in the future.

miRNAs in genetic dilated cardiomyopathy and hypertrophic cardiomyopathy

Dilated cardiomyopathy is a heart disorder characterized by enlarged diameter of the heart and weak pumping function. In fact, there are many causes such as chronic viral infection, chronic pressure overload, and genetic variants that can lead to a dilated heart [160]. We have previously elaborated regarding VMC and pressure overload-induced dilated cardiomyopathy. Therefore, in this section, we focus on reviewing the role of miRNAs in animal models of genetic disorder-induced dilated cardiomyopathy.

The inducible dilated cardiomyopathy mouse model carries a human truncation mutation in the sarcomeric protein, titin. miR-208b antagonization by LNA-modified antimiR-208b prevented the transition from adaptive to maladaptive remodeling in the dilated cardiomyopathy mouse model, whereas miR-208b overexpression resulted in cardiac hypertrophy [161]. Liu et al. showed that miR-133a-1 and miR-133a-2 double-mutant mice that survived to adulthood succumbed to dilated cardiomyopathy and HF, at least in part, through the regulation of SRF and cyclin D2 [162]. Wei et al. generated mice lacking miR-1-1 and miR-1-2 and found that all miR-1 dKO mice developed dilated cardiomyopathy and died before postnatal day 17. Estrogen-related receptor β (ERRβ) was identified as the direct target of miR-1 [163]. These data indicate that basal levels of miR-133a and miR-1 are necessary for normal structural and functional properties of the heart. Quattrocelli et al. demonstrated that long-term miR-669a therapy using an AAV vector alleviated chronic dilated cardiomyopathy in mice with Sgcb-null muscular dystrophy (MD). miR-669a treatment decreased hypertrophic

remodeling, fibrosis, and cardiomyocyte apoptosis [164]. Another animal model for dilated cardiomyopathy has been generated via the transgenic expression of mammalian sterile 20-like kinase 1 (Mst1). Inhibition of miR-34a by LNA-antimiR-34a attenuated heart enlargement and lung congestion, decreased the expression of genes associated with cardiac stress, and improved cardiac function [165].

HCM is a genetic disorder that is characterized by left ventricular hypertrophy unexplained by secondary causes and a non-dilated left ventricle with sustained or increased ejection fraction [166]. MYH7 mutations are found in approximately 20% of patients with HCM [167]. Transgenic mouse models of HCM have been designed based on MYH7 mutations. Although many dysregulated miRNAs have been reported in the human heart and mouse models [168], their functional relevance *in vivo* is largely unknown, which requires further study.

Dynamic interplay between pathways mediated by miRNAs

Signaling networks triggering apoptosis, hypertrophic growth, fibrosis, and oxidative stress are common mechanisms underlying different types of CVDs.

miRNAs involved in the apoptotic pathway can determine the progression of various diseases, including MI, DCM, myocarditis, and doxorubicin-induced HF (Figure 8). In MI, miR-320 and miR-24 overexpression increased apoptosis and promoted cardiac dysfunction, while mir-21, miR-140, miR-185, miR-133, miR-214, miR-1, miR-499, and miR-125b-5p protected against apoptosis and alleviated cardiac dysfunction [30, 31, 33, 36, 39–41, 44]. In DCM, miR-320 and miR-223 promoted apoptosis and cardiac dysfunction [78, 83]. In VMCs, miR-21, miR-1, miR-133,

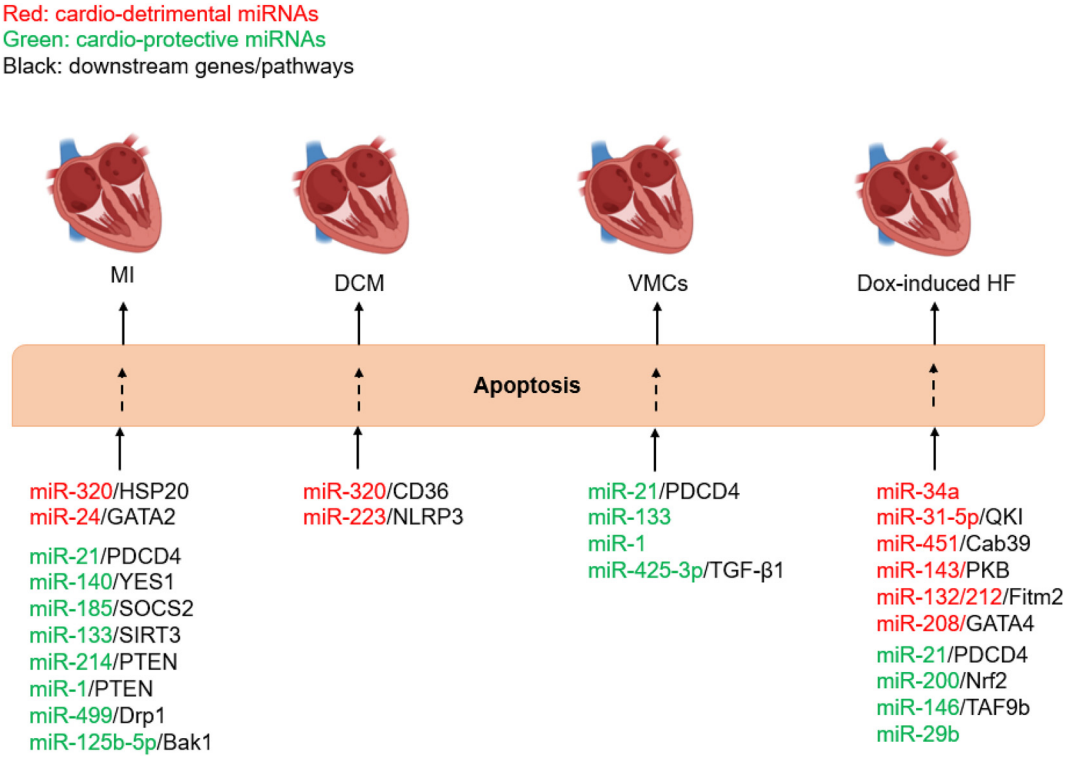

Figure 8: miRNAs involved in apoptosis pathway determining the progression of various diseases including MI, DCM, myocarditis and Dox induced heart failure. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. MI, myocardial infarction; DCM, diabetic cardiomyopathy; VMCs, viral myocarditis; Dox-induced HF, doxorubicin-induced heart failure; HSP20, heat-shock protein 20; PDCD4, programmed cell death 4; YES1, YES proto-oncogene 1; SOCS2, suppressor of cytokine signaling 2; SIRT3, Sirtuin 3; PTEN, phosphatase and tensin homolog; Drp1, dynamin-related protein-1; Bak1, BCL2 antagonist/killer 1; NLRP3, OD-like receptor pyrin domain containing 3; TGFβ1, transforming growth factor beta 1; QKI, Quaking; Cab39, calcium binding protein 39; PKB, protein kinase B; Fitm2, fat storage-inducing transmembrane protein 2; Nrf2, nuclear factor (erythroid-derived 2)-like 2; TAF9b, TBP associated factor 9b.

and miR-425-3p increased cardiac performance by reducing apoptosis [137, 139, 140]. In Dox-induced HF, miR-31-5p, miR-451, miR-143, miR-132/212, miR-208, and miR-34a increased while miR-29b, miR-146, miR-200, and miR-21 protected against apoptosis and HF [147, 149–151, 153, 154, 156–158]. Though described in different heart diseases, these apoptosis-related miRNAs might play similar roles in various diseases. Indeed, miR-320-mediated apoptosis attenuated cardiac function in MI and DCM. miR-21 reduced apoptosis and improved cardiac function in MI, VMC, and Dox-induced HF. miR-1 and miR-133 are cardio-protective in MI and VMC. Therefore, it is very likely that apoptosis-related miRNAs participate in all CVDs and show consistent effects. These miRNAs are intriguing targets for the treatment of CVDs. However, it should be noted that increased apoptosis in cardiomyocytes and non-cardiomyocytes (such as fibroblasts, ECs, and inflammatory cells) might exhibit different outcomes in CVDs. Targeting apoptosis-related miRNAs requires cardiac-specific overexpression or repression strategies. Notably, the cardiac-specific rAAV9 delivery system using the α-MHC promoter might be a good approach. However, lack of methods for high affinity, high specificity, and safe delivery of miRNAs to cardiomyocytes is still a major concern.

While apoptosis has been widely studied in MI, VMC, and Dox-induced HF, hypertrophic growth has been frequently investigated in pressure overload-induced HF, hypertensive cardiomyopathy, and DCM (Figure 9). Most hypertrophy-related miRNAs are also involved in the apoptotic process. For example, miR-212/132 and miR-208 increased cardiomyocyte hypertrophy in TAC pressure overload-induced HF and promoted apoptosis in Dox-induced HF [117, 120, 147, 157]. miR-1 protected against hypertrophy in the TAC model [122], however, it decreased apoptosis in MI and VMC [40, 139]. While miR-451 increased cardiomyocyte hypertrophy in DCM [81], it induced apoptosis in Dox-induced HF [153]. miR-133

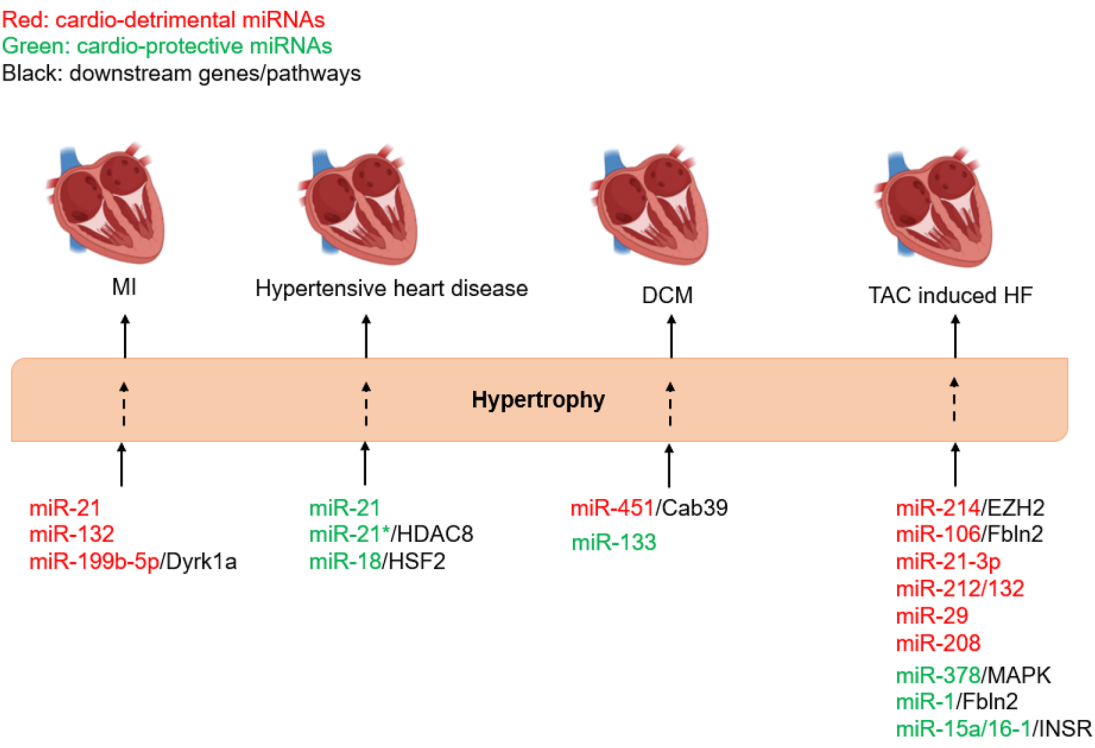

Figure 9: miRNAs involved in hypertrophic growth progression during multiple heart diseases including MI, hypertensive heart disease, DCM and TAC induced HF. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. MI, myocardial infarction; DCM, diabetic cardiomyopathy; TAC, transverse aortic constriction; Dyrk1a, specificity tyrosine-phosphorylation-regulated kinase 1A; HDAC8, histone deacetylase-8; HSF2, heat shock factor 2; Cab39, calcium binding protein 39; EZH2, zeste homolog 2; Fbln2, fibulin 2; MAPK, mitogen-activated protein kinase; INSR, insulin receptor.

prevented hypertrophy in DCM and decreased apoptosis in MI and VMC [36, 82, 139]. Although several hypertrophy-related miRNAs such as miR-378, miR-15a/16-1, and miR-106 are very likely to be involved in apoptosis, they need to be investigated in further animal studies. However, miR-214 promoted hypertrophy in TAC model by targeting E2H2 and decreased apoptosis in MI by targeting PTEN [39, 125], indicating that hypertrophy and apoptosis might be regulated by parallel or converging signaling pathways.

miRNAs associated with fibrosis are divided into two main categories: 1. fibroblast-localized miRNAs directly regulating fibrosis; 2. non-fibroblast (mainly cardiomyocytes)-localized miRNAs indirectly regulating fibrosis (Figure 10). In fact, most miRNAs are widely expressed in various types of cardiac cells. Fibroblast-specific miRNAs are rare. Wilson et al. reported that miR-21, miR-132, miR-29, and miR-222 were relatively more enriched in cardiac fibroblasts than in cardiomyocytes among the several detected miRNAs [169]. Systemic knockdown of these miRNAs seemed to directly affect fibroblast function and fibrosis. Functionally, miR-21 and miR-132 knockdown decreased fibrosis which further improved cardiac function in the MI model [26, 27]. Overexpression of miR-29 and miR-222 suppressed fibrosis and protected against cardiac dysfunction [24, 80]. Another cluster of miRNAs involved in fibrosis is cardiomyocyte-specific (such as miR-1, miR-133, miR-208) or relatively enriched miRNAs (such as miR-18 and miR-26). Cardiomyocyte-specific overexpression of these miRNAs either by rAAV9 delivery system or by generation of transgenic mice using α-MHC promoter predominantly affect myocyte apoptosis or hypertrophy, which indirectly lead to rearranged fibrosis. Specifically, cardiac-specific overexpression of miR-1, miR-133 and knockdown of miR-208 protected against fibrosis and cardiac dysfunction in MI or pressure overload-induced HF. Overexpression of miRNAs such as miR-18 and miR-26 that are relatively enriched in cardiac cells also decreased fibrosis [68, 70, 77, 120, 122]. Interestingly, in fibroblasts, miRNAs such as miR-21, miR-21*, let-7i, and miR-150 are relatively enriched [169]. Cardiomyocyte-specific down-regulation of these miRNAs (for example, miR-21 and miR-21*) promoted fibrosis [114], while fibroblast-specific or global knockdown of these miRNAs protected against fibrosis [112, 113]. These studies reinforce the need for cell-type-specific miRNA delivery systems to avoid side effects in unintended target cells.

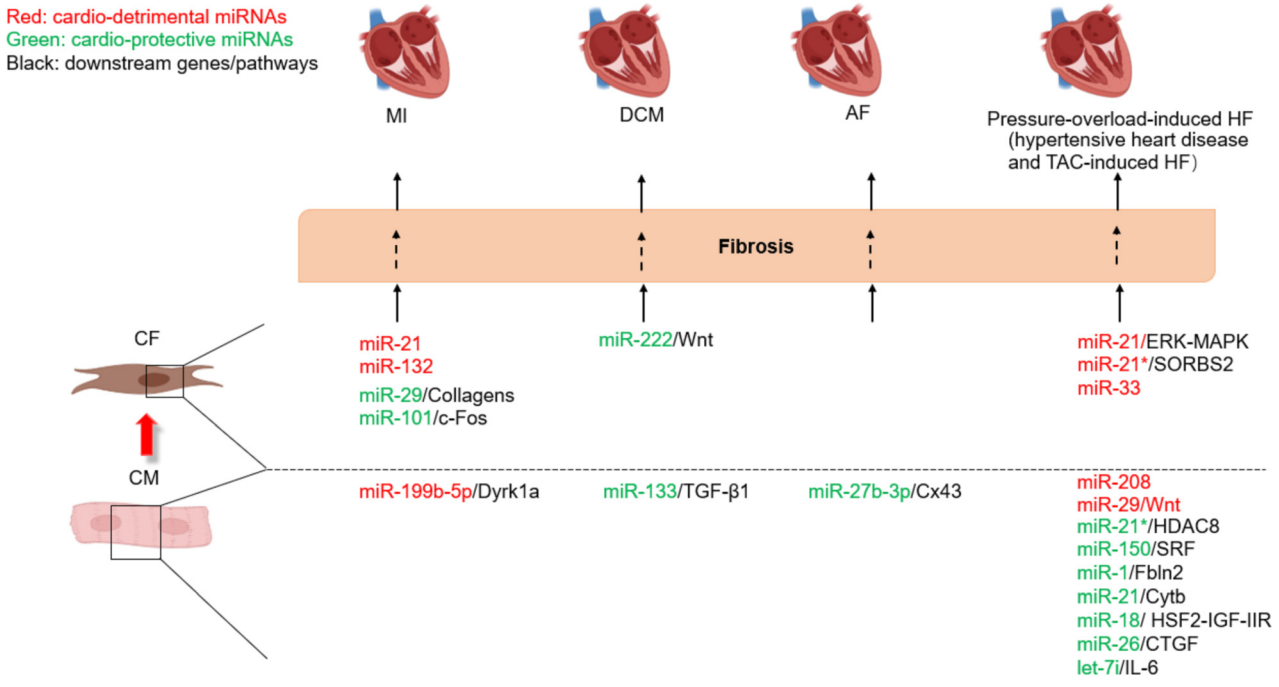

Figure 10: miRNAs involved in cardiac fibrosis pathway. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively, with their known targets. miRNAs are divided into two main categories including fibroblast localized miRNAs directly regulating fibrosis and non-fibroblast (mainly cardiomyocytes) localized miRNAs indirectly regulating fibrosis. MI, myocardial infarction; DCM, diabetic cardiomyopathy; AF, atrial fibrillation; HF, heart failure; Dyrk1, specificity tyrosine-phosphorylation-regulated kinase 1; TGF-β1, transforming growth factor beta 1; Cx43, Connexin 43; ERK-MAPK, extracellular signal regulated kinase-mitogen-activated protein kinase; SORBS2, sorbin and SH3 domain-containing protein 2; HDAC8, histone deacetylase-8; SRF, serum response factor; Fbln2, fibulin 2; Cytb, cytochrome *b*; HSF2-IGF-IIR, heat shock factor 2-insulin-like growth factor receptor II; CTGF, connective tissue growth factor; IL-6, interleukin 6.

The networks between apoptosis, hypertrophic growth, and fibrosis are complicated. miRNAs have provided new insights into these interactions (Figure 11). Overexpression of cardiomyocyte-specific miRNAs (such as miR-208) leads to increased myocyte hypertrophy and apoptosis, thereby indirectly promoting fibroblast activation and fibrosis [120, 161]. Conversely, knockdown of fibroblast-localized miRNAs (such as miR-21) decreased myocyte hypertrophy [112]. There seems to be a malignant link between cardiomyocyte dysfunction and fibroblast-related disorders. miRNAs are one of the initial factors that trigger these adverse events and therefore, targeting miRNAs might be a potent therapeutic strategy against CVDs. To summarize, cardiac-specific overexpression of miR-1, miR-133, miR-21, and miR-21* and cardiac-specific inhibition of miR-208, miR-212/132, and miR-29 might protect against myocyte apoptosis, hypertrophy, and fibrosis in various CVDs including MI, DCM, pressure overload-induced HF, Dox-induced HF, and VMC. In contrast, fibroblast-specific knockdown of miR-21, miR-21*, miR-132, and miR-33 and fibroblast-specific overexpression of miR-29, miR-101, and miR-222 might prevent the positive cycle between apoptosis, hypertrophy, and fibrosis.

Cell–cell crosstalk mediated by miRNAs

Many animal studies have revealed that cardiomyocyte-specific miRNA overexpression or downregulation can influence the functional properties of non-cardiomyocytes and vice versa. Exosomes are small vesicles (30–150 nm in diameter) enclosed by a lipid bilayer and are secreted by most cells in the body. miRNAs are one of the most abundant cargo molecules in the exosomes, in addition to other biological molecules [170]. Cell–cell crosstalk mediated by exosome-enriched miRNAs has been reported to be involved in various CVDs (Figure 12).

miRNAs secreted from non-myocytes translocate into recipient cardiomyocytes to regulate myocyte function. Bang et al. reported that treatment with Ang II increased the levels of miR-21* in fibroblast-derived exosomes. Moreover, cardiac fibroblast-secreted exosomes enriched with miR-21-3p induced cardiac hypertrophy by targeting recipient cardiomyocytes. *In vivo* systemic antagonism of miR-21* attenuated Ang II-induced cardiac hypertrophy [113]. Wang et al. demonstrated that macrophage-derived exosomes enriched with miR-155 promoted cardiomyocyte pyroptosis, cardiac hypertrophy, and fibrosis by directly targeting FoxO3a in uremic mice [171]. Fang et al. found that miR-200a delivered by exosomes from adipocytes downregulated TSC1 in recipient cardiomyocytes and enhanced mTOR activation, leading to cardiomyocyte hypertrophy [172]. Interestingly, Qiao et al. demonstrated downregulation of miR-21 in exosomes from explant-derived cardiac stromal cells (phenotypically resembling mesenchymal cells or fibroblasts) isolated from patients with HF (FEXO). Notably, FEXO therapy exacerbated cardiac function and left ventricular remodeling in the MI model. However, restoring miR-21-5p expression rescued the reparative function of FEXO

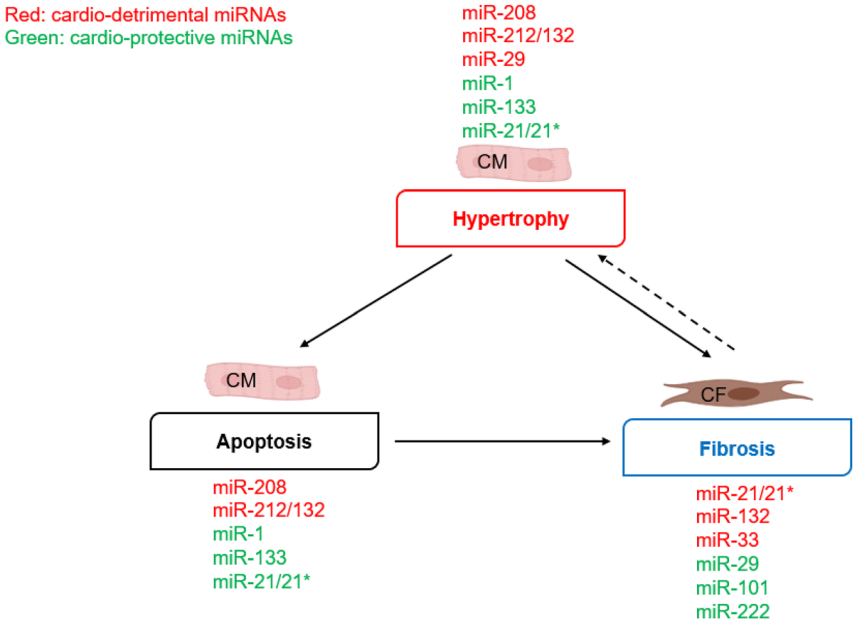

Figure 11: The networks between apoptosis, hypertrophic growth and fibrosis in heart diseases. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively. CF, cardiac fibroblast; CM,cardiomyocyte.

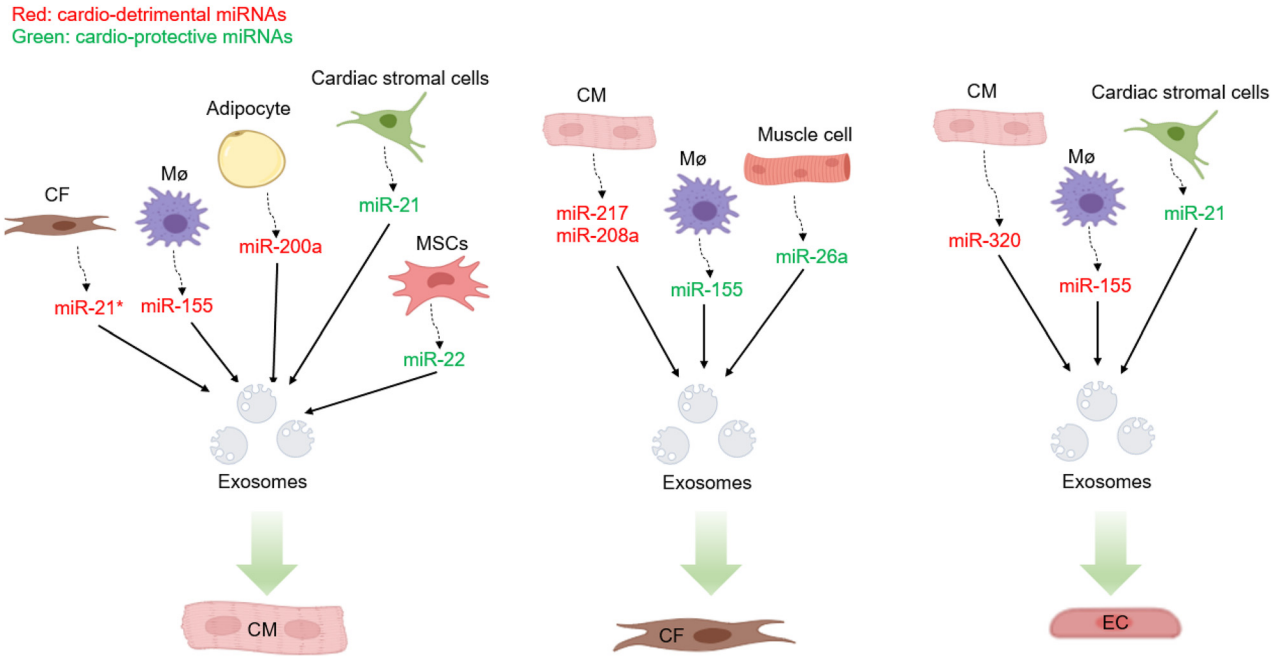

Figure 12: Cell–cell crosstalk mediated by miRNAs via exosomes. Exosomes localized miRNAs secreted from donor cells translocate into recipient cells (cardiomyocytes, cardiac fibroblasts and endothelial cells), regulating the progression of heart diseases. Cardio-detrimental miRNAs and cardio-protective miRNAs are presented as red and green, respectively. CF, cardiac fibroblast; CM, cardiomyocyte; Mø, macrophage; MSCs, mesenchymal stem cells; EC, endothelial cell.

by enhancing cardiomyocyte survival through the PTEN/Akt pathway [173]. Exosomes enriched with miR-22 secreted by MSCs reduced cardiomyocyte apoptosis due to ischemia. The anti-apoptotic effect of miR-22 was mediated by direct targeting of methyl CpG binding protein 2 (MeCP2) [174].

Fibroblasts are also regulated by exosome-containing miRNAs secreted from non-CF cell types. Our group reported that miR-217 aggravated pressure overload-induced cardiac hypertrophy and fibrosis by targeting PTEN, *in vivo*. Importantly, cardiomyocyte-derived miR-217-containing exosomes enhanced the proliferation of fibroblasts [175]. miR-208a-containing exosomes derived from cardiomyocytes contributed to increased fibroblast proliferation and differentiation into myofibroblasts. *In vivo* transfusion of miR-208a-containing exosomes into rats resulted in deteriorated cardiac function through the Dyrk2 pathway [176]. Macrophage-derived exosomes enriched with miR-155 can translocate into cardiac fibroblasts and inhibit cardiac fibroblast proliferation by decreasing the expression of sevenless 1 (Sos1). In addition, these exosomes activated inflammation by downregulating the expression of suppressor of cytokine signaling 1. Functionally, transfusion of WT macrophage-derived exosomes to mir-155(−/−) mice exacerbate cardiac rupture [177]. Overexpression of miR-26a in muscles prevent chronic kidney disease (CKD)-induced cardiac fibrosis via exosome-mediated miR-26a transfer by targeting the FoxO1 and the insulin pathway [178].

ECs can also receive miRNAs from non-ECs. Wang et al. reported that cardiomyocytes from type 2 diabetic rats secreted miR-320-containing exosomes into ECs to exert an anti-angiogenic effect. Mechanistically, exosomal miR-320 targets IGF-1, Hsp20, and Ets2 in recipient mouse cardiac ECs [179]. miR-21-containing exosomes from explant-derived cardiac stromal cells also translocate into ECs to promote angiogenesis and cardiac performance through the TEN/Akt pathway in MI [173]. Macrophage-derived exosomes containing miR-155 are transferred to ECs, leading to impaired angiogenesis and deteriorated cardiac dysfunction in MI by targeting Rac family small GTPase 1 (RAC1), p21 (RAC1)-activated kinase 2 (PAK2), sirtuin 1 (SIRT1), and protein kinase AMP-activated catalytic subunit alpha 2 (AMPKα2) [180].

These *in vitro* and *in vivo* studies strongly indicate the potent role of exosome-containing miRNAs in CVDs.

Exosomal miRNAs are novel tools for the diagnosis and treatment of CVDs.

Crosstalk between subcellular-localized miRNAs

miRNAs typically suppress gene expression at the post-transcriptional level in the cytoplasm. However, recent studies have shown that subcellular miRNAs, such as nucleus-localized miRNAs and mitochondria-localized miRNAs were dysregulated in CVDs such as DCM, hypertensive heart disease, and pressure overload-induced HF (Figure 13).

In fact, most miRNAs are present in both the nucleus and cytoplasm, with some showing selective nuclear enrichment [181]. Our recent study showed that approximately one-third of all miRNAs were more enriched in cultured H9c2 cardiomyocytes [83]. Functionally, we showed that miR-320 acts as a small activating RNA in the nucleus at the transcriptional level. CD36 is a key target gene of nuclear miR-320, but not cytoplasmic miR-320. Moreover, we found that induced expression of CD36 was responsible for increased cardiac fatty acid uptake and lipid accumulation, thereby causing cardiac lipotoxicity and increased myocyte apoptosis. This *in vivo* study is the first example demonstrating a natural small activating RNA to promote transcription, thereby uncovers a novel mechanism for diabetes-induced cardiac dysfunction. In the cytosol, miR-320 post-transcriptionally downregulated the expression of SRF and promoted atherogenesis in ApoE (−/−) mice [9]. We also reported upregulation of another nucleus-localized miRNA, miR-665, in a pressure overload-induced HF model. Overexpression of miR-665 aggravated TAC-induced cardiac dysfunction by targeting PTEN, while downregulation of miR-665 protected against cardiac dysfunction [182]. In ECs, cytoplasmic localized miR-665 suppressed CD34 expression and rAAV-mediated delivery of miR-665 reduced coronary microvessel angiogenesis and cardiac microvessel density, which further impaired cardiac function in the TAC model [183]. Interestingly, a study by Santovito et al. revealed that miR-126-5p, bound to argonaute-2 (Ago2), formed a complex with Mex-3 RNA binding family member A (Mex3a), which is present on the surface of autophagic vesicles and guides its transport into the nucleus. In the nucleus, miR-126-5p dissociated from Ago2 and targeted caspase-3 in an aptamer-like fashion with its seed sequence, preventing caspase dimerization and inhibiting its activity to limit apoptosis [184]. However, the mechanisms underlying the translocation of miRNAs into the nucleus need to be elucidated in detail.

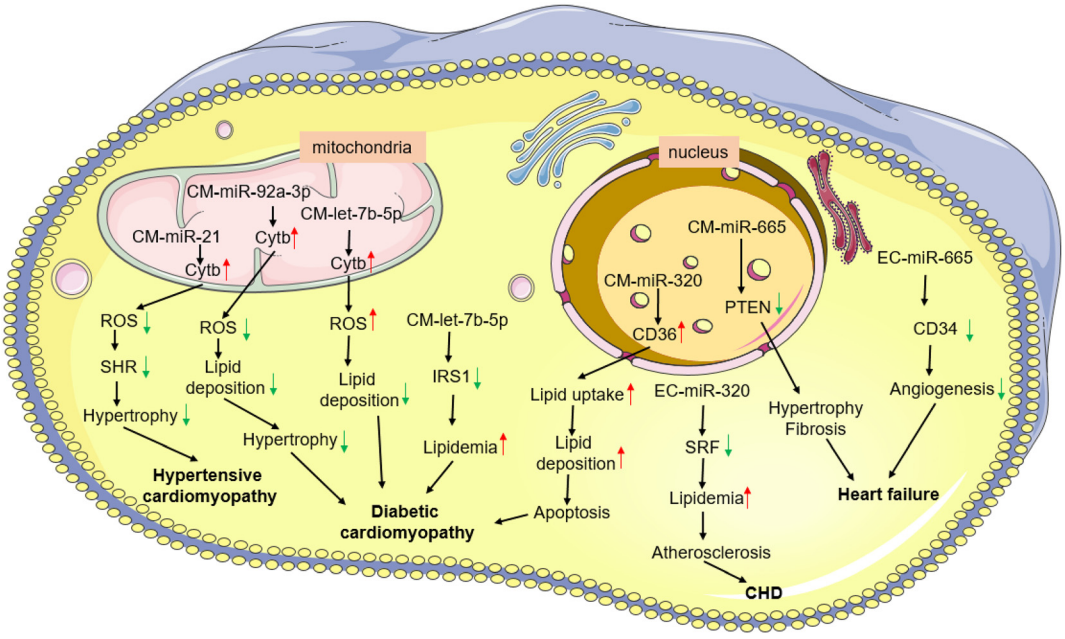

Figure 13: Subcellular localized miRNAs in heart diseases including hypertensive heart disease, DCM, CHD, and HF. DCM, diabetic cardiomyopathy; CHD, coronary heart disease; CF, heart failure; CM, cardiomyocyte; EC, endothelial cell; Cytb, cytochrome *b*; SHR, spontaneous hypertensive rat; IRS1, insulin receptor substrate 1; SRF, serum response factor; PTEN, phosphatase and tensin homolog.

miRNAs are also present in the mitochondria. As early as 2009, nuclear-coded miRNAs were reported in rat liver mitochondria and HeLa cells [185–187]. Das et al. reported that rat mitochondrial miR-181c targeted the mitochondrial gene, COX1 in cardiomyocytes [188]. Subsequently, their group demonstrated divergent effects of different miR-181 family members: cytosol miR-181a/b targeted PTEN and caused an increase in infarct size in miR-181a/b−/− mice due to increased PTEN signaling, whereas miR-181c targeted mt-COX1 in the mitochondria, resulting in decreased infarct size in miR-181c/d−/− mice [189]. These mice were also protected against HFD-induced DCM through the mitochondrial calcium uptake 1 protein (MICU1) pathway [190]. Our group has demonstrated the upregulation of miR-21 in the mitochondria of cardiomyocytes from SHRs. Delivery of miR-21 via rAAV9 was sufficient to reduce blood pressure and attenuate cardiac hypertrophy in SHRs. Mechanistically, miR-21 directly targeted mitochondrial-encoded cytochrome-b (mt-Cytb) to positively modulate mt-Cytb translation in the mitochondria, which further decreased mitochondrial ROS [61]. In db/db diabetic hearts, we found that 14 miRNAs were downregulated in the mitochondria [84]. Among these miRNAs, miR-92a-2-5p and let-7b-5p targeted mt-Cytb and positively modulated its expression. Interestingly, rAAV9-mediated delivery of miR-92a-2-5p, but not let-7b-5p, was sufficient to rescue diabetic cardiac diastolic dysfunction. Mechanistically, while let-7b-5p upregulated mt-Cytb, it also downregulated insulin receptor substrate 1 in the cytosol, resulting in the failure to improve diastolic dysfunction in db/db mice [61]. These investigations revealed a complicated regulatory pattern of miRNAs in subcellular organelles during CVDs.

Conclusion and perspectives

These *in vivo* animal-based studies strongly indicate that miRNAs are an attractive and promising target for the treatment of CVDs. Several chemically modified oligonucleotides have been used in clinical trials. For example, miR-122 antagomir is used to treat hepatitis C virus and delivery of MRX34, which mimics miR-34, is used for the treatment of primary liver cancer [3]. Recently, a first clinical trial of an antisense drug in HF targeting miR-132 induced significant QRS narrowing and encouraging positive trends for relevant cardiac fibrosis biomarkers [191]. These encouraging results of miRNA applications in these experiments suggest that these molecules have potent therapeutic potential and negligible toxicity.

In addition to miR-132, we have summarized a cluster of miRNAs suitable for the treatment of CVDs. As detailed previously, cardiac-specific overexpression of miR-1, miR-133, miR-21, and miR-21* while cardiac-specific inhibition of miR-208, miR-212/132, and miR-29 might protect against myocyte apoptosis, hypertrophy, and fibrosis in various CVDs. In contrast, fibroblast-specific knockdown of miR-21, miR-21*, miR-132, and miR-33 and fibroblast-specific overexpression of miR-29, miR-101, and miR-222 might prevent myocyte apoptosis, hypertrophy, and fibrosis in heart diseases. The challenge lies in developing a cell type-specific delivery system to overexpress or downregulate the miRNAs. For targeting myocytes, rAAV-based delivery using cardiac troponin T, or the α-MHC promoter might be a promising approach. Many studies including ours, have indicated that rAAV9 is nontoxic. In fact, several AAV-based gene therapies are currently FDA-approved. For example, Luxturna was approved in 2017 for rare inherited retinal dystrophy, and Zolgensma was approved in 2019 for spinal muscular atrophy. Therefore, rAAV9-based miRNA or tough decoy (TuD) delivery provides a considerable theoretical basis for developing therapeutics against CVDs. However, the production and formulation of AAV products requires specific conditions to ensure stability and yield. Furthermore, storing AAV products can prove to be quite challenging. Moreover, fibroblast-specific miRNA delivery by the rAAV system is challenging. To date, the cardiac fibroblast-specific promoter has not yet been identified. The suggested fibroblast markers including fibroblast-specific protein-1 (FSP-1), periostin, and collagen type I alpha 1 chain (Col1a1) are not unique to fibroblasts. For example, periostin and Col1a1 are also expressed in cardiac myocytes [192, 193]. As there is no consensus definition of the fibroblast and its markers, further studies are needed to identify cell type-specific promoters for cardiac fibroblasts and other cardiac cell types.

miRNAs are well known to mediate translational repression in the cytoplasm. miRNAs have also been detected in membrane-compartmentalized organelles such as mitochondria and nucleus. These subcellular miRNAs determined the outcomes of various CVDs [61, 83]. Very recently, Yang and colleagues found that endogenous miR-1 could physically bind with cardiac membrane proteins, including a potassium channel Kir2.1 in cardiomyocytes. Acute presence of miR-1 depolarized resting membrane potential and prolonged final repolarization of the action potential in cardiomyocytes [194]. Though the functional significance in cardiac diseases has remained elusive, this study revealed novel biophysical action of endogenous miRs in modulating cardiac electrophysiology and provided new insights into the pathogenesis of cardiac arrhythmias. Moreover, it is possible that miRNAs also

translocate into subcellular fractions such as endoplasmic reticulum, golgis apparatus and autophagosome to paly decisive roles in CVDs. However, one of the main drawbacks of studying subcellular miRNA is the lack of well-established mitochondrial-, membrane-, and nuclear localization signals identified for miRNAs. Strategies to directly and specifically overexpress or knockdown miRNA expression in the subcellular structures are needed urgently.

Mechanistically, subcellular-localized miRNAs seemed to function in a unique manner compared to the well-studied cytosol-localized miRNAs. For example, mitochondrial-localized miRNAs enhanced the translation of mitochondrial gene encoded transcripts, and nuclear miRNAs affected gene transcription. It is still unclear as to how miRNAs translocate into the mitochondria and the nucleus. Moreover, many studies have revealed the activation or repression effects of nuclear-localized miRNAs on gene transcription, but the mechanistic details remain unclear, and the full extent of nuclear miRNA function is currently unknown. A major drawback of current miRNA studies is the absence of strategies to study cytosolic, mitochondrial, and nuclear miRNAs independently and systemically. In the future, studies identifying interactions between cellular- and subcellular-localized miRNAs are required which employ more complicated and new biochemical assays, especially when performed in an unbiased manner. Thus, more efforts are required to deepen our understanding of the role of miRNAs in CVDs.

Research funding: This work was supported by grants from the National Natural Science Foundation of China (nos. 81822002, 91439203, 91839302, 81630010, 31771264, 81790624, 82170273, and 31800973). The funders had no role in the study design, data collection and analysis, manuscript preparation, or decision to publish.

Author contributions: (I) Conception and design: H Li, C Chen, and DW Wang; (II) Administrative support: DW Wang; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: H Li; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

References

1. Gomez LA. Cardiovascular diseases: a public health problem and a global challenge. Biomedica 2011;31:469–73.
2. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. J Am Coll Cardiol 2017;70:1–25.
3. Wojciechowska A, Braniewska A, Kozar-Kaminska K. Microrna in cardiovascular biology and disease. Adv Clin Exp Med 2017;26:865–74.
4. Cui G, Li Z, Li R, Huang J, Wang H, Zhang L, et al. A functional variant in apoa5/a4/c3/a1 gene cluster contributes to elevated triglycerides and severity of cad by interfering with microrna 3201 binding efficiency. J Am Coll Cardiol 2014;64:267–77.
5. Grech ED. Pathophysiology and investigation of coronary artery disease. BMJ 2003;326:1027–30.
6. Dzau VJ, Gibbons GH. Endothelium and growth factors in vascular remodeling of hypertension. Hypertension 1991;18:III115–21.
7. Gibbons GH. Endothelial function as a determinant of vascular function and structure: a new therapeutic target. Am J Cardiol 1997;79:3–8.
8. Wang H, Zhu HQ, Wang F, Zhou Q, Gui SY, Wang Y. Microrna-1 prevents high-fat diet-induced endothelial permeability in apoe knock-out mice. Mol Cell Biochem 2013;378:153–9.
9. Chen C, Wang Y, Yang S, Li H, Zhao G, Wang F, et al. Mir-320a contributes to atherogenesis by augmenting multiple risk factors and down-regulating srf. J Cell Mol Med 2015;19:970–85.
10. Sun X, He S, Wara AKM, Icli B, Shvartz E, Tesmenitsky Y, et al. Systemic delivery of microrna-181b inhibits nuclear factor-kappab activation, vascular inflammation, and atherosclerosis in apolipoprotein e-deficient mice. Circ Res 2014;114:32–40.
11. Schober A, Nazari-Jahantigh M, Wei Y, Bidzhekov K, Gremse F, Grommes J, et al. Microrna-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing dlk1. Nat Med 2014;20:368–76.
12. Wiese CB, Zhong J, Xu ZQ, Zhang Y, Ramirez Solano MA, Zhu W, et al. Dual inhibition of endothelial mir-92a-3p and mir-489-3p reduces renal injury-associated atherosclerosis. Atherosclerosis 2019;282:121–31.
13. de Yebenes VG, Briones AM, Martos-Folgado I, Mur SM, Oller J, Bilal F, et al. Aging-associated mir-217 aggravates atherosclerosis and promotes cardiovascular dysfunction. Arterioscler Thromb Vasc Biol 2020;40:2408–24.
14. Chen Z, Wen L, Martin M, Hsu CY, Fang L, Lin FM, et al. Oxidative stress activates endothelial innate immunity via sterol regulatory element binding protein 2 (srebp2) transactivation of microrna-92a. Circulation 2015;131:805–14.
15. Ulrich V, Rotllan N, Araldi E, Luciano A, Skroblin P, Abonnenc M, et al. Chronic mir-29 antagonism promotes favorable plaque remodeling in atherosclerotic mice. EMBO Mol Med 2016;8:643–53.
16. Randolph GJ. Mechanisms that regulate macrophage burden in atherosclerosis. Circ Res 2014;114:1757–71.
17. Bobryshev YV, Ivanova EA, Chistiakov DA, Nikiforov NG, Orekhov AN. Macrophages and their role in atherosclerosis: pathophysiology and transcriptome analysis. BioMed Res Int 2016;2016:9582430.
18. Di Gregoli K, Jenkins N, Salter R, White S, Newby AC, Johnson JL. Microrna-24 regulates macrophage behavior and retards atherosclerosis. Arterioscler Thromb Vasc Biol 2014;34:1990–2000.
19. Tian FJ, An LN, Wang GK, Zhu JQ, Li Q, Zhang YY, et al. Elevated microrna-155 promotes foam cell formation by targeting hbp1 in atherogenesis. Cardiovasc Res 2014;103:100–10.

20. Chen H, Li X, Liu S, Gu L, Zhou X. Mircrorna-19a promotes vascular inflammation and foam cell formation by targeting hbp-1 in atherogenesis. Sci Rep 2017;7:12089.
21. Jansen F, Stumpf T, Proebsting S, Franklin BS, Wenzel D, Pfeifer P, et al. Intercellular transfer of mir-126-3p by endothelial microparticles reduces vascular smooth muscle cell proliferation and limits neointima formation by inhibiting lrp6. J Mol Cell Cardiol 2017;104:43–52.
22. Gao L, Zeng H, Zhang T, Mao C, Wang Y, Han Z, et al. Microrna-21 deficiency attenuated atherogenesis and decreased macrophage infiltration by targeting dusp-8. Atherosclerosis 2019;291:78–86.
23. Porter KE, Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol Ther 2009;123:255–78.
24. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of micrornas after myocardial infarction reveals a role of mir-29 in cardiac fibrosis. Proc Natl Acad Sci U S A 2008;105:13027–32.
25. Pan Z, Sun X, Shan H, Wang N, Wang J, Ren J, et al. Microrna-101 inhibited postinfarct cardiac fibrosis and improved left ventricular compliance via the fbj osteosarcoma oncogene/transforming growth factor-beta1 pathway. Circulation 2012;126:840–50.
26. Foinquinos A, Batkai S, Genschel C, Viereck J, Rump S, Gyongyosi M, et al. Preclinical development of a mir-132 inhibitor for heart failure treatment. Nat Commun 2020;11:633.
27. Hinkel R, Ramanujam D, Kaczmarek V, Howe A, Klett K, Beck C, et al. Antimir-21 prevents myocardial dysfunction in a pig model of ischemia/reperfusion injury. J Am Coll Cardiol 2020;75:1788–800.
28. Lodrini AM, Goumans MJ. Cardiomyocytes cellular phenotypes after myocardial infarction. Front Cardiovasc Med 2021;8:750510.
29. Chiong M, Wang ZV, Pedrozo Z, Cao DJ, Troncoso R, Ibacache M, et al. Cardiomyocyte death: mechanisms and translational implications. Cell Death Dis 2011;2:e244.
30. Ren XP, Wu J, Wang X, Sartor MA, Jones K, Qian J, et al. Microrna-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20. Circulation 2009;119:2357–66.
31. Su Q, Li L, Liu Y, Zhou Y, Wang J, Wen W. Ultrasound-targeted microbubble destruction-mediated microrna-21 transfection regulated pdcd4/nf-kappab/tnf-alpha pathway to prevent coronary microembolization-induced cardiac dysfunction. Gene Ther 2015;22:1000–6.
32. Song Y, Zhang C, Zhang J, Jiao Z, Dong N, Wang G, et al. Localized injection of mirna-21-enriched extracellular vesicles effectively restores cardiac function after myocardial infarction. Theranostics 2019;9:2346–60.
33. Yang S, Li H, Chen L. Microrna-140 attenuates myocardial ischemia-reperfusion injury through suppressing mitochondria-mediated apoptosis by targeting yes1. J Cell Biochem 2019;120:3813–21.
34. Li Y, Zhou J, Zhang O, Wu X, Guan X, Xue Y, et al. Bone marrow mesenchymal stem cells-derived exosomal microrna-185 represses ventricular remolding of mice with myocardial infarction by inhibiting socs2. Int Immunopharm 2020;80:106156.
35. Garg A, Foinquinos A, Jung M, Janssen-Peters H, Biss S, Bauersachs J, et al. Mirna-181a is a novel regulator of aldosterone-mineralocorticoid receptor-mediated cardiac remodelling. Eur J Heart Fail 2020;22:1366–77.
36. Sun B, Liu S, Hao R, Dong X, Fu L, Han B. Rgd-peg-pla delivers mir-133 to infarct lesions of acute myocardial infarction model rats for cardiac protection. Pharmaceutics 2020;12:575.
37. Izarra A, Moscoso I, Levent E, Canon S, Cerrada I, Diez-Juan A, et al. Mir-133a enhances the protective capacity of cardiac progenitors cells after myocardial infarction. Stem Cell Rep 2014;3:1029–42.
38. Huang F, Li ML, Fang ZF, Hu XQ, Liu QM, Liu ZJ, et al. Overexpression of microrna-1 improves the efficacy of mesenchymal stem cell transplantation after myocardial infarction. Cardiology 2013;125:18–30.
39. Yang X, Qin Y, Shao S, Yu Y, Zhang C, Dong H, et al. Microrna-214 inhibits left ventricular remodeling in an acute myocardial infarction rat model by suppressing cellular apoptosis via the phosphatase and tensin homolog (pten). Int Heart J 2016;57:247–50.
40. Glass C, Singla DK. Microrna-1 transfected embryonic stem cells enhance cardiac myocyte differentiation and inhibit apoptosis by modulating the pten/akt pathway in the infarcted heart. Am J Physiol Heart Circ Physiol 2011;301:H2038–49.
41. Wang JX, Jiao JQ, Li Q, Long B, Wang K, Liu JP, et al. Mir-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. Nat Med 2011;17:71–8.
42. Duygu B, Poels EM, Juni R, Bitsch N, Ottaviani L, Olieslagers S, et al. Mir-199b-5p is a regulator of left ventricular remodeling following myocardial infarction. Noncoding RNA Res 2017;2:18–26.
43. Tang Y, Wang Y, Park KM, Hu Q, Teoh JP, Broskova Z, et al. Microrna-150 protects the mouse heart from ischaemic injury by regulating cell death. Cardiovasc Res 2015;106:387–97.
44. Bayoumi AS, Park KM, Wang Y, Teoh JP, Aonuma T, Tang Y, et al. A carvedilol-responsive microrna, mir-125b-5p protects the heart from acute myocardial infarction by repressing pro-apoptotic bak1 and klf13 in cardiomyocytes. J Mol Cell Cardiol 2018;114:72–82.
45. Chen JJ, Zhou SH. Mesenchymal stem cells overexpressing mir-126 enhance ischemic angiogenesis via the akt/erk-related pathway. Cardiol J 2011;18:675–81.
46. Icli B, Wara AK, Moslehi J, Sun X, Plovie E, Cahill M, et al. Microrna-26a regulates pathological and physiological angiogenesis by targeting bmp/smad1 signaling. Circ Res 2013;113:1231–41.
47. Bellera N, Barba I, Rodriguez-Sinovas A, Ferret E, Asin MA, Gonzalez-Alujas MT, et al. Single intracoronary injection of encapsulated antagomir-92a promotes angiogenesis and prevents adverse infarct remodeling. J Am Heart Assoc 2014;3:e000946.
48. Wang D, Deuse T, Stubbendorff M, Chernogubova E, Erben RG, Eken SM, et al. Local microrna modulation using a novel anti-mir-21-eluting stent effectively prevents experimental in-stent restenosis. Arterioscler Thromb Vasc Biol 2015;35:1945–53.
49. Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, et al. Microrna-24 regulates vascularity after myocardial infarction. Circulation 2011;124:720–30.
50. Tackling G, Borhade MB. Hypertensive heart disease. Treasure Island, FL: Statpearls; 2020.
51. Drazner MH. The progression of hypertensive heart disease. Circulation 2011;123:327–34.

52. Pichler G, Redon J, Martinez F, Solaz E, Calaforra O, Andres MS, et al. Cardiac magnetic resonance-derived fibrosis, strain and molecular biomarkers of fibrosis in hypertensive heart disease. J Hypertens 2020;38:2036–42.
53. Dampney RA, Horiuchi J, Killinger S, Sheriff MJ, Tan PS, McDowall LM. Long-term regulation of arterial blood pressure by hypothalamic nuclei: some critical questions. Clin Exp Pharmacol Physiol 2005;32:419–25.
54. Friese RS, Altshuler AE, Zhang K, Miramontes-Gonzalez JP, Hightower CM, Jirout ML, et al. Microrna-22 and promoter motif polymorphisms at the chga locus in genetic hypertension: functional and therapeutic implications for gene expression and the pathogenesis of hypertension. Hum Mol Genet 2013;22: 3624–40.
55. Zhu Q, Hu J, Wang L, Wang W, Wang Z, Li PL, et al. Inhibition of microrna-429 in the renal medulla increased salt sensitivity of blood pressure in sprague dawley rats. J Hypertens 2017;35: 1872–80.
56. Liu Y, Usa K, Wang F, Liu P, Geurts AM, Li J, et al. Microrna-214-3p in the kidney contributes to the development of hypertension. J Am Soc Nephrol 2018;29:2518–28.
57. Baker MA, Wang F, Liu Y, Kriegel AJ, Geurts AM, Usa K, et al. Mir-192-5p in the kidney protects against the development of hypertension. Hypertension 2019;73:399–406.
58. Carr G, Barrese V, Stott JB, Povstyan OV, Jepps TA, Figueiredo HB, et al. Microrna-153 targeting of kcnq4 contributes to vascular dysfunction in hypertension. Cardiovasc Res 2016; 112:581–9.
59. Li X, Cai W, Xi W, Sun W, Shen W, Wei T, et al. Microrna-31 regulates immunosuppression in ang ii (angiotensin ii)-induced hypertension by targeting ppp6c (protein phosphatase 6c). Hypertension 2019;73:e14–24.
60. Jackson KL, Gueguen C, Lim K, Eikelis N, Stevenson ER, Charchar FJ, et al. Neural suppression of mirna-181a in the kidney elevates renin expression and exacerbates hypertension in schlager mice. Hypertens Res 2020;43:1152–64.
61. Li H, Zhang X, Wang F, Zhou L, Yin Z, Fan J, et al. Microrna-21 lowers blood pressure in spontaneous hypertensive rats by upregulating mitochondrial translation. Circulation 2016;134: 734–51.
62. Syed M, Ball JP, Mathis KW, Hall ME, Ryan MJ, Rothenberg ME, et al. Microrna-21 ablation exacerbates aldosterone-mediated cardiac injury, remodeling, and dysfunction. Am J Physiol Endocrinol Metab 2018;315:E1154–67.
63. Wang F, Fang Q, Chen C, Zhou L, Li H, Yin Z, et al. Recombinant adeno-associated virus-mediated delivery of microrna-21-3p lowers hypertension. Mol Ther Nucleic Acids 2018;11:354–66.
64. Hori D, Dunkerly-Eyring B, Nomura Y, Biswas D, Steppan J, Henao-Mejia J, et al. Mir-181b regulates vascular stiffness age dependently in part by regulating tgf-beta signaling. PLoS One 2017;12:e0174108.
65. Huo KG, Richer C, Berillo O, Mahjoub N, Fraulob-Aquino JC, Barhoumi T, et al. Mir-431-5p knockdown protects against angiotensin ii-induced hypertension and vascular injury. Hypertension 2019;73:1007–17.
66. Gabani M, Liu J, Ait-Aissa K, Koval O, Kim YR, Castaneda D, et al. Mir-204 regulates type 1 ip3r to control vascular smooth muscle cell contractility and blood pressure. Cell Calcium 2019;80: 18–24.
67. Sun L, Zhang J, Li Y. Chronic central mir-29b antagonism alleviates angiotensin ii-induced hypertension and vascular endothelial dysfunction. Life Sci 2019;235:116862.
68. Huang CY, Pai PY, Kuo CH, Ho TJ, Lin JY, Lin DY, et al. P53-mediated mir-18 repression activates hsf2 for igf-iir-dependent myocyte hypertrophy in hypertension-induced heart failure. Cell Death Dis 2017;8:e2990.
69. Chiasson V, Takano APC, Guleria RS, Gupta S. Deficiency of microrna mir-1954 promotes cardiac remodeling and fibrosis. J Am Heart Assoc 2019;8:e012880.
70. Zhang W, Wang Q, Feng Y, Chen X, Yang L, Xu M, et al. Microrna-26a protects the heart against hypertension-induced myocardial fibrosis. J Am Heart Assoc 2020;9:e017970.
71. Wang X, Wang HX, Li YL, Zhang CC, Zhou CY, Wang L, et al. Microrna let-7i negatively regulates cardiac inflammation and fibrosis. Hypertension 2015;66:776–85.
72. Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM, et al. Therapeutic inhibition of mir-208a improves cardiac function and survival during heart failure. Circulation 2011;124:1537–47.
73. Wang Y, Huang Z, Zhong H, Wang L, Xi D, Shi Y, et al. Mir-1929-3p overexpression alleviates murine cytomegalovirus-induced hypertensive myocardial remodeling by suppressing ednra/ nlrp3 inflammasome activation. BioMed Res Int 2020;2020: 6653819.
74. Aneja A, Tang WH, Bansilal S, Garcia MJ, Farkouh ME. Diabetic cardiomyopathy: insights into pathogenesis, diagnostic challenges, and therapeutic options. Am J Med 2008;121: 748–57.
75. Feng B, Chen S, Gordon AD, Chakrabarti S. Mir-146a mediates inflammatory changes and fibrosis in the heart in diabetes. J Mol Cell Cardiol 2017;105:70–6.
76. Zheng D, Ma J, Yu Y, Li M, Ni R, Wang G, et al. Silencing of mir-195 reduces diabetic cardiomyopathy in c57bl/6 mice. Diabetologia 2015;58:1949–58.
77. Chen S, Puthanveetil P, Feng B, Matkovich SJ, Dorn GW 2nd, Chakrabarti S. Cardiac mir-133a overexpression prevents early cardiac fibrosis in diabetes. J Cell Mol Med 2014;18:415–21.
78. Xu D, Zhang X, Chen X, Yang S, Chen H. Inhibition of mir-223 attenuates the nlrp3 inflammasome activation, fibrosis, and apoptosis in diabetic cardiomyopathy. Life Sci 2020;256: 117980.
79. Tao L, Huang X, Xu M, Yang L, Hua F. Mir-144 protects the heart from hyperglycemia-induced injury by regulating mitochondrial biogenesis and cardiomyocyte apoptosis. Faseb J 2020;34: 2173–97.
80. Wang Z, Wang Z, Gao L, Xiao L, Yao R, Du B, et al. Mir-222 inhibits cardiac fibrosis in diabetic mice heart via regulating wnt/beta-catenin-mediated endothelium to mesenchymal transition. J Cell Physiol 2020;235:2149–60.
81. Kuwabara Y, Horie T, Baba O, Watanabe S, Nishiga M, Usami S, et al. Microrna-451 exacerbates lipotoxicity in cardiac myocytes and high-fat diet-induced cardiac hypertrophy in mice through suppression of the lkb1/ampk pathway. Circ Res 2015;116: 279–88.
82. Kambis TN, Shahshahan HR, Kar S, Yadav SK, Mishra PK. Transgenic expression of mir-133a in the diabetic akita heart prevents cardiac remodeling and cardiomyopathy. Front Cardiovasc Med 2019;6:45.

83. Li H, Fan J, Zhao Y, Zhang X, Dai B, Zhan J, et al. Nuclear mir-320 mediates diabetes-induced cardiac dysfunction by activating transcription of fatty acid metabolic genes to cause lipotoxicity in the heart. Circ Res 2019;125:1106–20.
84. Li H, Dai B, Fan J, Chen C, Nie X, Yin Z, et al. The different roles of mirna-92a-2-5p and let-7b-5p in mitochondrial translation in db/db mice. Mol Ther Nucleic Acids 2019;17:424–35.
85. Chen C, Yang S, Li H, Yin Z, Fan J, Zhao Y, et al. Mir30c is involved in diabetic cardiomyopathy through regulation of cardiac autophagy via becn1. Mol Ther Nucleic Acids 2017;7:127–39.
86. Dai B, Li H, Fan J, Zhao Y, Yin Z, Nie X, et al. Mir-21 protected against diabetic cardiomyopathy induced diastolic dysfunction by targeting gelsolin. Cardiovasc Diabetol 2018;17:123.
87. Jia C, Chen H, Wei M, Chen X, Zhang Y, Cao L, et al. Gold nanoparticle-based mir155 antagonist macrophage delivery restores the cardiac function in ovariectomized diabetic mouse model. Int J Nanomed 2017;12:4963–79.
88. Kim GH. Microrna regulation of cardiac conduction and arrhythmias. Transl Res 2013;161:381–92.
89. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, et al. The muscle-specific microrna mir-1 regulates cardiac arrhythmogenic potential by targeting gja1 and kcnj2. Nat Med 2007;13:486–91.
90. Terentyev D, Belevych AE, Terentyeva R, Martin MM, Malana GE, Kuhn DE, et al. Mir-1 overexpression enhances ca(2+) release and promotes cardiac arrhythmogenesis by targeting pp2a regulatory subunit b56alpha and causing camkii-dependent hyperphosphorylation of ryr2. Circ Res 2009;104:514–21.
91. Zhang Y, Sun L, Zhang Y, Liang H, Li X, Cai R, et al. Overexpression of microrna-1 causes atrioventricular block in rodents. Int J Biol Sci 2013;9:455–62.
92. Shan H, Zhang Y, Cai B, Chen X, Fan Y, Yang L, et al. Upregulation of microrna-1 and microrna-133 contributes to arsenic-induced cardiac electrical remodeling. Int J Cardiol 2013;167:2798–805.
93. Zhang J, Wu L, Li Z, Fu G. Mir-1231 exacerbates arrhythmia by targeting calciumchannel gene cacna2d2 in myocardial infarction. Am J Transl Res 2017;9:1822–33.
94. Jin Y, Zhou T, Feng Q, Yang J, Cao J, Xu X, et al. Inhibition of microrna-206 ameliorates ischemia-reperfusion arrhythmia in a mouse model by targeting connexin43. J Cardiovasc Transl Res 2020;13:584–92.
95. Osbourne A, Calway T, Broman M, McSharry S, Earley J, Kim GH. Downregulation of connexin43 by microrna-130a in cardiomyocytes results in cardiac arrhythmias. J Mol Cell Cardiol 2014;74:53–63.
96. Danielson LS, Park DS, Rotllan N, Chamorro-Jorganes A, Guijarro MV, Fernandez-Hernando C, et al. Cardiovascular dysregulation of mir-17-92 causes a lethal hypertrophic cardiomyopathy and arrhythmogenesis. Faseb J 2013;27:1460–7.
97. Liu X, Zhang Y, Du W, Liang H, He H, Zhang L, et al. Mir-223-3p as a novel microrna regulator of expression of voltage-gated k+ channel kv4.2 in acute myocardial infarction. Cell Physiol Biochem 2016;39:102–14.
98. Mazurek SR, Calway T, Harmon C, Farrell P, Kim GH. Microrna-130a regulation of desmocollin 2 in a novel model of arrhythmogenic cardiomyopathy. MicroRNA 2017;6:143–50.
99. Lv X, Li J, Hu Y, Wang S, Yang C, Li C, et al. Overexpression of mir-27b-3p targeting wnt3a regulates the signaling pathway of wnt/beta-catenin and attenuates atrial fibrosis in rats with atrial fibrillation. Oxid Med Cell Longev 2019;2019:5703764.
100. Lu Y, Zhang Y, Wang N, Pan Z, Gao X, Zhang F, et al. Microrna-328 contributes to adverse electrical remodeling in atrial fibrillation. Circulation 2010;122:2378–87.
101. Luo X, Pan Z, Shan H, Xiao J, Sun X, Wang N, et al. Microrna-26 governs profibrillatory inward-rectifier potassium current changes in atrial fibrillation. J Clin Invest 2013;123:1939–51.
102. Chiang DY, Kongchan N, Beavers DL, Alsina KM, Voigt N, Neilson JR, et al. Loss of microrna-106b-25 cluster promotes atrial fibrillation by enhancing ryanodine receptor type-2 expression and calcium release. Circ Arrhythm Electrophysiol 2014;7:1214–22.
103. Reilly SN, Liu X, Carnicer R, Recalde A, Muszkiewicz A, Jayaram R, et al. Up-regulation of mir-31 in human atrial fibrillation begets the arrhythmia by depleting dystrophin and neuronal nitric oxide synthase. Sci Transl Med 2016;8:340ra374.
104. Wang Y, Cai H, Li H, Gao Z, Song K. Atrial overexpression of microrna-27b attenuates angiotensin ii-induced atrial fibrosis and fibrillation by targeting alk5. Hum Cell 2018;31:251–60.
105. Cheng WL, Kao YH, Chao TF, Lin YK, Chen SA, Chen YJ. Microrna-133 suppresses zfhx3-dependent atrial remodelling and arrhythmia. Acta Physiol 2019;227:e13322.
106. Wang J, Bai Y, Li N, Ye W, Zhang M, Greene SB, et al. Pitx2-microrna pathway that delimits sinoatrial node development and inhibits predisposition to atrial fibrillation. Proc Natl Acad Sci U S A 2014;111:9181–6.
107. Callis TE, Pandya K, Seok HY, Tang RH, Tatsuguchi M, Huang ZP, et al. Microrna-208a is a regulator of cardiac hypertrophy and conduction in mice. J Clin Invest 2009;119:2772–86.
108. Simon AM, Goodenough DA, Paul DL. Mice lacking connexin40 have cardiac conduction abnormalities characteristic of atrioventricular block and bundle branch block. Curr Biol 1998;8:295–8.
109. D'Souza A, Pearman CM, Wang Y, Nakao S, Logantha S, Cox C, et al. Targeting mir-423-5p reverses exercise training-induced hcn4 channel remodeling and sinus bradycardia. Circ Res 2017;121:1058–68.
110. Yanni J, D'Souza A, Wang Y, Li N, Hansen BJ, Zakharkin SO, et al. Silencing mir-370-3p rescues funny current and sinus node function in heart failure. Sci Rep 2020;10:11279.
111. Zhang J, Wei F, Ding L, Wang L, Zhang X, Yu L, et al. Microrna-1976 regulates degeneration of the sinoatrial node by targeting cav1.2 and cav1.3 ion channels. J Mol Cell Cardiol 2019;134:74–85.
112. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, et al. Microrna-21 contributes to myocardial disease by stimulating map kinase signalling in fibroblasts. Nature 2008;456:980–4.
113. Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, et al. Cardiac fibroblast-derived microrna passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 2014;124:2136–46.
114. Yan M, Chen C, Gong W, Yin Z, Zhou L, Chaugai S, et al. Mir-21-3p regulates cardiac hypertrophic response by targeting histone deacetylase-8. Cardiovasc Res 2015;105:340–52.
115. Ramanujam D, Sassi Y, Laggerbauer B, Engelhardt S. Viral vector-based targeting of mir-21 in cardiac nonmyocyte cells reduces pathologic remodeling of the heart. Mol Ther 2016;24:1939–48.
116. Nishiga M, Horie T, Kuwabara Y, Nagao K, Baba O, Nakao T, et al. Microrna-33 controls adaptive fibrotic response in the

remodeling heart by preserving lipid raft cholesterol. Circ Res 2017;120:835–47.
117. Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, et al. The mirna-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. Nat Commun 2012;3:1078.
118. Sassi Y, Avramopoulos P, Ramanujam D, Gruter L, Werfel S, Giosele S, et al. Cardiac myocyte mir-29 promotes pathological remodeling of the heart by activating wnt signaling. Nat Commun 2017;8:1614.
119. Liu W, Liu Y, Zhang Y, Zhu X, Zhang R, Guan L, et al. Microrna-150 protects against pressure overload-induced cardiac hypertrophy. J Cell Biochem 2015;116:2166–76.
120. van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microrna. Science 2007;316:575–9.
121. Ganesan J, Ramanujam D, Sassi Y, Ahles A, Jentzsch C, Werfel S, et al. Mir-378 controls cardiac hypertrophy by combined repression of mitogen-activated protein kinase pathway factors. Circulation 2013;127:2097–106.
122. Karakikes I, Chaanine AH, Kang S, Mukete BN, Jeong D, Zhang S, et al. Therapeutic cardiac-targeted delivery of mir-1 reverses pressure overload-induced cardiac hypertrophy and attenuates pathological remodeling. J Am Heart Assoc 2013;2:e000078.
123. Guan X, Wang L, Liu Z, Guo X, Jiang Y, Lu Y, et al. Mir-106a promotes cardiac hypertrophy by targeting mitofusin 2. J Mol Cell Cardiol 2016;99:207–17.
124. Gurha P, Abreu-Goodger C, Wang T, Ramirez MO, Drumond AL, van Dongen S, et al. Targeted deletion of microrna-22 promotes stress-induced cardiac dilation and contractile dysfunction. Circulation 2012;125:2751–61.
125. Yang T, Gu H, Chen X, Fu S, Wang C, Xu H, et al. Cardiac hypertrophy and dysfunction induced by overexpression of mir-214 in vivo. J Surg Res 2014;192:317–25.
126. Raso A, Dirkx E, Philippen LE, Fernandez-Celis A, De Majo F, Sampaio-Pinto V, et al. Therapeutic delivery of mir-148a suppresses ventricular dilation in heart failure. Mol Ther 2019; 27:584–99.
127. Guo H, Ma K, Hao W, Jiao Y, Li P, Chen J, et al. Mir15a/mir16-1 cluster and its novel targeting molecules negatively regulate cardiac hypertrophy. Clin Transl Med 2020;10:e242.
128. Heymans S, Corsten MF, Verhesen W, Carai P, van Leeuwen RE, Custers K, et al. Macrophage microrna-155 promotes cardiac hypertrophy and failure. Circulation 2013;128:1420–32.
129. Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. Nat Rev Rheumatol 2020;16: 391–405.
130. Zhang Y, Wang Y, Zhang L, Xia L, Zheng M, Zeng Z, et al. Reduced platelet mir-223 induction in Kawasaki disease leads to severe coronary artery pathology through a mir-223/pdgfrbeta vascular smooth muscle cell axis. Circ Res 2020;127:855–73.
131. Wang X, Ding YY, Chen Y, Xu QQ, Qian GH, Qian WG, et al. Mir-223-3p alleviates vascular endothelial injury by targeting il6st in Kawasaki disease. Front Pediatr 2019;7:288.
132. Corsten MF, Papageorgiou A, Verhesen W, Carai P, Lindow M, Obad S, et al. Microrna profiling identifies microrna-155 as an adverse mediator of cardiac injury and dysfunction during acute viral myocarditis. Circ Res 2012;111:415–25.
133. Corsten MF, Heggermont W, Papageorgiou AP, Deckx S, Tijsma A, Verhesen W, et al. The microrna-221/-222 cluster balances the antiviral and inflammatory response in viral myocarditis. Eur Heart J 2015;36:2909–19.
134. Gou W, Zhang Z, Yang C, Li Y. Mir-223/pknox1 axis protects mice from cvb3-induced viral myocarditis by modulating macrophage polarization. Exp Cell Res 2018;366:41–8.
135. Zhang Y, Cai S, Ding X, Lu C, Wu R, Wu H, et al. Microrna-30a-5p silencing polarizes macrophages towards m2 phenotype to alleviate cardiac injury following viral myocarditis by targeting socs1. Am J Physiol Heart Circ Physiol 2021;320:H1348–60.
136. Liu YL, Wu W, Xue Y, Gao M, Yan Y, Kong Q, et al. Microrna-21 and -146b are involved in the pathogenesis of murine viral myocarditis by regulating th-17 differentiation. Arch Virol 2013; 158:1953–63.
137. He J, Yue Y, Dong C, Xiong S. Mir-21 confers resistance against cvb3-induced myocarditis by inhibiting pdcd4-mediated apoptosis. Clin Invest Med 2013;36:E103–111.
138. He F, Xiao Z, Yao H, Li S, Feng M, Wang W, et al. The protective role of microrna-21 against coxsackievirus b3 infection through targeting the map2k3/p38 mapk signaling pathway. J Transl Med 2019;17:335.
139. Li W, Liu M, Zhao C, Chen C, Kong Q, Cai Z, et al. Mir-1/133 attenuates cardiomyocyte apoptosis and electrical remodeling in mice with viral myocarditis. Cardiol J 2020;27:285–94.
140. Li J, Tu J, Gao H, Tang L. Microrna-425-3p inhibits myocardial inflammation and cardiomyocyte apoptosis in mice with viral myocarditis through targeting tgf-beta1. Immun Inflamm Dis 2021; 9:288–98.
141. Zhao S, Yang G, Liu PN, Deng YY, Zhao Z, Sun T, et al. Mir-590-3p is a novel microrna in myocarditis by targeting nuclear factor kappa-b in vivo. Cardiology 2015;132:182–8.
142. Chen L, Hou X, Zhang M, Zheng Y, Zheng X, Yang Q, et al. Microrna-223-3p modulates dendritic cell function and ameliorates experimental autoimmune myocarditis by targeting the nlrp3 inflammasome. Mol Immunol 2020;117: 73–83.
143. Pan A, Tan Y, Wang Z, Xu G. Stat4 silencing underlies a novel inhibitory role of microrna-141-3p in inflammation response of mice with experimental autoimmune myocarditis. Am J Physiol Heart Circ Physiol 2019;317:H531–40.
144. Mirna M, Paar V, Topf A, Kraus T, Sotlar PK, Aigner PA, et al. A new player in the game: treatment with antagomir-21a-5p significantly attenuates histological and echocardiographic effects of experimental autoimmune myocarditis. Cardiovasc Res 2021;cvab015. https://doi.org/10.1093/cvr/cvab015.
145. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation 2015;131: 1981–8.
146. Mukhopadhyay P, Batkai S, Rajesh M, Czifra N, Harvey-White J, Hasko G, et al. Pharmacological inhibition of cb1 cannabinoid receptor protects against doxorubicin-induced cardiotoxicity. J Am Coll Cardiol 2007;50:528–36.
147. Gupta SK, Garg A, Avramopoulos P, Engelhardt S, Streckfuss-Bomeke K, Batkai S, et al. Mir-212/132 cluster modulation prevents doxorubicin-mediated atrophy and cardiotoxicity. Mol Ther 2019;27:17–28.
148. Du Y, Liu G, Zhao L, Yao R. Protective effect of mir-204 on doxorubicin-induced cardiomyocyte injury via hmgb1. Oxid Med Cell Longev 2020;2020:8819771.

149. Hu X, Liu H, Wang Z, Hu Z, Li L. Mir-200a attenuated doxorubicin-induced cardiotoxicity through upregulation of nrf2 in mice. Oxid Med Cell Longev 2019;2019:1512326.
150. Pan JA, Tang Y, Yu JY, Zhang H, Zhang JF, Wang CQ, et al. Mir-146a attenuates apoptosis and modulates autophagy by targeting taf9b/p53 pathway in doxorubicin-induced cardiotoxicity. Cell Death Dis 2019;10:668.
151. Ji X, Ding W, Xu T, Zheng X, Zhang J, Liu M, et al. Microrna-31-5p attenuates doxorubicin-induced cardiotoxicity via quaking and circular rna pan3. J Mol Cell Cardiol 2020;140:56–67.
152. Zhang H, Tian Y, Liang D, Fu Q, Jia L, Wu D, et al. The effects of inhibition of microrna-375 in a mouse model of doxorubicin-induced cardiac toxicity. Med Sci Mon Int Med J Exp Clin Res 2020;26:e920557.
153. Li J, Wan W, Chen T, Tong S, Jiang X, Liu W. Mir-451 silencing inhibited doxorubicin exposure-induced cardiotoxicity in mice. BioMed Res Int 2019;2019:1528278.
154. Li XQ, Liu YK, Yi J, Dong JS, Zhang PP, Wan L, et al. Microrna-143 increases oxidative stress and myocardial cell apoptosis in a mouse model of doxorubicin-induced cardiac toxicity. Med Sci Mon Int Med J Exp Clin Res 2020;26:e920394.
155. Sun W, Zhao P, Zhou Y, Xing C, Zhao L, Li Z, et al. Ultrasound targeted microbubble destruction assisted exosomal delivery of mir-21 protects the heart from chemotherapy associated cardiotoxicity. Biochem Biophys Res Commun 2020;532:60–7.
156. Jing X, Yang J, Jiang L, Chen J, Wang H. Microrna-29b regulates the mitochondria-dependent apoptotic pathway by targeting bax in doxorubicin cardiotoxicity. Cell Physiol Biochem 2018; 48:692–704.
157. Tony H, Yu K, Qiutang Z. Microrna-208a silencing attenuates doxorubicin induced myocyte apoptosis and cardiac dysfunction. Oxid Med Cell Longev 2015;2015:597032.
158. Piegari E, Cozzolino A, Ciuffreda LP, Cappetta D, De Angelis A, Urbanek K, et al. Cardioprotective effects of mir-34a silencing in a rat model of doxorubicin toxicity. Sci Rep 2020;10:12250.
159. Yin Z, Zhao Y, Li H, Yan M, Zhou L, Chen C, et al. Mir-320a mediates doxorubicin-induced cardiotoxicity by targeting vegf signal pathway. Aging (Albany NY) 2016;8:192–207.
160. Garfinkel AC, Seidman JG, Seidman CE. Genetic pathogenesis of hypertrophic and dilated cardiomyopathy. Heart Fail Clin 2018; 14:139–46.
161. Zhou Q, Schotterl S, Backes D, Brunner E, Hahn JK, Ionesi E, et al. Inhibition of mir-208b improves cardiac function in titin-based dilated cardiomyopathy. Int J Cardiol 2017;230:634–41.
162. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, et al. Microrna-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev 2008;22:3242–54.
163. Wei Y, Peng S, Wu M, Sachidanandam R, Tu Z, Zhang S, et al. Multifaceted roles of mir-1s in repressing the fetal gene program in the heart. Cell Res 2014;24:278–92.
164. Quattrocelli M, Crippa S, Montecchiani C, Camps J, Cornaglia AI, Boldrin L, et al. Long-term mir-669a therapy alleviates chronic dilated cardiomyopathy in dystrophic mice. J Am Heart Assoc 2013;2:e000284.
165. Bernardo BC, Ooi JY, Matsumoto A, Tham YK, Singla S, Kiriazis H, et al. Sex differences in response to mirna-34a therapy in mouse models of cardiac disease: identification of sex-, disease- and treatment-regulated mirnas. J Physiol 2016;594:5959–74.
166. Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ Res 2017;121:749–70.
167. Coto E, Reguero JR, Palacin M, Gomez J, Alonso B, Iglesias S, et al. Resequencing the whole myh7 gene (including the intronic, promoter, and 3' utr sequences) in hypertrophic cardiomyopathy. J Mol Diagn 2012;14:518–24.
168. Kuster DW, Mulders J, Ten Cate FJ, Michels M, Dos Remedios CG, da Costa Martins PA, et al. Microrna transcriptome profiling in cardiac tissue of hypertrophic cardiomyopathy patients with mybpc3 mutations. J Mol Cell Cardiol 2013;65: 59–66.
169. Wilson KD, Hu S, Venkatasubrahmanyam S, Fu JD, Sun N, Abilez OJ, et al. Dynamic microrna expression programs during cardiac differentiation of human embryonic stem cells: role for mir-499. Circ Cardiovasc Genet 2010;3:426–35.
170. Zheng D, Huo M, Li B, Wang W, Piao H, Wang Y, et al. The role of exosomes and exosomal microrna in cardiovascular disease. Front Cell Dev Biol 2020;8:616161.
171. Wang B, Wang ZM, Ji JL, Gan W, Zhang A, Shi HJ, et al. Macrophage-derived exosomal mir-155 regulating cardiomyocyte pyroptosis and hypertrophy in uremic cardiomyopathy. JACC Basic Transl Sci 2020;5:148–66.
172. Fang X, Stroud MJ, Ouyang K, Fang L, Zhang J, Dalton ND, et al. Adipocyte-specific loss of ppargamma attenuates cardiac hypertrophy. JCI Insight 2016;1:e89908.
173. Qiao L, Hu S, Liu S, Zhang H, Ma H, Huang K, et al. Microrna-21-5p dysregulation in exosomes derived from heart failure patients impairs regenerative potential. J Clin Invest 2019; 129:2237–50.
174. Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting mecp2 via mir-22. PLoS One 2014;9:e88685.
175. Nie X, Fan J, Li H, Yin Z, Zhao Y, Dai B, et al. Mir-217 promotes cardiac hypertrophy and dysfunction by targeting pten. Mol Ther Nucleic Acids 2018;12:254–66.
176. Yang J, Yu X, Xue F, Li Y, Liu W, Zhang S. Exosomes derived from cardiomyocytes promote cardiac fibrosis via myocyte-fibroblast cross-talk. Am J Transl Res 2018;10:4350–66.
177. Wang C, Zhang C, Liu L, A X, Chen B, Li Y, et al. Macrophage-derived mir-155-containing exosomes suppress fibroblast proliferation and promote fibroblast inflammation during cardiac injury. Mol Ther 2017;25:192–204.
178. Wang B, Zhang A, Wang H, Klein JD, Tan L, Wang ZM, et al. Mir-26a limits muscle wasting and cardiac fibrosis through exosome-mediated microrna transfer in chronic kidney disease. Theranostics 2019;9:1864–77.
179. Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, et al. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of mir-320 into endothelial cells. J Mol Cell Cardiol 2014;74:139–50.
180. Liu S, Chen J, Shi J, Zhou W, Wang L, Fang W, et al. M1-like macrophage-derived exosomes suppress angiogenesis and exacerbate cardiac dysfunction in a myocardial infarction microenvironment. Basic Res Cardiol 2020;115:22.
181. Khudayberdiev SA, Zampa F, Rajman M, Schratt G. A comprehensive characterization of the nuclear microrna repertoire of post-mitotic neurons. Front Mol Neurosci 2013;6:43.

182. Fan J, Zhang X, Nie X, Li H, Yuan S, Dai B, et al. Nuclear mir-665 aggravates heart failure via suppressing phosphatase and tensin homolog transcription. Sci China Life Sci 2020;63:724–36.
183. Fan J, Li H, Nie X, Yin Z, Zhao Y, Zhang X, et al. Mir-665 aggravates heart failure via suppressing cd34-mediated coronary microvessel angiogenesis. Aging (Albany NY) 2018;10:2459–79.
184. Santovito D, Egea V, Bidzhekov K, Natarelli L, Mourao A, Blanchet X, et al. Noncanonical inhibition of caspase-3 by a nuclear microrna confers endothelial protection by autophagy in atherosclerosis. Sci Transl Med 2020;12:eaaz2294.
185. Kren BT, Wong PY, Sarver A, Zhang X, Zeng Y, Steer CJ. Microrna identified in highly purified liver-derived mitochondria may play a role in apoptosis. RNA Biol 2009;6:65–72.
186. Barrey E, Saint-Auret G, Bonnamy B, Damas D, Boyer O, Gidrol X. Pre-microrna and mature microrna in human mitochondria. PLoS One 2011;6:e20220.
187. Bandiera S, Ruberg S, Girard M, Cagnard N, Hanein S, Chretien D, et al. Nuclear outsourcing of rna interference components to human mitochondria. PLoS One 2011;6:e20746.
188. Das S, Ferlito M, Kent OA, Fox-Talbot K, Wang R, Liu D, et al. Nuclear mirna regulates the mitochondrial genome in the heart. Circ Res 2012;110:1596–603.
189. Das S, Kohr M, Dunkerly-Eyring B, Lee DI, Bedja D, Kent OA, et al. Divergent effects of mir-181 family members on myocardial function through protective cytosolic and detrimental mitochondrial microrna targets. J Am Heart Assoc 2017;6: e004694.
190. Roman B, Kaur P, Ashok D, Kohr M, Biswas R, O'Rourke B, et al. Nuclear-mitochondrial communication involving mir-181c plays an important role in cardiac dysfunction during obesity. J Mol Cell Cardiol 2020;144:87–96.
191. Taubel J, Hauke W, Rump S, Viereck J, Batkai S, Poetzsch J, et al. Novel antisense therapy targeting microrna-132 in patients with heart failure: results of a first-in-human phase 1b randomized, double-blind, placebo-controlled study. Eur Heart J 2021;42: 178–88.
192. O'Meara CC, Wamstad JA, Gladstone RA, Fomovsky GM, Butty VL, Shrikumar A, et al. Transcriptional reversion of cardiac myocyte fate during mammalian cardiac regeneration. Circ Res 2015;116: 804–15.
193. Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, et al. Mir-222 is necessary for exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell Metabolol 2015; 21:584–95.
194. Yang D, Wan X, Dennis AT, Bektik E, Wang Z, Costa MGS, et al. Microrna biophysically modulates cardiac action potential by direct binding to ion channel. Circulation 2021;143: 1597–613.