# Differential Expression of MicroRNAs in Different Disease States

**Maha Abdellatif**

Cardiovascular Research Institute, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103

## Abstract

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

## Keywords

## Introduction

Lin-4, the first miRNA to be discovered, is a 22 ribonucleotide long molecule that negatively regulates the translation of lin-14 and, thereby, the timing of the larva-to-adult switch of *Caenorhabditis elegans* (*C. elegans*) [1]. It functions by binding to complementary sequences in the 3' untranslated region (3'UTR) of lin-14 mRNA, via which it inhibits its translation without affecting its stability [2]. A similar mechanism was later ascribed to let-7 and its regulation of a set of genes that included lin-14, lin-28, lin-41, lin-42, and daf-12 in *C. elegans*, where if over- or under-expressed, results in premature development or reiteration of cell lineages, respectively [3]. These findings define 2 aspects of miRNA's unique functionality; 1) precise regulation of the timing of a cellular event, 2) via synchronous posttranscriptional inhibition of a cadre of genes that are functionally interdependent, thus, operating as an efficient molecular switch.

MiRNAs are predominantly known to negatively regulate mRNA translation through assembling into a complex known as miRNA RNA-inducing silencing complex (RISC or

Corresponding author: Maha Abdellatif, Cardiovascular Research Institute, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103. Tel: (973) 972-1254; Fax: (973) 972-7489; abdellma@umdnj.edu.

**Disclosures**
None

miRISC). This complex includes the RNA-specific endonuclease Dicer, which is involved in the processing of pre-miRNA into the mature form [4]; the argonaute (Ago) protein, onsisting of 4 isoforms, of which Ago2 or slicer, can cleave the target mRNA [5, 6]; the P-body protein PW182 [7]; the human immunodeficiency virus transactivating response RNA-binding protein (TRBP), known to recruit Ago2 to RISC [8]; and fragile X mental retardation protein (FMRP1) [9], which associates with polyribosomes [10]; among others whose RISC-related functions have not been fully elucidated yet [11]. This complex binds a target gene via partial sequence complementarity between the miRNA and, preferentially, a conserved site within the 3'UTR of the gene. Through this interaction they can inhibit translation by any of 3 different mechanisms: 1) inhibiting translation at a post-initiation step without reducing mRNA abundance, polyadenylation, or polyribsomal content [2]; 2) inhibiting translation initiation via a cap- [12–14] and poly(A)-dependent dependent mechanism [12]; 3) inducing deadenylation of mRNAs independently of inhibition of translation [15–17].

Perturbation in gene expression is one of the main underlying mechanisms that contribute to disease development. While transcriptional regulation of some genes has been recorded others are regulated by posttranscriptional means. The discovery of miRNAs has recently advanced our understanding regarding the latter mode of gene regulation in development and disease [18], as will be detailed below for cardiovascular diseases, in particular.

## MiRNAs in cardiac hypertrophy and failure

### Cardiac-enriched miR-1 and miR-133 in cardiac hypertrophy and failure

MiRNAs are differentially regulated during cardiac hypertrophy and failure in both rodents and humans [19–27] (Fig. 1). Reprogramming of gene expression in the heart to recapitulate the pattern of the fetal/neonatal heart is an established mechanism that contributes to the development of cardiac hypertrophy [28–30]. Therefore, an analogous change in the expression pattern of miRNAs supports their role in the underlying pathogenesis [31]. MiR-1 is one of the earliest miRNAs that are downregulated in the heart after the induction of pressure overload, which precedes the increase in cardiac mass, suggesting that it may potentially be involved in initiating the process [23]. In accordance, miR-1 levels are also lower in genetic models of hypertrophy that include the calcineurin- [32] and AKT-overexpressing [33] transgenic mice hearts. Furthermore, miR-1 is reduced in the hypertrophied left ventricle of acromegalic patients [23] and in those who suffer from aortic stenosis with normal ejection fraction [20]. Experiments in cultured myocytes [23] and those involving inhibition of miR-1 in vivo [33], suggest that a decrease in miR-1 is necessary for an increase in cell size and mass. Some of its targets that were validated in these studies include insulin-like growth factor-1 [34], calmodulin, and myocyte enhancer factor 2A [32], all of which are known to contribute to the development of cardiac hypertrophy. In contrast, the fluctuation in miR-1 levels were less consistent in the failing heart. For example, as Matkovich et al [21] and Thum et al [26] recorded an increase in miR-1, Ikeda et al [20], Sucharov et al [24], and Naga Prasad et al [22] detected a reduction in miR-1, in ischemic and non-ischemic dilated cardiomyopathies that were associated with a significant reduction in percent ejection fraction. Therefore, it would appear that an increase in cardiac mass is accompanied by downregulation of miR-1, however, as hypertrophy progresses into dilatation and contractile dysfunction, miR-1 concentrations return to normal or above normal levels.

MiR-133, which is also a muscle-enriched miRNA that shares the same primary transcript as miR-1, is likewise downregulated during cardiac hypertrophy [19, 27, 33]. Moreover, like miR-1, its knockdown via antagomirs was sufficient for inducing cardiac hypertrophy and the characteristic increase in some of the fetal genes [33]. In contrast to these results, genomic knockout of miR-133a-1 or miR-133a-2, each of which contribute to ~50% reduction in miR-133 levels, which is equivalent to the decrease observed in cardiac hypertrophy,

exhibited normal cardiac growth and function under quiescent or pressure overload conditions [35]. This discrepancy in outcomes is not readily explainable. On the other hand, a transgenic mouse model overexpressing miR-133 that compensates for its downregulation during hypertrophy failed to inhibit cardiac enlargement, suggesting that downregulation of miR-133 in the heart is not required for the induction of cardiac hypertrophy [36].

### The regulation of contractile fibers by myomiRs during cardiac hypertrophy and failure

While miR-1 and miR-133 are enriched in both skeletal and cardiac muscle, miR-208 is cardiac restricted [37]. It has 2 isoforms, miR-208a and miR-208b, that are contained within the introns of *Myh6* (α myosin heavy chain) and *Myh7* (β myosin heavy chain) genes, respectively. Homozygous miR-208a mice (miR-208a$^{-/-}$) are developmentally normal with the expected normal levels of α and β myosinheavychain (Myh) at birth, although the adult mice fail to upregulate βMyh in response to thyroid hormone inhibition [38]. Likewise, pressure overload on the heart did not induce the expected increase in βMyh. In addition, there was absence of hypertrophic growth and fibrosis following an increase in workload, accompanied by reduced levels of miR-499 [38]. Interestingly, transgenic overexpression of miR-499 in the miR-208a$^{-/-}$ background restored the normal response to thyroid hormone inhibition, suggesting that it is a downstream effector of miR-208 and is sufficient for mediating its effect [39]. MiR-499 was shown to target Sox6 [39], a negative regulator of βMyh [40], and accordingly the transgene reduced its levels in the miR-208$^{-/-}$ mice. Paradoxically, though, ablation of miR-499 did not recapitulate the miR-208a$^{-/-}$ phenotype.

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

In contrast to the absence of any spontaneous defects associated with the miR-208a/b knockout mice, cardiac specific transgenic expression of miR-208a induced cardiac hypertrophy within 4 months of age [42]. The phenotype, though, did not exhibit downregulation of αMyh, upregulation of atrial natriuretic factor, or changes in any of the hypertrophy-related miRNAs. It was, however, associated with a decrease in myostatin, a negative regulator of hypertrophy [43] and a validated target of miR-208a, which could potentially explain its anti-hypertrophic effect [42]. Additionally, these mice exhibited defects in conduction in the form of first- or second-degree atrioventricular bundle block, compared to the miR-208a$^{-/-}$ mice, which suffered from atrial fibrillation. The latter could be attributed to a reduction in connexin 40 and its transcriptional regulator Hop, however, these proteins were unchanged in the miR-208a transgenic mice. Meanwhile, GATA4, a validated target of miR-208a and a positive regulator of connexin 40 (Cx40) [44], is more abundant in the miR-208a$^{-/-}$ mice, although this obviously does not reconcile with the observed downregulation of Cx40 in this model. Similar to the miR-208 transgenic, a cardiac-specific transgenic mouse model of miR-208's downstream effector, miR-499, is sufficient for inducing cardiac hypertrophy [45]. However, under these conditions, its validated target, Sox6, was not altered in the transgenic hearts.

## MiRNAs that regulate cardiac fibrosis

One of the most highly and consistently increased miRNAs during cardiac hypertrophy is miR-21 [19, 23, 25, 27]. Its function in this context has been elusive, as cardiac-specific transgenic mice appeared structurally and functionally normal, and did not differ from wild type mice in its response to pressure overload [46, 47]. While miR-21 increases in the whole heart during pathological hypertrophy, it was suggested that its increase in myofibroblasts is more pronounced than it is in the myocytes [47]. In the former cell type, it was shown to directly target and suppress sprouty1, resulting in enhanced extracellular signal-regulated kinase 1/2 phosphorylation and myofibroblast survival, which, in turn, indirectly contributes to the increase in fibrosis during cardiac hypertrophy [47]. This was confirmed by treating mice with a miR-21 antagomir, which resulted in the reduction of both fibrosis and cardiac hypertrophy. A recent report, though, disputes these results, as it shows that global genomic ablation of miR-21, or knockdown with LNA-modified antisense oligonucleotides, has no impact on the development of fulminant cardiac hypertrophy in response to pressure overload, angiotensin II, calcineurin, or infarction [48]. It is plausible that an unidentified gene/mechanism compensated for the genomic ablation of miR-21. Thus, the jury is still out regarding the role of miR-21 during hypertrophy.

Although cardiac hypertrophy is not inhibited as a result of normalizing miR-133 levels; apoptosis and fibrosis were restored to normal levels [36]. Connective tissue growth factor (CTGF) is one of the main factors that mediate fibrosis in the heart [49, 50]. While one study did show that it is a direct target of miR-133, counter to prediction, the miR-133 transgenic mouse exhibits an increase in CTGF mRNA in the heart [51]. Alternatively, miR-133 directly targets collagen 1A1 and is downregulated during angiotensin II-induced myocardial fibrosis, along with miR-29b [52]. MiR-29b was also shown to target multiple collagen isoforms and its inhibition in vivo was sufficient for inducing fibrosis in the heart [53]. On the other hand, CTFG was also identified as a target of the miR-17~92 cluster [54], as miR-18 and -19 inversely correlate with the expression of CTGF, thrombospondin, and fibrosis in the, aging, failing heart in mice and human subjects [55]. However, there is no data that show that normalizing the levels of miR-29, miR-18, or miR-19 in the heart during hypertrophy or failure would inhibit the development of fibrosis.

## MiRNAs that influence cardiac size

MiR-23a is clustered with miR-27a and miR-24, and increases during pressure overload- [19, 23, 25, 27] and isoproterenol-induced cardiac hypertrophy [56]. It was found to be directly regulated via nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 NFATc3 and is both required and sufficient for mediating the effects of the calcineurin-3 (NFATc3) pathway during cardiac hypertrophy [56]. Its effects are partly mediated via the direct targeting and suppression of the negative regulator, muscle ring finger 1 (MuRF1). In accordance, inhibiting miR-23a with antagomirs results in the upregulation of MuRF1 in the heart, and virtually complete inhibition of isoproterenol-induced cardiac hypertrophy and the accompanied changes in gene expression and cardiac functions. This reconciles with a MuRF1-dependent function that was revealed in the MuRF1 knockout mice, as they developed an exaggerated form of pressure-induced hypertrophy [57]. The other member of the cluster, miR-27a, is also upregulated during cardiac hypertrophy, and, likewise, was found both necessary and sufficient for the induction of cardiac hypertrophy and contractile dysfunction in a mouse model [58]. In this context it targets peroxisome proliferator-activated receptor gamma (PPARγ), a well-known regulator of fatty acid and glucose metabolism [59]. The finding is consistent with the role of PPARγ in inhibiting cardiac hypertrophy [60]. It also has been shown to positively regulate βMyh through targeting the thyroid hormone receptor β1 in cardiac myocytes [61]. However, there is no evidence yet as to whether this contributes to the upregulation of βMyh and contractile dysfunction during cardiac hypertrophy in vivo.

More recently, da Costa Martins *et al* demonstrated that mir-199b, which is upregulated during cardiac hypertrophy, is also a direct transcriptional target of NFATc [62]. In this case, miR-199b targets and suppresses the NFATc kinase dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a (Dyrk1a), which is a negative regulator of NFAT. Accordingly, mutant mice overexpressing miR-199b in the heart, or those hemizygous for Dyrk1a, exhibited exaggerated hypertrophy in response to pressure overload. Conversely, antisense 'antagomir' treatment normalized miR-199a levels, and not only prevented hypertrophy and failure, but also almost completely reversed them.

The transcription factor GATA4 plays an essential role in the development of cardiac hypertrophy [63, 64] as it regulates the expression of some of the fetal genes that are re-expressed in this condition [65]. In accordance, its DNA-binding activity and abundance increase during hypertrophy [66, 67]. Recent data utilizing state-of-the-art RNA polymerase II immunoprecipitation followed by deep sequencing established that the increase in GATA4 expression in cardiac myocytes is not due to an increase in the transcription of its gene [68]. This prompted the search for miRNAs, which were downregulated during cardiac hypertrophy that targeted GATA4 3'UTR. MiR-26a/b, which is highly expressed in the heart and other organs, inversely correlated with the expression of GATA4 levels in the developing and hypertrophied hearts. It was proven to directly target GATA4 and, subsequently, reduce cardiac growth. However, although knockdown of miR-26 was sufficient for inducing GATA4 in the heart in vivo, it was insufficient for inducing cardiac hypertrophy. Another validated target of miR-26b is phospholipase C beta1, which proved to be a major negative regulator of miR-26b, creating a double negative feedback loop characteristic of many miRNAs and their targets [68]. This is the first proven example of a gene that is primarily regulated by a posttranscriptional miRNA-dependent mechanism during disease, underscoring the functional relevance of miRNAs.

## MiRNAs in ischemic heart disease

### MiRNAs that regulate apoptosis during ischemia

Myocardial ischemia results from insufficient blood flow to the myocardium, which leads to apoptotic, autophagic, and necrotic cell death, with subsequent inflammatory cell infiltration, fibrosis and contractile dysfunction [69]. MiRNAs' expression is rapidly perturbed during myocardial ischemia or cellular hypoxia (Fig. 2). MiR-1 and miR-133, which are reduced during cardiac hypertrophy, are upregulated when myocytes are subjected to hypoxia. Under these conditions, miR-1 was found to target heat shock proteins (HSP) HSP60 and HSP70, while miR-133 targeted caspase-9 [70]. In accordance, miR-1 has a proapoptotic effect, whereas miR-133 is antiapoptotic. In contrast, miR-320 is downregulated during ischemia/reperfusion (I/R) and is responsible for upregulation of its target HSP20 [71]. Further suppression of this miRNA with an antagomir successfully reduces infarct size after I/R.

Notably, miR-21, which is ubiquitously expressed, has emerged as a major inhibitor of cell apoptosis in myocytes as well as a plethora of other cell types. It is acutely downregulated during myocardial ischemia, specifically within the ischemia zone, where replenishing it reduces the infarct size and retards the development of heart failure [46, 72]. These results were corroborated by experiments conducted in isolated cardiac myocytes, in which miR-21 was proven to inhibit hypoxia-induced apoptosis by targeting Fas ligand (FasL) [46] and programmed cell death 4 (PDCD4) [73]. Furthermore, miR-21 is induced by the AKT pathway and partly mediates its antiapoptotic effect via suppression of FasL [46]. Conversely, miR-21 activates AKT through suppression of phosphatase and tensin homolog (PTEN) via a double negative feedback loop [46]. In addition to myocytes, miR-21 is enriched in myofibroblasts and infiltrating cells within the infarct zone. In these cells it was shown to inhibit PTEN and

induce upregulation of metalloprotease-2 [74], thereby, promoting fibrosis. As we discussed earlier, miR-21also has a profibrotic effect during cardiac hypertrophy, via promoting the survival of myofibroblast [47], however, this particular mechanism was not examined in the ischemia heart.

Similar to miR-21, miR-494 is also reduced in the infarct zone, where normalizing its levels by transgenic overexpression reduces infarct size and improves contractility [75]. Intriguingly, miR-494 targets both proapoptotic genes that include PTEN, Rho-associated coiled-coil containing protein kinase 1, and calcium/calmodulin-dependent protein kinase II delta, as well as, antiapoptotic genes, such as fibroblast growth factor receptor 2 and leukemia inhibitory factor. However, as mentioned, the net result is a cardioprotective effect that is associated with enhanced AKT phosphorylation, possibly due to inhibition of PTEN. Likewise, miR-24 is downregulated in the peri-infarct zone, where replenishing it reduces myocyte apoptosis, infarct size, and cardiac dysfunction after myocardial ischemia by targeting Bim [76]. Additionally, miR-24, along with miR-21 and miR-221, inhibit Bim in cardiac progenitor cells and improve their survival and contribution to functional recovery after coronary artery ligation [77].

Other miRNAs are upregulated within the infarct zone. One example includes miR-15b, which is upregulated in the infarct zone of a porcine model after ischemia (60 min) / reperfusion (24 h) [78]. When neutralized in cardiac myocytes it enhances their viability during hypoxia in association with an increase of 2 of its validated targets, ADP-ribosylation factor-like 2 [79] and Bcl2 [80]. In vivo, suppressing miR-15b with locked nucleic acid-modified "tiny" antimiRs resulted in reducing the infarct size after ischemia (75 min) / reperfusion (24 h) and improved % ejection fraction and left ventricular end diastolic volume measured 2 weeks after I/R [78]. The mechanism underlying this protection is presumed to be improved myocyte survival possibly due to an increase in the expression of suppressors of cell death.

The response of a cell to hypoxia or ischemia is biphasic; initially ischemia induces an adaptive response, which then transitions into a damaging effect upon the persistence of ischemia. Accordingly, exposing cells to brief periods of hypoxia or ischemia elicits a protective response, which involves activation of the AKT pathway [81]. This process is known as ischemia preconditioning (IPC) and has been proven to protect organs against subsequent damaging periods of ischemia [82]. MiRNAs are involved in early preconditioning, which involves posttranscriptional vs. transcriptional events [83], including the upregulation of hypoxia-inducible factor 1 alpha (Hif-1α)[84, 85]. In specific, miR-199a-5p is downregulated within minutes of applying IPC to the porcine or mouse hearts or hypoxia preconditioning (HPC) to cardiac myocytes. At least in myocytes, this reduction proved to be both required and sufficient for upregulation of its target Hif-1α, to the same extent as that observed after HPC[86]. Sirt1 was also validated as a direct conserved target of miR-199a-5p, which is required for downregulation of prolyl hydroxylase 2 and, thus, stabilization of Hif-1α[86]. These effects are mediated via an a AKT-dependent pathway, which is sufficient for inducing downregulation of miR-199a-5p and upregulation of Hif-1α and Sirt1 [87]. This is a unique example of a miRNA, which functions as an immediate early switch for the translation of a gene required for immediate adaptation to an insult such as hypoxia. In addition, AKT induces upregulation of miR-21 [46], which is upregulated during IPC of the heart [88, 89]. It targets and inhibits proapoptotic genes that include PTEN, FasL, and PDCD4, thus, assuming a powerful antiapoptotic function during ischemia. The results implicate miRNAs in mediating the prosurvival effects of the AKT pathway through both increasing prosurvival proteins like Hif-1a and Sirt1 and suppressing proapoptotic genes such as PTEN, FasL, and PDCD4.

## MiRNAs that regulate angiogenesis during ischemia

In addition to directly regulating cell viability during myocardial ischemia, miRNAs could also indirectly influence cell survival and the size of infarct zone or scar formation via regulating angiogenesis. MiR-126 is one that is highly expressed in murine lung and heart and to a lesser extent in the brain, liver, and kidney, where it is localized to endothelial [90] and epithelial cells [91]. Deletion of both its alleles results in ~50% embryonic lethality due to systemic edema and widespread hemorrhage from ruptured vessels [92, 93]. Those embryos that escaped the lethal phenotype developed normally with no apparent abnormalities until they were challenged with ischemia, which elicits reparative angiogenesis. In particular, when the left coronary artery was occluded, angiogenesis was compromised in the ischemic hearts, which resulted in precipitous cardiac failure [93]. Similar results were observed when miR-126 antagomir was injected in mice [94]. One of miR-126's relevant and validated targets include Spred-1, for which gain- and loss-of-function experiments in an aortic ring sprouting assay proved that it is sufficient for inhibiting vascular outgrowths [93]. In an opposing function, however, miR-126 was found to target stromal cell-derived factor-1 [95]. This study shows that silencing miR-126 during ischemia results in an increase in this protein, which mobilizes Sca-1(+)/Lin(−) progenitor cells and enhances angiogenesis in a hind limb ischemia model. It is possible that the extent of silencing of miR-126 in different studies determines which of its functions predominates.

In contrast to miR-126, forced expression of miR-92a inhibits tube formation, cell migration, and adhesion of endothelial cells [96]. Conversely, inhibition of miR-92a with antagomirs enhances angiogenesis during hind limb or myocardial ischemia in mice, and improves blood flow and tissue viability in the region. MiR-92a targets alpha5 integrin, which plausibly plays a role in mediating its function, although, most likely other targets are also involved. MiR-92 is a member of the miR-17~92 cluster of miRNA. Recently, it was shown that other members of this cluster, including miR-17, miR-18a, and miR-20, also exhibited an antiangiogenic effect both in vitro and in vivo, where Janus kinase 1 was identified as a miR-17 target [97]. In contrast, another study reported that myc induces upregulation of miR-17~92 and that overexpression of the cluster with Ras enhanced tumor growth by increasing neovascularization [54]. In this case, it was suggested that the targeting of thrombospondin by miR-19 mediates the cluster's effects. This does agree with the fact that c-myc induces angiogenesis by posttranscriptionally inhibiting the expression of thrombospondin-1 [98–100], and provides an explanatory mechanism for it. The discrepancy between may reflect differences in the models that were investigated.

Whereas miR-24 is downregulated in the peri-infarct zone and contributes to myocyte apoptosis and cardiac dysfunction during myocardial ischemia through an increase in its target Bim [76], another study reports that miR-24 is upregulated in endothelial cells, in specific, after cardiac ischemia, where it induces apoptosis of these cells [101]. Accordingly, blocking its upregulation by an antagomir, rescues endothelial cells from apoptosis, increases angiogenesis, and limits both infarct size and cardiac dysfunction. In this context, it was shown to target GATA2 and p21-activated kinase 4. Thus, it would appear that miR-24 has different targets and opposite effects in cardiac myocytes vs. endothelial cells. It should be noted here that the dose of antagomir used in vivo was relatively low and appeared to be preferentially taken up by the endothelial cells, thus, purportedly sparing the myocytes from any apoptotic effect. What should also be taken into account, especially if used for therapeutic targeting, is that miR-24 also targets histone 2Ax in differentiating hematopoietic cells and renders them more susceptible to DNA damaging agents [102]. Thus, silencing miR-24 will have an beneficial effect on these cells as well.

Mir-424 is also upregulated during hypoxia in endothelial cells, in particular, and induces upregulation of Hif-1α via targetng cullin 2 [103]. The latter is one of the components of the

von Hipple-Lindau-elongin B/C-Nedd8 complex that is responsible for the degradation of Hif-1α[104]. Thus, miR-424 overexpression induces migration, proliferation, and tubulogenesis of human umbilical vein endothelial cells (HUVEC). In vivo, miR-424-overexpressing HUVEC were preferentially integrated into the neovasculature of an ovarian carcinoma, however, it did not increase vascular formation. In the mouse heart, miR-322, the mouse homolog of miR-424, is upregulated after 1 wk of coronary artery ligation in the periinfarct and remote areas, although its role in promoting angiogenesis under these conditions has not been investigated yet.

Hif-1α is an established angiogenic factor that functions through directly enhancing the transcription of vascular endothelial growth factor. In was also found to induce the expression of miR-210 during hypoxia [105, 106]. This miRNA was found to be sufficient for inducing tubulogenesis of endothelial cells [106]. Moreover, recent findings show that supplementing the myocardium with miR-210 increases angiogenesis in the periinfarct zone, reduces apoptosis and infarct size, and results in improved cardiac function following left anterior descending coronary artery ligation [107]. Two of its targets include ephrin-A3 and protein tyrosine phosphatase, non-receptor type 1, which are involved in inhibition of angiogenesis and apoptosis, respectively.

In addition to the intracellular function of miRNAs, it was recently noted that some have a paracrine quality. In specific, the saphenous vein-derived pericyte progenitor cells mediate their angiogenic effect via secretion of miR-132 [108]. This was confirmed when silencing of miR-132 by an antimiR reduced the ability of these progenitor cells to induce angiogenesis and reduce infarct size after coronary artery occlusion. This function is potentially mediated by repression of RasGAP and subsequently activation of Ras. Furthermore, by targeting methyl CpG binding protein 2, miR-132 may also inhibits fibrosis.

## MiRNAs that regulate arrhythomogenesis

Abnormalities in electrical conduction and/or repolarization is an underlying cause of arrhythmias manifest during coronary/ischemic heart disease [109]. MiRNAs not only regulate cell viability during ischemia, but have also been found to regulate the expression of molecules involved in mounting an action potential and in conductivity. MiR-1, for example, is upregulated during coronary artery disease in humans and ischemic rat hearts, which are accompanied by an increase in the incidence of arrhythmias [110]. As proof of miR-1's role in arrhythmogenesis, overexpressing or inhibiting miR-1 in the ischemic rat heart resulted in an equivalent effect on the frequency of arrhythmias. MiR-1's validated targets include the gap junction protein Cx43 and Kir2.1 subunit of the K+ channel that mediates $I_{K1}$, both of which could potentially be mediating its effects. Indeed, deletion or missense mutation of Cx43 [111] or $I_{K1}$ [112], respectively, were shown to increase the incidence of arrhythmias. Moreover, their ablation resulted in the reversal of miR-1's knockdown anti-arrhythmogenic effect [110]. On the other hand, the downregulation of miR-133 during cardiac hypertrophy is responsible for a reduction in the $I_{to,f}$ accessory subunit, *Kcnip2*, through an indirect effect, which could be a cause of prolonged QT-interval [36].

An increase in miR-1 in adult myocytes has been shown to regulate inward calcium current ($I_{Ca}$) and calcium release from the sarcoplasmic reticulum (SR) [113]. Overexpression of miR-1 increases $I_{Ca}$, however, this does not elicit an increase in maximum SR $Ca^{2+}$ release, as it possibly counteracted by a 25% lower SR $Ca^{2+}$ content. Increased spark frequency and SR $Ca^{2+}$ leak in the miR-1-overexpressing myocytes might be a contributing factor to the lower SR $Ca^{2+}$. These effects of miR-1 could be attributed to its targeting and suppression of the protein phosphatase 2A (PP2A) regulatory subunit B56α and consequently an increase in the phosphorylation of the ryanodine (RyR) and dihydropyridine receptors. Indeed,

phosphorylation of RyR2 by CAMKII has been linked to SR diastolic $Ca^{2+}$ leakiness and lower SR $Ca^{2+}$ content [114] and to ventricular tachycardiac [115]. In concordance, overexpression of miR-1 dramatically increased the frequency of extrasystolic $Ca^{2+}$ transients and afterdepolarizations in isoproterenol-stimulated, paced, rat adult myocytes [113], which was reversed by a CAMKII inhibitor. MiR-133, which is co-expressed with miR-1, collaborates in this function by targeting both the alpha and beta subunits of the catalytic subunit of PP2A, and has a similar capacity to increase arrhythmogenesis in isolated rat myocytes [116]. This mechanism has not been validated in vivo, however, supportive evidence for that includes an increase in miR-1 and miR-133 and a decrease in PP2A catalytic and regulatory subunits in a canine heart failure model [116]. These results would suggest that higher levels of both miR-1 and miR-133 in the failing or ischemic heart contribute to arrhythmogenesis via perturbation of the expression of genes involved in conduction, depolarization, and calcium handling.

On the other hand, miR-328, but not miR-1 or miR-133, was reported to be upregulated in a canine model of atrial fibrillation (AF) and in human AF patients with rheumatic heart disease [117]. Intramyocardial injection of miR-328 into the right atrium free wall increased susceptibility to AF. Similarly, transgenic overexpression of the miRNA in the mouse heart induced spontaneous AF 28 d after birth, and vice versa, knockdown of miR-328 reduced the incidence of pacing-induced AF. In this case miR-328 targeted and suppressed both the α1c and β1 subunits of the L-type calcium channel, which accordingly explained the dramatic reduction in the L-type $Ca^{2+}$ current observed in the transgenic model. Thus, it would appear that the miRNA-regulated mechanisms underlying arrhythmogenesis in the failing or ischemic heart differ from those involved with AF.

## MiRNAs in vascular disease and remodeling

### MiRNAs in atherosclerogenesis

Perturbation of cholesterol homeostasis is a main underlying cause of atherosclerosis. One of the regulatory factors in cholesterol metabolism include sterol regulatory element-binding protein (SREBP) transcription factors, which regulate the expression of cholesterogenic genes such as low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase [118]. The human SREBP-1 and -2 genes harbor miR-33b and miR-33a, respectively, which appear to be co-expressed with their host genes [119]. Interestingly, miR-33 was found to target adenosine triphosphate–binding cassette A1 (ABCA1) cholesterol transporter [119], which mediates intracellular cholesterol efflux from cells to form plasma high-density lipoprotein that is protective against atherosclerosis [120]. Thus, as expected, when antisense miR-33 is injected in western diet-fed mice, serum high-density lipoprotein (HDL) is significantly elevated. The clinical relevance of this was tested in low-density lipoprotein receptor-deficient mice with atherosclerosis. Treatment of these mice with antimiR-33 for 4 weeks resulted in upregulation of ABCA1 in the liver and macrophages, an increase in HDL levels, and ultimately reduced plaques size and inflammatory gene expression [121]. Similar results were observed in African green monkeys, in which antimiR-33 resulted in elevated HDL, in addition to reduced levels of very-low-density lipoprotein-associated triglycerides [122].

Other miRNAs such as miR-145/143 cluster, miR-133, and miR-221, have a more direct role in atherogenesis. MiR-145/miR-143 cluster is enriched in visceral and vascular smooth muscle cells (VSMC) as early as E11.5 [123] and throughout adulthood [123–125]. Genomic deletion of this cluster resulted in viable mice with a mild vascular phenotype but no cardiac abnormalities [123–125]. In particular, the smooth muscle cells were smaller and exhibited an increase in rough endoplasmic reticulum and a decrease in actin stress fibers, with a resultant thinner tunica media. In addition, there were small neointimal lesions and a

megacolon phenotype, which suggested that knockout of miR-145/143 may inhibit differentiation and, subsequently, enhance proliferation and migration of smooth muscle cells [125]. However, the failure to detect a consistent increase in VSMC proliferation or apoptosis, argued that the miRNA may not be directly required for differentiation but rather for the ability of VSMC to differentiate in response to the physiological contractile demand [124].

MiR-145 is downregulated in response to vascular injury [126]. While the knockout models dispute the fact that downregulation of miR-145 is sufficient for reducing α-actin and myosin heavy chain [123, 124], they do show that abrogation of miR-145 induces limited neointimal lesion formation [124, 125]. In particular, one study has shown that these lesions are only observed in the femoral artery of older knockout mice (18 mo), which suggested that although downregulation of miR-145/143 does not result in fulminant neointimal formation, it does promote it [124]. In consensus, restoring miR-145 levels during vascular injury inhibits neointimal formation [123, 125], with promising therapeutic prospects. MiR-145's effect in this context could be, at least partially, explained by the suppression of its target, Krupple-lik factor 5 (Klf5). Indeed, KLF5, first identified as a transcription factor that induces embryonic smooth muscle myosin heavy chain [127], is involved in enhancing smooth muscle proliferation by upregulating cyclin D1 [128].

MiR-133 is mainly known to be enriched in the heart and skeletal muscle, but a recent report shows that it is also expressed in smooth muscle cells, albeit in relatively low levels [129]. After balloon injury, miR-133 is downregulated in the tunica media and becomes undetectable in the neointimal layer. Overexpression of miR-133 in the carotid artery halts VSMC proliferation and prevents neointimial hyperplasia. The levels of miR-133 in this tissue inversely correlate with its targets Sp1 and moesin. The prediction is that by inhibiting Sp1, Klf4 is deactivated, which in-turn, prevents downregulation of Myh11, and VSMC phenotype switching and proliferation.

In contrast to miR-145 and miR-133, miR-221 [130, 131], but not the co-clustered miR-222 [130], positively regulates smooth muscle proliferation. It is induced by platelet-derived growth factor (PDGF), which is known to stimulate VSMC switching and proliferation during angiogenesis and neointimal formation [130, 131]. Gain- and loss-of-function experiments proved that it is indeed required for mediating the effects of PDGF via targeting and suppressing the cell cycle inhibitors $p27^{Kip1}$ [130, 131] and $p57^{Kip2}$ [131], as well as, c-kit [130]. In concordance, knockdown of miR-221 during vascular injury reduced neointimal thickness by 40% [131]. The effects of miR-221 are likely to be reinforced by the concurrent upregulation of miR-21 in neointimal lesions [132]. MiR-21, which is a ubiquitously expressed prosurvival miRNA, was shown to inhibit PTEN expression under these conditions [132–134]. Accordingly, its abrogation increased apoptotic VSMC death and reduced neoitimal thickness. Thus, neointimal formation is the result of a combinatorial effect of changes in the expression of several miRNAs and their targets that regulate VSMC differentiation, proliferation, and survival.

### MiRNAs in aneurysm formation

Patients with thoracic aortic aneurysm have reduced levels of miR-1, miR-133, miR-21, and miR-29a, all of which significantly inversely correlated with the aortic diameter [135]. It was confirmed that miR-29a targeted metalloproteinase 2 (MMP2) but not MMP9, which showed a positive correlation with aneurysm size in these patients. In contrast, Boon et al found no change in miR-29a, but an increase in miR-29b in thoracic aortic aneurysm specimens obtained from a similar patient demographic (30 patients undergoing tricuspid valve replacement vs. control samples from patients undergoing coronary artery bypass) [136]. The one difference, though, was that the former study's control group included some

samples from the aorta of heart donors, which may account for the difference, considering that coronary artery disease in the control group might have been associated with changes in miRNA expression in the aorta. In rodents, miR-29b was also upregulated in the dilated aorta of angiotensin II-treated aged mice and in Fibulin-4 knockdown mice [136]. Treatment of the former mice with antimiR-29 induced an increase in miR-29's targets elastic collagen 1A1 and 3A1 and reversed the phenotype. In this model MMP9 was decreased but the levels of MMP2 were undetermined. Marfan's syndrome, which is characterized by the development of aortic aneurysms due to a defect in connective tissue, is also associated with an increase in miR-29b and a decrease in elastin in the aorta of the Fbn1$^{C1039G/+}$ mouse model [137]. In contrast, though, MMP2, which is also a miR-29 target, was increased, suggesting that miR-29b may not be the main regulator of its expression under these conditions. Similar to the angiotensin II-treated aged model, prenatal treatment of these mice with antimiR-29 completely inhibited aortic dilation at 4 wks of age. Overall, miR-29 emerges as a major regulator of extracellular matrix protein expression with potential therapeutic utility.

## Conclusion

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

## Non-standard Abbreviations and Acronyms

| | |
|---|---|
| **3'UTR** | 3-prime untranslated region |
| **ABCA1** | adenosine triphosphate-binding cassette A1 |
| **AF** | atrial fibrillation |
| **CTGF** | connective tissue growth factor |
| **Cx** | connexin |
| **Dyrk1a** | dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1a |
| **FasL** | Fas ligand |
| **HDL** | high-density lipoprotein |
| **Hif-1α** | hypoxia-inducible factor 1 alpha |
| **HPC** | hypoxia preconditioning |
| **HUVEC** | human umbilical vein endothelial cells |
| **I/R** | ischemia/reperfusion |
| **IPC** | ischemia preconditioning |
| **Klf** | Krupple-like factor |

| | |
|---|---|
| **miRNA** | microrRNA |
| **MuRF1** | muscle ring finger 1 |
| **Myh** | myosin heavy chain |
| **NFATc** | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent |
| **PDCD4** | programmed cell death 4 |
| **PDGF** | platelet-derived growth factor |
| **PP2A** | protein phosphatase 2A |
| **PPARγ** | peroxisome proliferator-activated receptor gamma |
| **PTEN** | phosphatase and tensin homolog |
| **RyR** | ryanodine receptor |
| **SR** | sarcoplasmic reticulum |
| **SREBP** | sterol regulatory element-binding protein |
| **VSMC** | vascular smooth muscle cells |

## Acknowledgments

## References

1. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75:843–854. [PubMed: 8252621]

2. Olsen PH, Ambros V. The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking Lin-14 protein synthesis after the initiation of translation. Dev Biol. 1999; 216:671–680. [PubMed: 10642801]

3. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000; 403:901–906. [PubMed: 10706289]

4. Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. Science. 2002; 297:2056–2060. [PubMed: 12154197]

5. O'Carroll D, Mecklenbrauker I, Das PP, Santana A, Koenig U, Enright AJ, Miska EA, Tarakhovsky A. A Slicer-independent role for Argonaute 2 in hematopoiesis and the microRNA pathway. Genes Dev. 2007; 21:1999–2004. [PubMed: 17626790]

6. Meister G, Landthaler M, Patkaniowska A, Dorsett Y, Teng G, Tuschl T. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. Mol Cell. 2004; 15:185–197. [PubMed: 15260970]

7. Liu J, Rivas FV, Wohlschlegel J, Yates JR 3rd, Parker R, Hannon GJ. A role for the P-body component GW182 in microRNA function. Nat Cell Biol. 2005; 7:1261–1266. [PubMed: 16284623]

8. Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature. 2005; 436:740–744. [PubMed: 15973356]

9. Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J, Jongens TA, Nelson DL, Moses K, Warren ST. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat Neurosci. 2004; 7:113–117. [PubMed: 14703574]

10. Feng Y, Gutekunst CA, Eberhart DE, Yi H, Warren ST, Hersch SM. Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. J Neurosci. 1997; 17:1539–1547. [PubMed: 9030614]

11. Hock J, Weinmann L, Ender C, Rudel S, Kremmer E, Raabe M, Urlaub H, Meister G. Proteomic and functional analysis of Argonaute-containing mRNA-protein complexes in human cells. EMBO Rep. 2007; 8:1052–1060. [PubMed: 17932509]

12. Humphreys DT, Westman BJ, Martin DI, Preiss T. MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. Proc Natl Acad Sci U S A. 2005; 102:16961–16966. [PubMed: 16287976]

13. Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, Filipowicz W. Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science. 2005; 309:1573–1576. [PubMed: 16081698]

14. Thermann R, Hentze MW. Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. Nature. 2007; 447:875–878. [PubMed: 17507927]

15. Wu L, Fan J, Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. Proc Natl Acad Sci U S A. 2006; 103:4034–4039. [PubMed: 16495412]

16. Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P, Izaurralde E. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. Genes Dev. 2006; 20:1885–1898. [PubMed: 16815998]

17. Wakiyama M, Takimoto K, Ohara O, Yokoyama S. Let-7 microRNA-mediated mRNA deadenylation and translational repression in a mammalian cell-free system. Genes Dev. 2007; 21:1857–1862. [PubMed: 17671087]

18. Sayed D, Abdellatif M. MicroRNAs in Development and Disease. Physiol Rev. 2011; 91:827–887. [PubMed: 21742789]

19. Cheng Y, Ji R, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C. MicroRNAs are aberrantly expressed in hypertrophic heart: do they play a role in cardiac hypertrophy? Am J Pathol. 2007; 170:1831–1840. [PubMed: 17525252]

20. Ikeda S, Kong SW, Lu J, Bisping E, Zhang H, Allen PD, Golub TR, Pieske B, Pu WT. Altered microRNA expression in human heart disease. Physiol Genomics. 2007; 31:367–373. [PubMed: 17712037]

21. Matkovich SJ, Van Booven DJ, Youker KA, Torre-Amione G, Diwan A, Eschenbacher WH, Dorn LE, Watson MA, Margulies KB, Dorn GW 2nd. Reciprocal regulation of myocardial microRNAs and messenger RNA in human cardiomyopathy and reversal of the microRNA signature by biomechanical support. Circulation. 2009; 119:1263–1271. [PubMed: 19237659]

22. Naga Prasad SV, Duan ZH, Gupta MK, Surampudi VS, Volinia S, Calin GA, Liu CG, Kotwal A, Moravec CS, Starling RC, Perez DM, Sen S, Wu Q, Plow EF, Croce CM, Karnik S. Unique microRNA profile in end-stage heart failure indicates alterations in specific cardiovascular signaling networks. J Biol Chem. 2009; 284:27487–27499. [PubMed: 19641226]

23. Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. MicroRNAs play an essential role in the development of cardiac hypertrophy. Circ Res. 2007; 100:416–424. [PubMed: 17234972]

24. Sucharov C, Bristow MR, Port JD. miRNA expression in the failing human heart: functional correlates. J Mol Cell Cardiol. 2008; 45:185–192. [PubMed: 18582896]

25. Tatsuguchi M, Seok HY, Callis TE, Thomson JM, Chen JF, Newman M, Rojas M, Hammond SM, Wang DZ. Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. J Mol Cell Cardiol. 2007; 42:1137–1141. [PubMed: 17498736]

26. Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, Doevendans PA, Mummery CL, Borlak J, Haverich A, Gross C, Engelhardt S, Ertl G, Bauersachs J. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. Circulation. 2007; 116:258–267. [PubMed: 17606841]

27. van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, Richardson JA, Olson EN. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc Natl Acad Sci U S A. 2006; 103:18255–18260. [PubMed: 17108080]

28. Izumo S, Nadal-Ginard B, Mahdavi V. Protooncogene induction and reprogramming of cardiac gene expression produced in pressure overload. PNAS. 1988; 85:339–343. [PubMed: 2963328]

29. Mahdavi V, Lompre AM, Chambers AP, Nadal-Ginard B. Cardiac myosin heavy chain isozymic transitions during development and under pathological conditions are regulated at the level of mRNA availability. Eur Heart J. 1984; 5 Suppl F:181–191. [PubMed: 6241892]

30. Johnatty SE, Dyck JR, Michael LH, Olson EN, Abdellatif M. Identification of Genes Regulated During Mechanical Load-induced Cardiac Hypertrophy. J Mol Cell Cardiol. 2000; 32:805–815. [PubMed: 10775485]

31. Rane S, Sayed D, Abdellatif M. MicroRNA with a MacroFunction. Cell Cycle. 2007; 6:1850–1855. [PubMed: 17660716]

32. Ikeda S, He A, Kong SW, Lu J, Bejar R, Bodyak N, Lee KH, Ma Q, Kang PM, Golub TR, Pu WT. MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes. Mol Cell Biol. 2009; 29:2193–2204. [PubMed: 19188439]

33. Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G. MicroRNA-133 controls cardiac hypertrophy. Nat Med. 2007; 13:613–618. [PubMed: 17468766]

34. Elia L, Contu R, Quintavalle M, Varrone F, Chimenti C, Russo MA, Cimino V, De Marinis L, Frustaci A, Catalucci D, Condorelli G. Reciprocal Regulation of MicroRNA-1 and Insulin-Like Growth Factor-1 Signal Transduction Cascade in Cardiac and Skeletal Muscle in Physiological and Pathological Conditions. Circulation. 2009; 120:2377–2385. [PubMed: 19933931]

35. Liu N, Bezprozvannaya S, Williams AH, Qi X, Richardson JA, Bassel-Duby R, Olson EN. MicroRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev. 2008; 22:3242–3254. [PubMed: 19015276]

36. Matkovich SJ, Wang W, Tu Y, Eschenbacher WH, Dorn LE, Condorelli G, Diwan A, Nerbonne JM, Dorn GW 2nd. MicroRNA-133a Protects Against Myocardial Fibrosis and Modulates Electrical Repolarization Without Affecting Hypertrophy in Pressure-Overloaded Adult Hearts. Circ Res. 2009; 106:166–175. [PubMed: 19893015]

37. Beuvink I, Kolb FA, Budach W, Garnier A, Lange J, Natt F, Dengler U, Hall J, Filipowicz W, Weiler J. A novel microarray approach reveals new tissue-specific signatures of known and predicted mammalian microRNAs. Nucleic Acids Res. 2007; 35:e52-. [PubMed: 17355992]

38. 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; 316:575–579. [PubMed: 17379774]

39. van Rooij E, Quiat D, Johnson BA, Sutherland LB, Qi X, Richardson JA, Kelm RJ Jr, Olson EN. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Dev Cell. 2009; 17:662–673. [PubMed: 19922871]

40. Hagiwara N, Yeh M, Liu A. Sox6 is required for normal fiber type differentiation of fetal skeletal muscle in mice. Dev Dyn. 2007; 236:2062–2076. [PubMed: 17584907]

41. Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM, Stack C, Latimer PA, Olson EN, van Rooij E. Therapeutic Inhibition of miR-208a Improves Cardiac Function and Survival During Heart Failure. Circulation. 2011; 124:1537–1547. [PubMed: 21900086]

42. Callis TE, Pandya K, Seok HY, Tang RH, Tatsuguchi M, Huang ZP, Chen JF, Deng Z, Gunn B, Shumate J, Willis MS, Selzman CH, Wang DZ. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. J Clin Invest. 2009; 119:2772–2786. [PubMed: 19726871]

43. Morissette MR, Cook SA, Foo S, McKoy G, Ashida N, Novikov M, Scherrer-Crosbie M, Li L, Matsui T, Brooks G, Rosenzweig A. Myostatin regulates cardiomyocyte growth through modulation of Akt signaling. Circ Res. 2006; 99:15–24. [PubMed: 16763166]

44. Linhares VL, Almeida NA, Menezes DC, Elliott DA, Lai D, Beyer EC, Campos de Carvalho AC, Costa MW. Transcriptional regulation of the murine Connexin40 promoter by cardiac factors Nkx2-5, GATA4 and Tbx5. Cardiovasc Res. 2004; 64:402–411. [PubMed: 15537493]

45. Shieh JT, Huang Y, Gilmore J, Srivastava D. Elevated miR-499 levels blunt the cardiac stress response. PLoS One. 2011; 6

46. Sayed D, He M, Hong C, Gao S, Rane S, Yang Z, Abdellatif M. MicroRNA-21 is a downstream effector of AKT that mediates its antiapoptotic effects via suppression of Fas ligand. J Biol Chem. 2010; 285:20281–20290. [PubMed: 20404348]

47. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature. 2008; 456:980–984. [PubMed: 19043405]

48. Patrick DM, Montgomery RL, Qi X, Obad S, Kauppinen S, Hill JA, van Rooij E, Olson EN. Stress-dependent cardiac remodeling occurs in the absence of MicroRNA-21 in mice. J Clin Invest. 2010; 120:3912–3916. [PubMed: 20978354]

49. Ahmed MS, Oie E, Vinge LE, Yndestad A, Oystein Andersen G, Andersson Y, Attramadal T, Attramadal H. Connective tissue growth factor--a novel mediator of angiotensin II-stimulated cardiac fibroblast activation in heart failure in rats. J Mol Cell Cardiol. 2004; 36:393–404. [PubMed: 15010278]

50. Chen MM, Lam A, Abraham JA, Schreiner GF, Joly AH. CTGF expression is induced by TGF-beta in cardiac fibroblasts and cardiac myocytes: a potential role in heart fibrosis. J Mol Cell Cardiol. 2000; 32:1805–1819. [PubMed: 11013125]

51. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. Circ Res. 2009; 104:170–178. [PubMed: 19096030]

52. Castoldi G, di Gioia CR, Bombardi C, Catalucci D, Corradi B, Gualazzi MG, Leopizzi M, Mancini M, Zerbini G, Condorelli G, Stella A. MiR-133a regulates collagen 1A1: potential role of miR-133a in myocardial fibrosis in angiotensin II dependent hypertension. J Cell Physiol. 2011; 18:22939.

53. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A. 2008; 105:13027–13032. [PubMed: 18723672]

54. Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, Furth EE, Lee WM, Enders GH, Mendell JT, Thomas-Tikhonenko A. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet. 2006; 38:1060–1065. [PubMed: 16878133]

55. van Almen GC, Verhesen W, van Leeuwen RE, van de Vrie M, Eurlings C, Schellings MW, Swinnen M, Cleutjens JP, van Zandvoort MA, Heymans S, Schroen B. MicroRNA-18 and MicroRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. Aging Cell. 2011; 10:769–779. [PubMed: 21501375]

56. Lin Z, Murtaza I, Wang K, Jiao J, Gao J, Li PF. miR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy. Proc Natl Acad Sci U S A. 2009; 106:12103–12108. [PubMed: 19574461]

57. Willis MS, Ike C, Li L, Wang DZ, Glass DJ, Patterson C. Muscle ring finger 1, but not muscle ring finger 2, regulates cardiac hypertrophy in vivo. Circ Res. 2007; 100:456–459. [PubMed: 17272810]

58. Wang J, Song Y, Zhang Y, Xiao H, Sun Q, Hou N, Guo S, Wang Y, Fan K, Zhan D, Zha L, Cao Y, Li Z, Cheng X, Yang X. Cardiomyocyte overexpression of miR-27b induces cardiac hypertrophy and dysfunction in mice. Cell Res. 2011; 16:132.

59. Pineda Torra I, Gervois P, Staels B. Peroxisome proliferator-activated receptor alpha in metabolic disease, inflammation, atherosclerosis and aging. Curr Opin Lipidol. 1999; 10:151–159. [PubMed: 10327283]

60. Asakawa M, Takano H, Nagai T, Uozumi H, Hasegawa H, Kubota N, Saito T, Masuda Y, Kadowaki T, Komuro I. Peroxisome proliferator-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. Circulation. 2002; 105:1240–1246. [PubMed: 11889020]

61. Nishi H, Ono K, Horie T, Nagao K, Kinoshita M, Kuwabara Y, Watanabe S, Takaya T, Tamaki Y, Takanabe-Mori R, Wada H, Hasegawa K, Iwanaga Y, Kawamura T, Kita T, Kimura T. MicroRNA-27a regulates beta cardiac myosin heavy chain gene expression by targeting thyroid hormone receptor beta1 in neonatal rat ventricular myocytes. Mol Cell Biol. 2011; 31:744–755. [PubMed: 21149577]

62. da Costa Martins PA, Salic K, Gladka MM, Armand AS, Leptidis S, El Azzouzi H, Hansen A, Coenen-de Roo CJ, Bierhuizen MF, van der Nagel R, van Kuik J, de Weger R, de Bruin A, Condorelli G, Arbones ML, Eschenhagen T, De Windt LJ. MicroRNA-199b targets the nuclear kinase Dyrk1a in an auto-amplification loop promoting calcineurin/NFAT signalling. Nat Cell Biol. 1220; 12:1220–1227. [PubMed: 21102440]

63. Oka T, Maillet M, Watt AJ, Schwartz RJ, Aronow BJ, Duncan SA, Molkentin JD. Cardiac-specific deletion of Gata4 reveals its requirement for hypertrophy, compensation, and myocyte viability. Circ Res. 2006; 98:837–845. [PubMed: 16514068]

64. Liang Q, De Windt LJ, Witt SA, Kimball TR, Markham BE, Molkentin JD. The transcription factors GATA4 and GATA6 regulate cardiomyocyte hypertrophy in vitro and in vivo. J Biol Chem. 2001; 276:30245–30253. [PubMed: 11356841]

65. Belaguli NS, Sepulveda JL, Nigam V, Charron F, Nemer M, Schwartz RJ. Cardiac tissue enriched factors serum response factor and GATA-4 are mutual coregulators. Mol Cell Biol. 2000; 20:7550–7558. [PubMed: 11003651]

66. Hautala N, Tokola H, Luodonpaa M, Puhakka J, Romppanen H, Vuolteenaho O, Ruskoaho H. Pressure overload increases GATA4 binding activity via endothelin-1. Circulation. 2001; 103:730–735. [PubMed: 11156886]

67. van Berlo JH, Elrod JW, van den Hoogenhof MM, York AJ, Aronow BJ, Duncan SA, Molkentin JD. The transcription factor GATA-6 regulates pathological cardiac hypertrophy. Circ Res. 2010; 107:1032–1040. [PubMed: 20705924]

68. Han M, Yang Z, Sayed D, He M, Gao S, Lin L, Yoon S, Abdellatif M. GATA4 Expression is Primarily Regulated via a miR-26b-Dependent Posttranscriptional Mechanism During Cardiac Hypertrophy. Cardiovasc Res. 2012 In press.

69. Frangogiannis NG, Entman ML. Chemokines in myocardial ischemia. Trends Cardiovasc Med. 2005; 15:163–169. [PubMed: 16165012]

70. Xu C, Lu Y, Pan Z, Chu W, Luo X, Lin H, Xiao J, Shan H, Wang Z, Yang B. The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. J Cell Sci. 2007; 120:3045–3052. [PubMed: 17715156]

71. Ren XP, Wu J, Wang X, Sartor MA, Qian J, Jones K, Nicolaou P, Pritchard TJ, Fan GC. MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20. Circulation. 2009; 119:2357–2366. [PubMed: 19380620]

72. Dong S, Cheng Y, Yang J, Li J, Liu X, Wang X, Wang D, Krall TJ, Delphin ES, Zhang C. MicroRNA expression signature and the role of MicroRNA-21 in the early phase of acute myocardial infarction. J Biol Chem. 2009; 284:29514–29525. [PubMed: 19706597]

73. Cheng Y, Liu X, Zhang S, Lin Y, Yang J, Zhang C. MicroRNA-21 protects against the H(2)O(2)-induced injury on cardiac myocytes via its target gene PDCD4. J Mol Cell Cardiol. 2009; 47:5–14. [PubMed: 19336275]

74. Roy S, Khanna S, Hussain SR, Biswas S, Azad A, Rink C, Gnyawali S, Shilo S, Nuovo GJ, Sen CK. MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. Cardiovasc Res. 2009; 82:21–29. [PubMed: 19147652]

75. Wang X, Zhang X, Ren XP, Chen J, Liu H, Yang J, Medvedovic M, Hu Z, Fan GC. MicroRNA-494 targeting both proapoptotic and antiapoptotic proteins protects against ischemia/reperfusion-induced cardiac injury. Circulation. 1308; 122:1308–1318. [PubMed: 20837890]

76. Qian L, Van Laake LW, Huang Y, Liu S, Wendland MF, Srivastava D. miR-24 inhibits apoptosis and represses Bim in mouse cardiomyocytes. J Exp Med. 2011; 208:549–560. [PubMed: 21383058]

77. Hu S, Huang M, Nguyen PK, Gong Y, Li Z, Jia F, Lan F, Liu J, Nag D, Robbins RC, Wu JC. Novel MicroRNA Prosurvival Cocktail for Improving Engraftment and Function of Cardiac Progenitor Cell Transplantation. Circulation. 2011; 124:S27–S34. [PubMed: 21911815]

78. Hullinger TG, Montgomery RL, Seto AG, Dickinson BA, Semus HM, Lynch JM, Dalby CM, Robinson K, Stack C, Latimer PA, Hare JM, Olson EN, van Rooij E. Inhibition of miR-15 Protects Against Cardiac Ischemic Injury. Circ Res. 2011; 3:3.

79. Nishi H, Ono K, Iwanaga Y, Horie T, Nagao K, Takemura G, Kinoshita M, Kuwabara Y, Mori RT, Hasegawa K, Kita T, Kimura T. MicroRNA-15b modulates cellular ATP levels and degenerates mitochondria via Arl2 in neonatal rat cardiac myocytes. J Biol Chem. 2010; 285:4920–4930. [PubMed: 20007690]

80. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005; 102:13944–13949. [PubMed: 16166262]

81. Hausenloy DJ, Tsang A, Mocanu MM, Yellon DM. Ischemic preconditioning protects by activating prosurvival kinases at reperfusion. Am J Physiol Heart Circ Physiol. 2005; 288:H971–H976. [PubMed: 15358610]

82. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation. 1986; 74:1124–1136. [PubMed: 3769170]

83. Rowland RT, Meng X, Cleveland JC, Meldrum DR, Harken AH, Brown JM. Cardioadaptation induced by cyclic ischemic preconditioning is mediated by translational regulation of de novo protein synthesis. J Surg Res. 1997; 71:155–160. [PubMed: 9299284]

84. Cai Z, Zhong H, Bosch-Marce M, Fox-Talbot K, Wang L, Wei C, Trush MA, Semenza GL. Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1{alpha}. Cardiovasc Res. 2008; 77:463–470. [PubMed: 18006459]

85. Eckle T, Kohler D, Lehmann R, El Kasmi K, Eltzschig HK. Hypoxia-inducible Factor-1 is central to cardioprotection: a new paradigm for ischemic preconditioning. Circulation. 2008; 118:166–175. [PubMed: 18591435]

86. Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, Vatner DE, Vatner SF, Abdellatif M. Downregulation of MiR-199a Derepresses Hypoxia-Inducible Factor-1{alpha} and Sirtuin 1 and Recapitulates Hypoxia Preconditioning in Cardiac Myocytes. Circ Res. 2009; 104:879–886. [PubMed: 19265035]

87. Rane S, He M, Sayed D, Yan L, Vatner D, Abdellatif M. An antagonism between the AKT and beta-adrenergic signaling pathways mediated through their reciprocal effects on miR-199a-5p. Cell Signal. 2010; 22:1054–1062. [PubMed: 20193759]

88. Yin C, Salloum FN, Kukreja RC. A Novel Role of MicroRNA in Late Preconditioning. Upregulation of Endothelial Nitric Oxide Synthase and Heat Shock Protein 70. Circ Res. 2009; 104:572–575. [PubMed: 19213952]

89. Cheng Y, Zhu P, Yang J, Liu X, Dong S, Wang X, Chun B, Zhuang J, Zhang C. Ischaemic preconditioning-regulated miR-21 protects heart against ischaemia/reperfusion injury via anti-apoptosis through its target PDCD4. Cardiovasc Res. 2010; 87:431–439. [PubMed: 20219857]

90. Harris TA, Yamakuchi M, Ferlito M, Mendell JT, Lowenstein CJ. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc Natl Acad Sci U S A. 2008; 105:1516–1521. [PubMed: 18227515]

91. Guo C, Sah JF, Beard L, Willson JK, Markowitz SD, Guda K. The noncoding RNA, miR-126, suppresses the growth of neoplastic cells by targeting phosphatidylinositol 3-kinase signaling and is frequently lost in colon cancers. Genes Chromosomes Cancer. 2008; 47:939–946. [PubMed: 18663744]

92. Kuhnert F, Mancuso MR, Hampton J, Stankunas K, Asano T, Chen CZ, Kuo CJ. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. Development. 2008; 135:3989–3993. [PubMed: 18987025]

93. Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, Richardson JA, Bassel-Duby R, Olson EN. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell. 2008; 15:261–271. [PubMed: 18694565]

94. van Solingen C, Seghers L, Bijkerk R, Duijs JM, Roeten MK, van Oeveren-Rietdijk AM, Baelde HJ, Monge M, Vos JB, de Boer HC, Quax PH, Rabelink TJ, van Zonneveld AJ. Antagomir-mediated silencing of endothelial cell specific MicroRNA-126 impairs ischemia-induced angiogenesis. J Cell Mol Med. 2009; 13:1577–1585. [PubMed: 19120690]

95. van Solingen C, de Boer HC, Bijkerk R, Monge M, van Oeveren-Rietdijk AM, Seghers L, de Vries MR, van der Veer EP, Quax PH, Rabelink TJ, van Zonneveld AJ. MicroRNA-126 modulates endothelial SDF-1 expression and mobilization of Sca-1+/Lin- progenitor cells in ischaemia. Cardiovasc Res. 2011; 27:27.

96. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, Burchfield J, Fox H, Doebele C, Ohtani K, Chavakis E, Potente M, Tjwa M, Urbich C, Zeiher AM, Dimmeler S. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science. 2009; 324:1710–1713. [PubMed: 19460962]

97. Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C, Hofmann WK, Zeiher AM, Dimmeler S. Members of the MicroRNA-17-92 cluster exhibit a cell intrinsic anti-angiogenic function in endothelial cells. Blood. 2010; 2010:18.

98. Janz A, Sevignani C, Kenyon K, Ngo CV, Thomas-Tikhonenko A. Activation of the myc oncoprotein leads to increased turnover of thrombospondin-1 mRNA. Nucleic Acids Res. 2000; 28:2268–2275. [PubMed: 10871348]

99. Ngo CV, Gee M, Akhtar N, Yu D, Volpert O, Auerbach R, Thomas-Tikhonenko A. An in vivo function for the transforming Myc protein: elicitation of the angiogenic phenotype. Cell Growth Differ. 2000; 11:201–210. [PubMed: 10775037]

100. Tikhonenko AT, Black DJ, Linial ML. Viral Myc oncoproteins in infected fibroblasts down-modulate thrombospondin-1, a possible tumor suppressor gene. J Biol Chem. 1996; 271:30741–30747. [PubMed: 8940053]

101. Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, Galuppo P, Kneitz S, Pena JT, Sohn-Lee C, Loyer X, Soutschek J, Brand T, Tuschl T, Heineke J, Martin U, Schulte-Merker S, Ertl G, Engelhardt S, Bauersachs J, Thum T. MicroRNA-24 Regulates Vascularity After Myocardial Infarction. Circulation. 2011; 2011:25.

102. Lal A, Pan Y, Navarro F, Dykxhoorn DM, Moreau L, Meire E, Bentwich Z, Lieberman J, Chowdhury D. miR-24-mediated downregulation of H2AX suppresses DNA repair in terminally differentiated blood cells. Nat Struct Mol Biol. 2009; 16:492–498. [PubMed: 19377482]

103. Ghosh G, Subramanian IV, Adhikari N, Zhang X, Joshi HP, Basi D, Chandrashekhar YS, Hall JL, Roy S, Zeng Y, Ramakrishnan S. Hypoxia-induced MicroRNA-424 expression in human endothelial cells regulates HIF-alpha isoforms and promotes angiogenesis. J Clin Invest. 2010; 120:4141–4154. [PubMed: 20972335]

104. Pause A, Lee S, Worrell RA, Chen DY, Burgess WH, Linehan WM, Klausner RD. The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. Proc Natl Acad Sci U S A. 1997; 94:2156–2161. [PubMed: 9122164]

105. Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H, Harris AL, Gleadle JM, Ragoussis J. hsa-miR-210 Is induced by hypoxia and is an independent prognostic factor in breast cancer. Clin Cancer Res. 2008; 14:1340–1348. [PubMed: 18316553]

106. Fasanaro P, D'Alessandra Y, Di Stefano V, Melchionna R, Romani S, Pompilio G, Capogrossi MC, Martelli F. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J Biol Chem. 2008; 283:15878–15883. [PubMed: 18417479]

107. Hu S, Huang M, Li Z, Jia F, Ghosh Z, Lijkwan MA, Fasanaro P, Sun N, Wang X, Martelli F, Robbins RC, Wu JC. MicroRNA-210 as a novel therapy for treatment of ischemic heart disease. Circulation. 2010; 122:S124–S131. [PubMed: 20837903]

108. Katare R, Riu F, Mitchell K, Gubernator M, Campagnolo P, Cui Y, Fortunato O, Avolio E, Cesselli D, Beltrami AP, Angelini G, Emanueli C, Madeddu P. Transplantation of Human Pericyte Progenitor Cells Improves the Repair of Infarcted Heart Through Activation of an Angiogenic Program Involving Micro-RNA-132. Circ Res. 2011; 109:894–906. [PubMed: 21868695]

109. Lazzara R, El-Sherif N, Hope RR, Scherlag BJ. Ventricular arrhythmias and electrophysiological consequences of myocardial ischemia and infarction. Circ Res. 1978; 42:740–749. [PubMed: 350449]

110. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G, Wang Z. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med. 2007; 13:486–491. [PubMed: 17401374]

111. Lerner DL, Yamada KA, Schuessler RB, Saffitz JE. Accelerated onset and increased incidence of ventricular arrhythmias induced by ischemia in Cx43-deficient mice. Circulation. 2000; 101:547–552. [PubMed: 10662753]

112. Plaster NM, Tawil R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, Donaldson MR, Iannaccone ST, Brunt E, Barohn R, Clark J, Deymeer F, George AL Jr, Fish FA, Hahn A, Nitu A, Ozdemir C, Serdaroglu P, Subramony SH, Wolfe G, Fu YH, Ptacek LJ. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. Cell. 2001; 105:511–519. [PubMed: 11371347]

113. Terentyev D, Belevych AE, Terentyeva R, Martin MM, Malana GE, Kuhn DE, Abdellatif M, Feldman DS, Elton TS, Gyorke S. 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; 104:514–521. [PubMed: 19131648]

114. Ai X, Curran JW, Shannon TR, Bers DM, Pogwizd SM. Ca2+/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca2+ leak in heart failure. Circ Res. 2005; 97:1314–1322. [PubMed: 16269653]

115. Lehnart SE, Wehrens XH, Laitinen PJ, Reiken SR, Deng SX, Cheng Z, Landry DW, Kontula K, Swan H, Marks AR. Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. Circulation. 2004; 109:3208–3214. [PubMed: 15197150]

116. Belevych AE, Sansom SE, Terentyeva R, Ho HT, Nishijima Y, Martin MM, Jindal HK, Rochira JA, Kunitomo Y, Abdellatif M, Carnes CA, Elton TS, Gyorke S, Terentyev D. MicroRNA-1 and-133 Increase Arrhythmogenesis in Heart Failure by Dissociating Phosphatase Activity from RyR2 Complex. PLoS One. 2011; 6:6.

117. Lu Y, Zhang Y, Wang N, Pan Z, Gao X, Zhang F, Shan H, Luo X, Bai Y, Sun L, Song W, Xu C, Wang Z, Yang B. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. Circulation. 2010; 122:2378–2387. [PubMed: 21098446]

118. Vallett SM, Sanchez HB, Rosenfeld JM, Osborne TF. A direct role for sterol regulatory element binding protein in activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene. J Biol Chem. 1996; 271:12247–12253. [PubMed: 8647822]

119. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, Naar AM. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science. 2010; 328:1566–1569. [PubMed: 20466882]

120. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Genest J Jr, Hayden MR. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet. 1999; 22:336–345. [PubMed: 10431236]

121. Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, van Gils JM, Rayner AJ, Chang AN, Suarez Y, Fernandez-Hernando C, Fisher EA, Moore KJ. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. J Clin Invest. 2011; 121:2921–2931. [PubMed: 21646721]

122. Rayner KJ, Esau CC, Hussain FN, McDaniel AL, Marshall SM, van Gils JM, Ray TD, Sheedy FJ, Goedeke L, Liu X, Khatsenko OG, Kaimal V, Lees CJ, Fernandez-Hernando C, Fisher EA, Temel RE, Moore KJ. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature. 2011; 478:404–407. [PubMed: 22012398]

123. Xin M, Small EM, Sutherland LB, Qi X, McAnally J, Plato CF, Richardson JA, Bassel-Duby R, Olson EN. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev. 2009; 23:2166–2178. [PubMed: 19720868]

124. Boettger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L, Braun T. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Invest. 2009; 119:2634–2647. [PubMed: 19690389]

125. Elia L, Quintavalle M, Zhang J, Contu R, Cossu L, Latronico MV, Peterson KL, Indolfi C, Catalucci D, Chen J, Courtneidge SA, Condorelli G. The knockout of miR-143 and-145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. Cell Death Differ. 2009; 16:1590–1598. [PubMed: 19816508]

126. Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, Deitch EA, Huo Y, Delphin ES, Zhang C. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circ Res. 2009; 105:158–166. [PubMed: 19542014]

127. Watanabe N, Kurabayashi M, Shimomura Y, Kawai-Kowase K, Hoshino Y, Manabe I, Watanabe M, Aikawa M, Kuro-o M, Suzuki T, Yazaki Y, Nagai R. BTEB2, a Kruppel-like transcription factor, regulates expression of the SMemb/Nonmuscle myosin heavy chain B (SMemb/NMHC-B) gene. Circ Res. 1999; 85:182–191. [PubMed: 10417400]

128. Suzuki T, Sawaki D, Aizawa K, Munemasa Y, Matsumura T, Ishida J, Nagai R. Kruppel-like factor 5 shows proliferation-specific roles in vascular remodeling, direct stimulation of cell growth, and inhibition of apoptosis. J Biol Chem. 2009; 284:9549–9557. [PubMed: 19189969]

129. Torella D, Iaconetti C, Catalucci D, Ellison GM, Leone A, Waring CD, Bochicchio A, Vicinanza C, Aquila I, Curcio A, Condorelli G, Indolfi C. MicroRNA-133 Controls Vascular Smooth Muscle Cell Phenotypic Switch In Vitro and Vascular Remodeling In Vivo. Circ Res. 2011; 109:880–893. [PubMed: 21852550]

130. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. Induction of MicroRNA-221 by platelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype. J Biol Chem. 2009; 284:3728–3738. [PubMed: 19088079]

131. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. Circ Res. 2009; 104:476–487. [PubMed: 19150885]

132. Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ Res. 2007; 100:1579–1588. [PubMed: 17478730]

133. Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology. 2006; 130:2113–2129. [PubMed: 16762633]

134. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007; 133:647–658. [PubMed: 17681183]

135. Jones JA, Stroud RE, O'Quinn EC, Black LE, Barth JL, Elefteriades JA, Bavaria JE, Gorman JH 3rd, Gorman RC, Spinale FG, Ikonomidis JS. Selective microRNA Suppression in Human Thoracic Aneurysms: Relationship of miR-29a to Aortic Size and Proteolytic Induction. Circ Cardiovasc Genet. 2011; 18:18.

136. Boon RA, Seeger T, Heydt S, Fischer A, Hergenreider E, Horrevoets AJ, Vinciguerra M, Rosenthal N, Sciacca S, Pilato M, van Heijningen P, Essers J, Brandes RP, Zeiher AM, Dimmeler S. MicroRNA-29 in aortic dilation: implications for aneurysm formation. Circ Res. 2011; 109:1115–1119. [PubMed: 21903938]

137. Merk DR, Chin JT, Dake BA, Maegdefessel L, Miller MO, Kimura N, Tsao PS, Iosef C, Berry GJ, Mohr FW, Spin JM, Alvira CM, Robbins RC, Fischbein MP. miR-29b Participates in Early Aneurysm Development in Marfan Syndrome. Circ Res. 2011; 23:23.
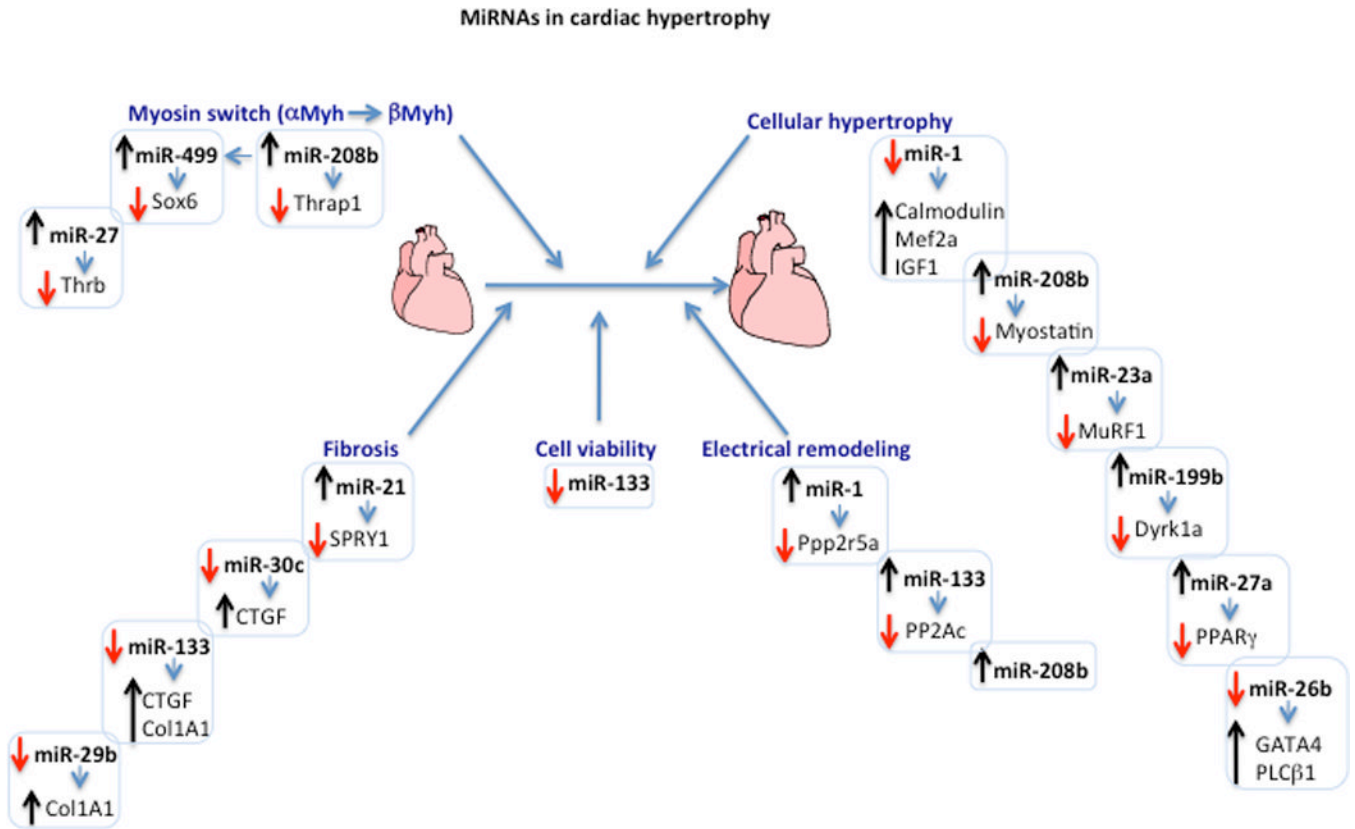
**Figure 1. A diagram showing miRNAs and their targets in cardiac hypertrophy**
The diagram displays the different miRNAs and their targets that are involved in gene switching, cellular hypertrophy, fibrosis, and electrical remodeling during cardiac hypertrophy. Upregulation or downregulation of a specific miRNA is represented by an upward (black) or a downward (red) arrow, respectively. The change in the expression levels of target genes inversely correlates with that of the targeting miRNA and is similarly represented by an up or down arrow. All listed targets have been validated. The listed targets include: thyroid hormone receptor-associated protein 1 (Thrap1), SRY-box containing gene 6 (Sox6), thyroid hormone receptor beta (Thrb), calmodulin, myocyte enhancer factor 2A (Mef2a), insulin-like growth factor 1 (IGF1), myostatin, muscle-specific RING finger protein 1 (MuRF1), dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (Dyrk1a), sprouty 2/4 (SPRY2/4), connective tissue growth factor (CTGF), collagen IA1 (Col1A1), GATA binding protein 4 (GATA4), phospholipase C beta 1 (PLCβ1), protein phosphatase 2, regulatory subunit B (B56) alpha isoform (Ppp2r5a), protein phosphatase 2 catalytic subunit (PP2Ac) and, the indirect targets alpha and beta myosin heavy chain (αand βMyh),

MiRNAs in cardiac ischemia
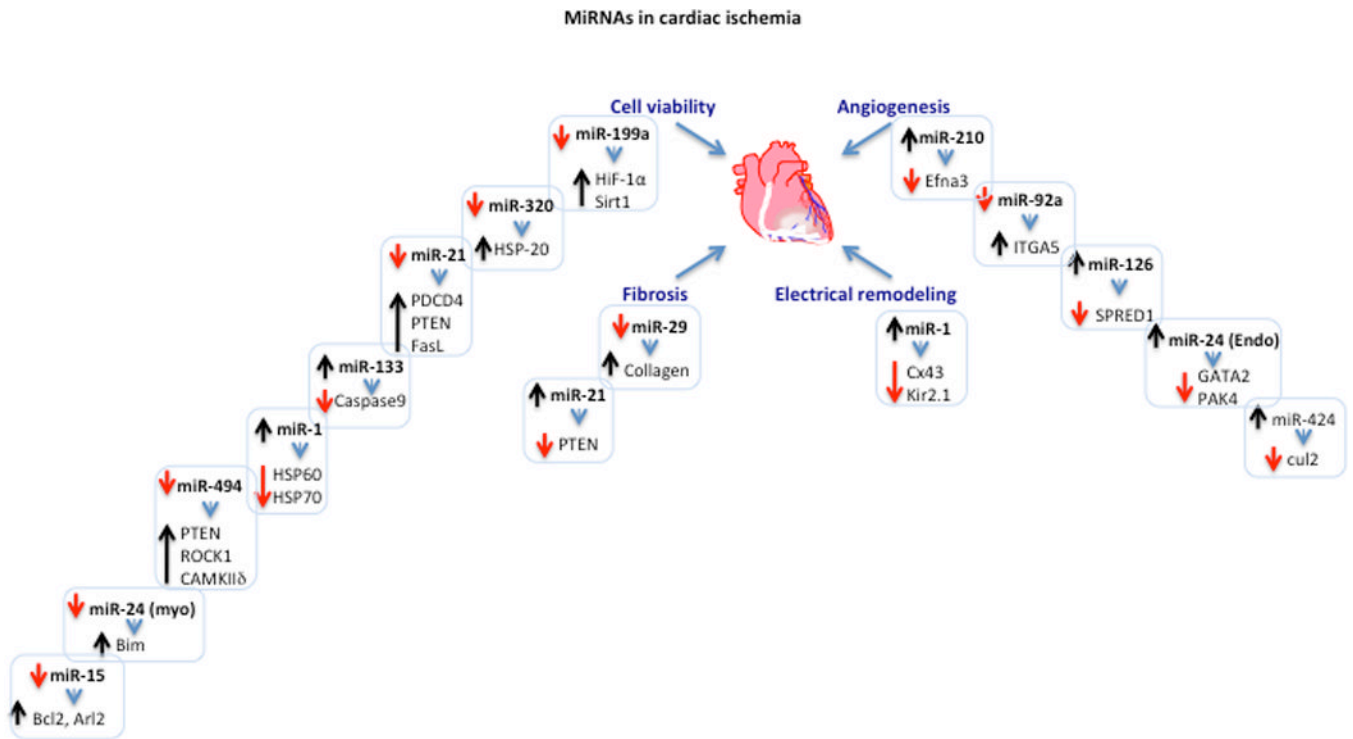


**Figure 2. A diagram showing miRNAs and their targets in cardiac ischemia**
The diagram displays the different miRNAs and their targets that are involved in cell
viability, angiogenesis, fibrosis, and electrical remodeling during cardiac ischemia.
Upregulation or downregulation of a specific miRNA is represented by an upward (black) or
a downward (red) arrow, respectively. The change in the expression levels of target genes
inversely correlates with that of the targeting miRNA and is similarly represented by an up
or down arrow. All listed targets have been validated. The listed targets include: hypoxia-
inducible factor 1 alpha (Hif-1α), sirtuin 1 (Sirt1), heat shock protein 20, 60, and 70 (HSP20,
HSP60, HSP70), programmed cell death 4 (PDCD4), phosphatase and tensin homolog
(PTEN), fas ligand (FasL), caspase 9, Rho-associated coiled-coil containing protein kinase 1
(Rock1), calmodulin kinase II delta (CAMKIIδ), BCL2-like 11 (Bim), ADP-ribosylation
factor-like 2 (Arl2), B-cell CLL/lymphoma 2 (Bcl2), ephrin A3 (Ephna3), integrin, alpha 5
(ITGA5), sprouty-related, EVH1 domain containing 1 (SPRED1), GATA binding protein 2
(GATA2), p21 protein (Cdc42/Rac)-activated kinase 4 (PAK4), cullin 2 (cul2), connexin 43
(Cx43), inward rectifier potassium channel 2 (Kir2.1).