Minireview

microRNAs in hypertrophy and heart failure

Michael V G Latronico[1] and Gianluigi Condorelli[2,3]

[1]Scientific and Technological Park, Casa di Cura MultiMedica – Istituto di Ricovero e Cura a Carattere Scientifico; [2]Department of Medicine, Consiglio Nazionale delle Ricerche, Milan 20138, Italy; [3]Department of Medicine, University of California San Diego, La Jolla, CA 92093-0613, USA

Corresponding author: Gianluigi Condorelli, Department of Medicine, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0613, USA. Emails: gcondorelli@ucsd.edu; gcondorelli@yahoo.com

Abstract

microRNAs (miRNAs) are highly conserved, short non-coding RNAs that have become established as having an important role in regulatory networks. We review here the progress made in our understanding of heart-related microRNAs over the last two years, focusing mainly on hypertrophic growth and heart failure.

Keywords: cardiac muscle, microRNA, gene expression, cardiovascular disease, heart hypertrophy, heart failure

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Introduction

microRNAs (miRNAs or miRs) are short, highly conserved, non-coding RNAs that have become established as having an important role in regulatory networks. They participate in a wide range of biological processes, including cardiovascular development and pathophysiology.[1] Briefly, miRNAs negatively regulate gene expression post-transcriptionally by directing protein complexes to binding sites present on the 3′ untranslated region (UTR) of targeted mRNAs, suppressing translation and/or inducing mRNA degradation. We outline here the progress made in our understanding of heart-related miRNA over the last two years, focusing mainly on the new targets reported for hypertrophic growth and heart failure (Table 1). Recent updates focusing on the role of miRNA in myocardial infarction[2] and heart development[3] can be found elsewhere.

microRNA profiling studies

Rao *et al.*[4] examined the miRNA signature of adult mouse heart using deep sequencing, a technology with unparalleled throughput that allows more accurate and sensitive genome-wide profiling of miRNAs than more conventional methods such as microarrays. They found that the miR-1/miR-206, let-7/miR-98 and miR-30 families were the most abundant families in cardiac tissue. miR-1 accounted for nearly 40% of all known miRNA reads in cardiac tissue and might be present in an even greater proportion in the cardiomyocyte fraction; some miRNAs, including miR-29a, miR-26a and let-7 family members, were more abundant than miR-133a, which is found in a clustered, bicistronic unit with miR-1. Another muscle-specific miRNA, miR-208a, which is encoded within an intron of myosin heavy chain 6 (*Myh6*) and, therefore, should presumably be highly expressed in heart, was also much less abundant than miR-1. These findings provided further indirect evidence that as yet unknown post-transcriptional mechanisms govern the stability of miRNAs. Rao *et al.*[4] also reported that cardiac-specific knockout of *Dgcr8* – the gene encoding the dsRNA-binding RNA polymerase partner of Drosha, the enzyme involved in miRNA maturation in the nucleus – was responsible for the generation of a fully penetrant and dilated heart phenotype in adult mice. Lethality was probably due to ectopic expression of fast skeletal muscle genes – which determine a deterioration of cardiac function – resulting from the downregulation of a limited number of miRNAs that are particularly important in the maintenance of cardiac muscle phenotype.

In another genome-wide profiling study – this time using microarray technology with probes for 288 human miRNAs and subsequent validation with semi-quantitative realtime–polymerase chain reaction (PCR) – that analyzed myocardium from end-stage dilated cardiomyopathy (DCM) patients, Naga Prasad *et al.*[5] found eight significantly dysregulated miRNAs in heart failure: miR-1, miR-7, miR-29b and miR-378 were downregulated, while miR-181b, miR-125b, miR-214 and miR-342 were upregulated in heart failure patients. Because miR-7, miR-181b, miR-214 and miR-378 were found to be dysregulated during the initial states of cardiac dysfunction in the heart of pressure-overloaded mice, these miRNAs were regarded as being causative, rather than an effect, of pathology. They then used bioinformatics to highlight the signaling networks regulated by the

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Table 1 Recently found dysregulated miRNA targets involved in cardiac hypertrophy and heart failure (HF)

microRNA	Dysregulation of the miRNA upon hypertrophy and/or HF	Targeted mRNA(s)	Function of target's protein	Model(s)/ pathology	Refs
miR-1	↓	Calmodulin-1 and −2; Myocyte enhancer factor-2	Prohypertrophic signaling	Cn-Tg	6
miR-1	↓	Insulin-like growth factor 1 (Igf1); Igf1 receptor	Prohypertrophic signaling	m, POH; AKT-Tg; h, AM	7
miR-1	↓	Twinfilin 1	Cytoskeleton regulation	r, PHE; r, POH	10
miR-133	↓	Cyclin D2	Cell cycle control	miR-133-dKO	11
miR-133	↓	Calcineurin	Prohypertrophic signaling	m, POH	15
miR-133	↓	Nuclear factor of activated T cells 4	Prohypertrophic signaling	r, PHE	16
miR-133; miR-30	↓	Connective tissue growth factor	Profibrotic signaling	m, POH; Ren2-Tg; h, AO	17
miR-133; miR-590	↓	Transforming growth factor β (Tgfb); Tgfb receptor 2	Profibrotic signaling	d, NIF; h, AF	18
miR-208b	↑	Thyroid hormone-associated protein 1; myostatin	Antihypertrophic signaling	m, POH	21
miR-208; miR-499	n/a	Sox6; Purβ; Sp3; HP-1β	Transcriptional repressors	miR-208a-KO	22
miR-30	n/a	Protein 53	Proapoptotic signaling	r, HOS	23
miR-23a	↑	Muscle specific ring finger protein 1	Antihypertrophic signaling	r, ISO/ALD; m, POH	25
miR-9	↓	Myocardin	Prohypertrophic transcriptional coactivator	r, ISO/ALD; m, IIH	26
miR-199a	↑	Hypoxia-inducible factor 1 alpha	Transcription factor for hypoxia-related genes	m, POH; βAR-Tg	27,30

↓, downregulated; ↑, upregulated; n/a, not applicable to the model used; Cn-Tg, transgenic calcineurin mouse model of heart failure; m, mouse; POH, pressure overload hypertrophy (through either transverse or abdominal aortic constriction); AKT-Tg, transgenic AKT mouse model of hypertrophy; h, humans; AM, acromegaly; r, rat; PHE, phenylephrine treatment of cultured cardiomyocytes; dKO, double-knockout mouse; Ren2-Tg, rat with inducible expression of the Ren2 renin gene; AO, aortic stenosis; d, dog; NIF, nicotine-induced fibrosis; AF, atrial fibrillation; KO, knockout mouse; HOS, hydrogen peroxide-induced oxidative stress; ISO/ALD, isoproterenol or aldosterone treatment of cultured cardiomyocytes; IHH, isoproterenol-induced hypertrophy of the heart; βAR-Tg, beta-adrenergic transgenic mouse model of heart failure

predicted targets of these eight miRNAs and to identify nodal molecules, i.e. molecules that occupy key positions in the signaling networks and that could account for the global changes associated with heart failure if dysregulated. Of the 1785 predicted targets found with bioinformatics for the differentially expressed miRNA genes, 1716 mapped to a total of 75 different signaling networks. The top canonical functional networks found affected with the highest level of significance were related to cardiovascular development and function, such as the NFκB signaling network. Interestingly, NFκB-related pathways were targeted by the dysregulated miRNAs at multiple peripheral points rather than just at the nodal molecule NFκB itself. Moreover, >40 of these networks were predicted to cross-talk with each other. These results give a hint of the intricate manner in which cardiac miRNAs are integrated into complex regulatory networks and how dysregulation of a limited number of miRNAs can effect multiple processes in ways that are conducive to the establishment of cardiac dysfunction.

The main cardiac expressed microRNAs

miR-1

Following mechanical and neurohormonal stimuli, calmodulin (Calm) binds to Ca^{2+} and activates the phosphatase calcineurin (Cn). Activated Cn dephosphorylates nuclear factor of activated T-cells (Nfat), which then translocates to the nucleus where – in cooperation with myocyte enhancer factor 2 (Mef2), GATA binging protein 4 (Gata4) and other transcription factors – it initiates gene expression. Inappropriate activation of the Ca^{2+}/Calm-Cn-Nfat axis is a key mechanism underlying cardiac hypertrophy and heart failure. Because nearly all Calm in the cardiomyocyte is protein bound, Calm levels are limiting and precisely control the regulation of signaling from Ca^{2+}. Maybe not surprisingly, Calm was recently reported to be regulated post-transcriptionally by miRNAs.[6] Computational algorithms identified *Calm1* and *Calm2* – the mRNAs of the two isoforms that comprise the great majority of Calm-encoding transcripts in the heart – as well as *Mef2* as having highly conserved miR-1 recognition sequences in their 3′UTRs. When these 3′UTRs were cloned downstream of a luciferase reporter, cotransfection of miR-1 caused downregulation of the luciferase activity. Moreover, adenovirally mediated overexpression of miR-1 in cultured rat cardiomyocytes downregulated the expression of Calm and Mef2 and inhibited an Nfat-dependent luciferase reporter. These findings were corroborated in an *in vivo* heart-failure model, the Cn-overexpressing mouse. At two months of age, a time at which these mice have severe systolic dysfunction, Calm and Mef2 were found to be significantly upregulated, while miR-1 was significantly downregulated. Gata4 was also found to be upregulated in Cn transgenic mouse heart, but its mRNA was not found to harbor any miR-1 binding sites, suggestive of indirect modulation of Gata4 expression by miR-1.

Insulin-like growth factor 1 (*Igf1*) and Igf1 receptor were also reported to be targets of miR-1.[7] miR-1 and Igf1 levels were inversely correlated in models of cardiac

hypertrophy and failure as well as in the C2C12 skeletal muscle-cell model of differentiation. The activation state of the Igf1 signal transduction cascade was found to reciprocally regulate miR-1 expression through the forkhead box O3a (FoxO3a) transcription factor. Interestingly, miR-1 expression correlated inversely with heart mass and chamber wall thickness in patients with acromegaly, a syndrome in which IGF1 is overproduced by aberrant synthesis of growth hormone. Thus, miR-1 has a critical role in mediating the effects of the Igf1 pathway in that it is part of a feedback loop controlling this signal transduction cascade.

miR-1 was also inversely correlated with expression of the antiapoptotic protein Bcl-2 in cardiomyocytes from ischemia/reperfused (I/R) rats – a model of acute ischemic heart disease (IHD) generated by transitorily ligating a coronary artery:[8] after I/R, miR-1 was found significantly increased, while Bcl-2 decreased. *In vitro*, exposure to hydrogen peroxide (H_2O_2) – which simulates oxidative stress and induces apoptosis – dramatically increased expression of miR-1 in cardiomyocytes. Overexpression of miR-1 facilitated H_2O_2-induced apoptosis in cardiomyocytes, whereas inhibition of miR-1 with an antisense inhibitory oligonucleotide caused marked resistance to H_2O_2. Bioinformatics predicted potential target sites for miR-1 on the 3′UTR of *Bcl*2, and overexpressing miR-1 in cultured cardiomyocytes significantly reduced the expression of Bcl-2 mRNA and protein. The role of miRNA in apoptosis has been reviewed extensively elsewhere.[9]

The mRNA of twinfilin1 (*Twf1*) was identified recently as another novel target of miR-1.[10] Twf1 is a cytoskeleton regulatory protein that binds to actin monomers, preventing their assembly into filaments. Expression of Twf1 is low in the heart, Twf2 being the major isoform in striated muscle. *Twf1* was found through bioinformatic algorithms to have two miR-1 binding sites. Overexpression of miR-1 significantly reduced the luciferase activity of a chimera reporter containing the 3′UTR of *Twf1*, and hypertrophic cardiomyocytes were found to express more of its protein. Interestingly, overexpression of Twf1 was sufficient to cause hypertrophy of cardiomyocytes *in vitro*. Therefore, miR-1 suppresses hypertrophy under basal conditions also by acting directly on cytoskeletal components.

miR-133

The miR-133 family comprises miR-133a-1, miR-133a-2 and miR-133b. miR-133a-1 and miR-133a-2 are identical miRNAs encoded by distinct genes (found, respectively, on chromosomes 18 and 2 in mouse) and differ by only two nucleotides from miR-133b (which is not found in cardiac muscle). miR-133a is regulated during muscle development by the transcription factor serum response factor (Srf)[11] and was found to regulate hypertrophy.[12] Deletion of either one of the genes encoding miR-133a was tolerated in mice, whereas deletion of both resulted in late embryonic or neonatal lethality: in the heart of double-knockout mouse, ventricular-septal defects, upregulation of cardiomyocyte proliferation and apoptosis, and ectopic expression of Srf-targeted smooth muscle genes, such as smooth muscle α-actin, transgelins and caldesmon 1, were found.[13] Approximately one in four double-knockout mice, however, survived to adulthood: these mice had severe deficits in cardiac contractility and died from heart failure. Heart defects were attributed, at least in part, to inappropriate expression of Srf and cyclin D2 (Ccnd2), the mRNAs of which were found to be direct targets of miR-133a. Thus, miR-133a-1 and miR-133a-2 redundantly regulate the gene expression programs required for normal cardiac growth and function, inhibiting expression of non-cardiac genes.

miR-133a has also been experimentally overexpressed in the heart.[14] Increasing miR-133a (13-fold) in postnatal mouse heart was not found to alter normal cardiac growth, but did significantly decrease density of the fast component of the transient outward potassium repolarizing current, $I_{to,f}$, and modestly increase QT duration. Only 1.5% of >22,000 assayed mRNAs were found to be dysregulated in miR-133a-overexpressing heart: of these dysregulated mRNAs, approximately half were downregulated, indicating that translational silencing rather than mRNA destabilization is the dominant miRNA mechanism in animals. Pressure overload – produced by partially constricting the aorta in order to increase hemodynamic afterload and, therefore, increase the workload imposed on the heart – causes hypertrophy, fibrosis, downregulation of miR-133 and eventually heart failure in wildtype mice. When transgenic miR-133a mice were subjected to pressure overload, there was no downregulation of miR-133a and less myocardial fibrosis and diastolic dysfunction; however, hypertrophy was not blunted, nor was the mRNA profile change that usually occurs with hypertrophy substantially different from that of wildtype mice. Downregulation of $I_{to,f}$, which also usually occurs with pressure overload, was prevented in miR-133a transgenic mice, although action potential duration and QT intervals did not reflect this benefit. Intriguingly, many of the published targets of miR-133a were not found to be downregulated in transgenic mice heart, for example Srf, caspase 9 and Ccnd2; Ctgf (see below) was even found increased. KChIP2 – the accessory subunit of the potassium channel responsible for $I_{to,f}$ – was found decreased, but its mRNA did not harbor miR-133a binding sites, indicating an indirect mechanism linking it to miR-133a.

miR-133 was demonstrated to target Cn, too.[15] Co-transfection of miR-133 and a luciferase reporter gene linked to the wildtype 3′UTR of Cn resulted in a significant decrease of luciferase activity, and co-application of miR-133 with a miR-133 antisense inhibitory oligoribonucleotide (AMO-133) alleviated the decrease of luciferase activity. In cultured cardiomyocytes, transfection of AMO-133 significantly increased both mRNA and protein levels of Cn, whereas transfection of miR-133 decreased the elevated protein and mRNA levels of Cn induced by treatment with phenylephrine (PHE), a prohypertrophic α-adrenergic receptor agonist. Thus, the normally high expression of miR-133 found in the heart inhibits hypertrophy under usual conditions; however, when miR-133 is downregulated, such as with pressure overload, post-transcriptional repression of Cn mRNA is lifted, expression of its protein is increased and hypertrophy can be induced through nuclear translation of Nfat. Moreover, similarly to Srf, Cn was found to be not

only a target but also a regulator of miR-133. In fact, the dysregulated level of miR-133, but not of other miRNAs, occurring in cardiomyocytes *in vivo* after pressure overload and *in vitro* after exposure to PHE was normalized with concomitant administration of the Cn inhibitor cyclosporine A. In addition, an antisense oligodeoxynucleotide directed against the catalytic subunit of Cn and an Nfat-specific decoy oligodeoxynucleotide were both capable of increasing the expression of miR-133 in cultured cardiomyocytes.

Almost contemporaneously, miR-133 was demonstrated to target Nfatc4 mRNA, but not that of Nfatc2 or Nfatc3[16] Two conserved base-pairing sites between the *Nfatc4* 3′UTR and miR-133a were found and verified with luciferase reporter experiments. Adenovirally mediated transduction of miR-133a reduced Nfatc4 mRNA and protein levels and blocked hypertrophy of PHE-treated cultured neonatal cardiomyocytes.

miR-133, along with miR-30c – another highly expressed miRNA in the heart – was also reported to target the mRNA of connective tissue growth factor (Ctgf), a profibrotic protein.[17] Cardiomyocytes produce Ctgf in response to pathological stimuli, such as pressure overload, and thereby participate in fibrosis by providing the signal to surround themselves with increased extracellular matrix. Cardiac expression of miR-133 and miR-30 was observed to decrease in the Ren2 rat – a model of hypertension-induced heart failure – in mice subjected to transverse aortic constriction, and in patients with left ventricular hypertrophy caused by aortic stenosis.[17] Downregulation of these two miRNAs, and the consequential lifting of post-transcriptional inhibition on Ctgf, was suggested to be a contributory cause of fibrosis, rather than a secondary effect, because it was observed early on, before any signs of loss of heart function.

In addition, miR-133 was reported to be involved in another profibrotic pathway, this time in conjunction with miR-590.[18] Chronic nicotine abuse has been linked to the development and progression of atherosclerosis, myocardial infarction, heart failure and the occurrence of cardiac arrhythmias. Exposure of cardiac fibroblasts to nicotine was found to cause the upregulation of transforming growth factor (Tgf)-β1 and Tgf-β receptor type II, and the downregulation of miR-133 and miR-590. These effects were abrogated by co-treatment with the nicotinic acetylcholine receptor antagonist α-bungarotoxin. The findings were corroborated *in vivo* in nicotine-infused dogs – a model of atrial fibrillation – and in heart samples from smokers with a history of atrial fibrillation. *Tgfb1* and *Tgfbr2* were found to harbor multiple binding sites for miR-133 and miR-590, respectively. 3′UTR-luciferase-reporter chimera assays carried out in HEK293 cells confirmed that miR-133 and miR-590 were capable of binding to and repressing their corresponding targets. A more in-depth review on the regulation of fibrosis by miRNA can be found elsewhere.[19]

miR-208

This miRNA, encoded by an intron of the fast myosin heavy chain gene *Myh6*, was demonstrated to be required for stress-dependent cardiac growth.[20] The related miRNA miR-208b, which differs from miR-208a by only three nucleotides at the 3′ end, was also found to be encoded in an intron of a myosin gene, in this case that of the slow myosin *Myh7*.[21] In mouse heart, myosin-7 is fetal specific, whereas a switch toward the adult isoform, myosin-6, occurs shortly after birth. A similar switch from miR-208b to miR-208a was also found to occur, suggesting that these miRNAs are transcribed with their host genes and, thus, constitute protein–gene/miRNA–gene units. Treatment of neonatal rat cardiomyocytes with thyroid hormone repressed expression of the *Myh7/miR-208b* unit and activated that of the *Myh6/miR-208a* unit. In addition, transgenic overexpression of miR-208a in adult mouse heart was observed to be sufficient to induce hypertrophic growth. Cardiac expression of miR-208b was found to be induced both in miR-208a transgenic mice and in mice subjected to pressure overload, in agreement with re-expression of myosin-7 being a characteristic of hypertrophy. However, in miR-208a transgenic mice re-expression of myosin-7 was found only in areas of the heart associated with interstitial fibrosis.[21] Thus, miR-208a overexpression induces hypertrophy independently of re-activation of *Myh7*. Thyroid hormone-associated protein 1 and myostatin, two negative regulators of muscle growth and hypertrophy, were found to be upregulated. Furthermore, miR-208a was found to target Gata4 and to be necessary for proper cardiac conduction.

Similarly to *Myh6* and *Myh7*, a third myosin gene, *Myh7b*, was also established to contain an intronic miRNA, identified as miR-499.[22] These three protein–gene/miRNA–gene units (i.e. *Myh6/miR-208a*, *Myh7/miR-208b* and *Myh7b/miR-499*) were discovered to be involved in a network regulating muscle function. Myh6 is a fast myosin that constitutes most of the myosin protein expressed in adult mouse heart. miR-208a, the miRNA co-expressed with Myh6, is also highly expressed and was found to precisely control the levels of expression of the slow myosins Myh7 and Myh7b and, consequently, of miR-208b and miR-499. miR-208b and miR-499 were found to play roles in the specification of muscle fiber identity by activating slow and repressing fast myofiber gene programs. Several transcriptional repressors known to be involved in myosin gene expression were identified and validated as miR-208 and miR-499 targets. The authors concluded that myosin genes not only encode the major contractile proteins of muscle, but can influence myofiber identity and, thus, muscle performance through the expression of a network of co-expressed miRNAs.

miR-30

Apoptosis is increased in hypertrophic myocardium and contributes to the progression to heart failure. The fusion of mitochondria can inhibit apoptosis, whereas mitochondrial fission – which requires the activation of dynamin-related protein-1 (Drp1), a GTPase that causes scission of the mitochondrial outer membrane – is involved in the initiation of apoptosis. In rat cardiomyocytes *in vitro*, H_2O_2 was demonstrated to upregulate p53, a protein that transcriptionally regulates proapoptotic proteins.[23] p53 was found to bind to the promoter region of *Drp1* and, therefore, increased expression of p53 could initiate mitochondrial

fission. miR-30 family members were found downregulated in H_2O_2-treated cells, and this miRNA family was demonstrated to target and suppress expression of p53. Therefore, downregulation of miR-30 is proapoptotic. Incidentally, miR-30 members have been found reduced in heart failure,[6] so could be involved in the induction of apoptosis in this setting.

Other dysregulated cardiac miRNAs

miR-23a

After two weeks of pressure overload, miR-23a was found to be the most induced of only four miRNAs (the others being miR-27b, miR-125b and miR-195) upregulated out of a total of 13 miRNAs analyzed in rat heart by Busk and Cirera.[24] Nfatc3 was found to regulate the expression of miR-23a.[25] miR-23a, miR-24 and miR-27a (members of the miR-23a ≈ 27a ≈ 24-2 cluster) were all upregulated in neonatal rat cardiomyocytes after exposure to either isoproterenol (ISO), a β-adrenergic receptor agonist, or the hormone aldosterone (ALD), both of which stimulate hypertrophic growth. However, hypertrophy could be blunted *in vitro* and *in vivo* only by knockdown of miR-23a. Transduction of cardiomyocytes with an adenovirus harboring an active form of Cn, known to mediate ISO- and ALD-stimulated hypertrophies, produced upregulation of miR-23a, and the Cn inhibitor cyclosporin A inhibited ISO-mediated upregulation of miR-23a. Cn's downstream target Nfatc3 was found to directly activate miR-23a expression through the transcriptional machinery by binding to the promoter region of the miR-23a ≈ 27a ≈ 24-2 cluster gene. Muscle-specific ring finger protein 1(MuRF1), an antihypertrophic protein, was identified as a target of miR-23a with the commonplace bioinformatics and luciferase-3′UTR chimera experiments. MuRF1 overexpression in cardiomyocytes was capable of inhibiting hypertrophy mediated by miR-23a, ISO and ALD.

miR-9

The same group also implicated miR-9 in Nfatc3-mediated hypertrophy signaling.[26] Exposure of rat cardiomyocytes to either ISO or ALD *in vitro* and their infusion in mice led to reduced phosphorylation of Nfatc3, its translocation into the nucleus and increased expression of the prohypertrophic protein myocardin, whose gene was found to harbor an Nfatc3 consensus sequence on its promoter. miR-9, found through bioinformatics to target myocardin mRNA, became downregulated upon exposure of cardiomyocytes to either ISO or ALD, and its overexpression was demonstrated to reduce the level of myocardin and blunt hypertrophy *in vitro* and *in vivo*. The mechanism leading to downregulation of miR-9, unfortunately, was not elucidated.

miR-199

Rane *et al.*[27] demonstrated upregulation of miR-199a-5p in hypertrophic hearts from pressure overloaded mice. Upon the onset of heart failure, miR-199a-5p expression was found to decline significantly. miR-199a-5p was also found increased in the hearts of both β1-adrenergic receptor (AR)- and β2-AR-overexpressing mice – two transgenic models of heart failure – before the onset of DCM. In neonatal cardiomyocytes *in vitro*, ISO was demonstrated to increase the expression of miR-199a-5p, whereas insulin receptor-stimulated activation of the Akt pathway induced miR-199a-5p downregulation along with upregulation of hypoxia-inducible factor 1-alpha (Hif-1α), a transcription factor, and sirtuin 1 (Sirt1), a histone deacetylase that induces modest hypertrophy and protects cardiomyocytes from apoptosis.[28] The mRNAs of these two proteins were previously reported by the same group as targets of miR-199a-5p in cardiomyocytes subjected to hypoxia or ischemia: under these conditions, miR-199a was found to be downregulated, releasing Hif-1α-mediated inhibition on hypoxia-induced proapoptotic pathways.[29] Interestingly, the activation of β-ARs was demonstrated to antagonize the protective effect of the Akt pathway induced by stimuli such as insulin, precisely through miR-199a-5p-mediated inhibition of Hif-1α and Sirt1 and, thus, shedding new light on the positive effects of the use of beta-blockers in patients with heart failure and IHD.

Quantitative reverse transcriptase–PCR analysis of heart tissue from rats subjected to pressure overload was used by Song *et al.*[30] to track the time course of expression of a number of miRNAs found expressed in human heart. After one week of pressure overload (i.e. in hypertrophic hearts), miR-1, miR-133 and miR-499 were found to be downregulated, miR-199a was found significantly upregulated and no alteration was found in miR-214 compared with the levels in sham-operated mouse heart; miR-126, an endothelial–specific miRNA, was also found upregulated, suggesting that the hearts were undergoing angiogenesis at this point. Similar alterations in the expression of some of these miRNAs had been previously reported in pressure-overloaded mouse heart.[31–33] In hearts subjected to pressure overload for four weeks, the expression of miR-1, miR-133, miR-199a and miR-499 had returned to basal levels, while miR-21, miR-24 and miR-214 were upregulated. After 12 weeks of pressure overload, a time at which heart failure had ensued, miR-199a had increased once more. Overexpression of miR-199a with an adenoviral vector in cardiomyocytes *in vitro* was sufficient to increase the cell size and decrease myosin-6 expression, whereas antisense oligonucleotide-mediated knockdown of miR-199a decreased cardiomyocyte size and attenuated hypertrophy induced by PHE, suggesting that the alteration of miR-199a expression might be a cause for hypertrophy and not a consequence of it. Overexpression of miR-199a decreased the activity of a luciferase reporter encoding the 3′UTR of rat *Hif1a* in 293T cells, confirming it as a target of miR-199a. Downregulation of Hif-1α has been reported to cause the transition from hypertrophy to heart failure two weeks after pressure overload in mice.[34] Incidentally, Sirt1 was not found dysregulated in the report by Song *et al.*

Reversal of altered miRNA signatures

The normal gene expression pattern of the heart is significantly altered during hypertrophy and heart failure.

Profiling studies have found that mRNA signatures can differentiate IHD from non-IHD heart pathology, but vary little between different etiologies during the final stages of the disease. Moreover, mRNA signatures do not reflect the improvements provided by biomechanical support, such as that afforded by left ventricular assist devices (LVADs),[35,36] which are used as a last resort before heart transplantation.

LVAD support induces reversal of left ventricular remodeling and leads to recovery of ventricular function through normalization of biochemical parameters. To determine whether LVAD-induced benefits to the heart are also accompanied by changes in miRNA expression, Schipper *et al.*[37] measured the expression of four main cardiac miRNAs (miR-1, miR-133a, miR-133b and miR-208) by quantitative real-time (qRT)-PCR in patients presenting with refractory end-stage heart failure (caused by either IHD or DCM) before and after LVAD support. They found that the reduced expression of miR-1, miR-133a and miR-133b, which they observed in all patients regardless of heart-failure etiology, was partially restored by LVAD support, but only in patients with IHD. On the contrary, LVAD support was responsible for further downregulation of miRNA of DCM patients.

Although that report demonstrated that LVAD could normalize disease-altered miRNA expression, it is in contrast with a later comprehensive microarray profiling study conducted on IHD and non-IHD heart-failure patients by Matkovich *et al.*[38] These authors confidently detected 28 upregulated miRNAs, but no downregulated ones, in heart failure. This is consistent with the notion that more mRNAs are downregulated than upregulated in heart failure and that miRNAs usually repress mRNA expression. A number of the miRNAs found dysregulated (i.e. miR-21, miR-23a, miR-24, miR-26b, miR-27, miR-125b, miR-195 and mir-199a-3p) have been more consistently observed to become upregulated than others in the various studies conducted to date on the heart-failure miRNA profile. Intriguingly, 20 of the 28 upregulated miRNAs were either normalized or at least significantly decreased in the myocardium of patients on LVAD support; LVAD treatment did not alter the expression of any miRNA not found already dysregulated in heart failure. With regard to the mRNA profile, 65% of transcripts dysregulated upon heart failure were downregulated, and only ~8% of these were normalized by at least 25% in samples from LVAD patients. Thus, whereas the mRNA signature differentiated non-failing from failing myocardium and did not distinguish failing myocardium from that of LVAD-supported (i.e. recovering) hearts, the miRNA signature was exquisitely sensitive to the mechanical unloading of end-stage hearts. However, the miRNA signature of LVAD-unloaded hearts was not always distinguishable from that of non-failing ones and, therefore, was not totally effective as a biomarker for classification of the samples. This shortcoming was largely overcome by combining miRNA and mRNA profiles. The authors concluded that LVAD support extensively normalizes the miRNA signature, irrespective of etiology, and the more reactive nature of miRNAs compared with mRNAs in acute alterations of pathophysiological status makes them better molecular markers of stress and the response to it.

Final comments

The foregoing has highlighted the new knowledge that has been gained over the last couple of years on the way single miRNAs might affect individual mRNAs in the cardiovascular system. Much of these data have provided interesting, if not important, new insight into heart pathophysiology. For example, a signaling cascade can modulate the expression of miRNAs that act in concert to enhance prohypertrophic pathways (e.g. downregulation of miR-9 lifts transcriptional repression on myocardin) on the one hand while contemporaneously inhibiting antihypertrophic ones (e.g. upregulation of miR-23a inhibits MuRF1) on the other; in addition, the cascade itself can also be regulated by miRNAs (e.g. downregulation of miR-1 removes its repression on Calm and its downstream partner MEF2, simultaneously).[6,25,26] Control of the expression pattern of myosins by the miRNAs harbored within their genes is another elegant example.[22] However, the intricacy inherent in the post-transcriptional control mechanism actuated by miRNAs will inevitably entail the use of faster high-throughput/output investigational tools in order to obtain the encompassing and integrated outlook needed for molecular cardiology today. We need to improve our knowledge on how the binding of multiple miRNAs affects the expression of individual targeted mRNAs, and how multiple miRNA targets are interlinked to affect the various pathways and processes of the heart. The subtlety of miRNA-mediated regulation calls for an improvement in the way we measure and experiment with miRNAs. Studies using deep-sequencing techniques have recently taken off and will help to overcome some of the present problems.[39] The future certainly holds the necessary change of gear needed to fully understand the role of microRNAs in hypertrophy and heart failure.

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REFERENCES

1 Callis TE, Deng Z, Chen JF, Wang DZ. Muscling through the microRNA world. *Exp Biol Med (Maywood)* 2008;**233**:131–8
2 Frost RJ, van Rooij E. miRNAs as therapeutic targets in ischemic heart disease. *J Cardiovasc Transl Res* 2010;**3**:280–9
3 Liu N, Olson EN. MicroRNA regulatory networks in cardiovascular development. *Dev Cell* 2010;**18**:510–25
4 Rao PK, Toyama Y, Chiang HR, Gupta S, Bauer M, Medvid R, Reinhardt F, Liao R, Krieger M, Jaenisch R, Lodish HF, Blelloch R. Loss of cardiac microRNA-mediated regulation leads to dilated cardiomyopathy and heart failure. *Circ Res* 2009;**105**:585–94
5 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**:27487–99

6 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**:2193–204

7 Elia L, Contu R, Quintavalle M, Varrone F, Chimenti C, Russo MA, Cimino V, De 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**:2377–85

8 Tang Y, Zheng J, Sun Y, Wu Z, Liu Z, Huang G. MicroRNA-1 regulates cardiomyocyte apoptosis by targeting Bcl-2. *Int Heart J* 2009;**50**:377–87

9 Li P. MicroRNAs in cardiac apoptosis. *J Cardiovasc Transl Res* 2010;**3**:219–24

10 Li Q, Song XW, Zou J, Wang GK, Kremneva E, Li XQ, Zhu N, Sun T, Lappalainen P, Yuan WJ, Qin YW, Jing Q. Attenuation of microRNA-1 derepresses the cytoskeleton regulatory protein twinfilin-1 to provoke cardiac hypertrophy. *J Cell Sci* 2010;**123**:2444–52

11 Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 2006;**38**:228–33

12 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**:613–8

13 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**:3242–54

14 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* 2010;**106**:166–75

15 Dong DL, Chen C, Huo R, Wang N, Li Z, Tu YJ, Hu JT, Chu X, Huang W, Yang BF. Reciprocal repression between microRNA-133 and calcineurin regulates cardiac hypertrophy: a novel mechanism for progressive cardiac hypertrophy. *Hypertension* 2010;**55**:946–52

16 Li Q, Lin X, Yang X, Chang J. NFATc4 is negatively regulated in miR-133a-mediated cardiomyocyte hypertrophic repression. *Am J Physiol Heart Circ Physiol* 2010;**298**:H1340–7

17 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**:170–8, 6p following 178

18 Shan H, Zhang Y, Lu Y, Pan Z, Cai B, Wang N, Li X, Feng T, Hong Y, Yang B. Downregulation of miR-133 and miR-590 contributes to nicotine-induced atrial remodelling in canines. *Cardiovasc Res* 2009;**83**:465–72

19 Jiang X, Tsitsiou E, Herrick SE, Lindsay MA. MicroRNAs and the regulation of fibrosis. *FEBS J* 2010;**277**:2015–21

20 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

21 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**:2772–86

22 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**:662–73

23 Li J, Donath S, Li Y, Qin D, Prabhakar BS, Li P. miR-30 regulates mitochondrial fission through targeting p53 and the dynamin-related protein-1 pathway. *PLoS Genet* 2010;**6**:e1000795

24 Busk PK, Cirera S. MicroRNA profiling in early hypertrophic growth of the left ventricle in rats. *Biochem Biophys Res Commun* 2010;**396**:989–93

25 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 USA* 2009;**106**:12103–8

26 Wang K, Long B, Zhou J, Li PF. miR-9 and NFATc3 regulate myocardin in cardiac hypertrophy. *J Biol Chem* 2010;**285**:11903–12

27 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**:1054–62

28 Alcendor RR, Kirshenbaum LA, Imai S, Vatner SF, Sadoshima J. Silent information regulator 2alpha, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes. *Circ Res* 2004;**95**:971–80

29 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-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. *Circ Res* 2009;**104**:879–86

30 Song XW, Li Q, Lin L, Li DF, Wang GK, Ren AJ, Qin YW, Yuan WJ, Jing Q. MicroRNAs are dynamically regulated in hypertrophic hearts, and miR-199a is essential for the maintenance of cell size in cardiomyocytes. *J Cell Physiol* 2010;**255**:437–43

31 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**:416–24

32 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 USA* 2006;**103**:18255–60

33 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**:1831–40

34 Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkentin JD, Zou Y, Komuro I. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature* 2007;**446**:444–8

35 Margulies KB, Matiwala S, Cornejo C, Olsen H, Craven WA, Bednarik D. Mixed messages: transcription patterns in failing and recovering human myocardium. *Circ Res* 2005;**96**:592–9

36 Dorn GW 2nd, Matkovich SJ. Put your chips on transcriptomics. *Circulation* 2008;**118**:216–8

37 Schipper ME, van Kuik J, de Jonge N, Dullens HF, de Weger RA. Changes in regulatory microRNA expression in myocardium of heart failure patients on left ventricular assist device support. *J Heart Lung Transplant* 2008;**27**:1282–5

38 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**:1263–71

39 Creighton CJ, Reid JG, Gunaratne PH. Expression profiling of microRNAs by deep sequencing. *Brief Bioinform* 2009;**10**:490–7