Chapter 14
MicroRNA Therapeutics in Cardiovascular Disease

Antoine Bril

Abstract MicroRNAs are endogenous single-stranded RNAs of approximately 22 nucleotides in length which decrease the level of expression of specific proteins. miRNAs are involved in many pathologic conditions such as cancers and neuropsychiatric and metabolic diseases. In cardiovascular diseases, miRNAs have been shown to be involved in the proliferation of cardiomyocytes and non-myocyte cardiac cells, in cardiac hypertrophy and apoptosis, in the occurrence of atrial and ventricular arrhythmias, in vascular angiogenesis and smooth muscle cell pathologies, and in atherosclerosis. In heart failure, miRNAs have been shown to promote or inhibit hypertrophy and remodeling as well as to modulate cardiac pump function. By considering a few specific examples illustrating the value and complexity of targeting miRNAs, this chapter will describe the key data demonstrating the potential of miRNAs either to prevent cardiac hypertrophy (miR-208) or to improve cardiac function (miR-25) in heart failure. In the biogenesis of miRNAs, a passenger strand miRNA, called miRNA*, is usually degraded. The effects of miR-21* illustrate a situation where miRNA* exhibits a biological activity. Finally, miRNA-based therapeutics is nearly reaching human trials in the cardiovascular disease area. However, several questions remain to be fully investigated to progress such therapeutic approaches successfully. These include strategies to improve druggability parameters and delivery characteristics of selected candidates as well as approaches required to demonstrate clearly the efficacy/safety ratio of such novel therapies.

Keywords miRNA • Heart failure • Cardiovascular disease • Therapy • Oligonucleotide • Drug discovery

A. Bril, PharmD, PhD
Research and Partnerships, Institut de Recherches Internationales Servier,
50 rue Carnot, 92284 Suresnes Cedex, France
e-mail: antoine.bril@fr.netgrs.com

G. Jagadeesh et al. (eds.), *Pathophysiology and Pharmacotherapy of Cardiovascular Disease*, DOI 10.1007/978-3-319-15961-4_14

14.1 Introduction

Since their initial discovery in *C. elegans*, microRNAs (miRNAs) have been extensively studied, and their biology has been widely investigated. Many studies performed in plants, viruses, bacteria, and mammals including humans have described their genesis, degradation, and regulation.

MicroRNAs are endogenous single-stranded RNAs of approximately 22 nucleotides in length. They decrease the level of expression of specific proteins by annealing to specific sequences located in the 3′ untranslated regions of target messenger RNAs (mRNAs). Many review articles have summarized the biology of the formation and action of miRNAs [1–3]. Briefly, miRNA genes are transcribed in the nucleus by RNA polymerase II into a hairpin structure called primary microRNAs (pri-miRNAs) and then processed by the RNase III Drosha in a complex with the RNA-binding protein DiGeorge syndrome critical region 8 (DGCR8). The resulting pri-miRNA is a double-stranded 70-nucleotide-long structure exported to the cytoplasm by exportin-5. In the cytoplasm, the pre-miRNA forms a complex with the endonuclease DICER, the transactivation responsive RNA-binding protein (TRBP), and the adenosine deaminase action on RNA-1 (ADAR1) and is cleaved in a resulting miRNA-miRNA* duplex. The passenger strand, i.e., miRNA* or miRNA star, is then degraded, and the mature miRNA could associate with Argonaute to bind its mRNA target and degrade it.

In the cytoplasm, the association of miRNAs with their mRNA targets is within a multiprotein complex called the RNA-induced silencing complex (RISC). It is now well established that a given miRNA targets several mRNAs and that individual mRNAs are often simultaneously targeted by many miRNAs. Therefore, the regulatory signaling network resulting from miRNA biology is very complex and requires well-defined strategies for developing therapeutic interventions. Today, more than 2,000 miRNA sequences identified within the human genome are reported in specialized databases (www.mirbase.org), and almost all cellular processes are regulated by miRNAs. miRNAs have been involved in most pathologies such as cancers and neuropsychiatric and metabolic diseases [4–7]. Many review articles have described the many miRNAs that have been shown to play a role in various cardiovascular diseases [8–13]. This review will summarize the efficacy of miRNAs as potential therapeutic targets for heart failure by considering specific examples illustrating the importance and complexity of targeting miRNAs from a drug discovery perspective. In a second part, some of the questions that remain to be fully investigated to enable the delivery of novel therapeutic strategies will be described.

14.2 miRNAs as Molecular Therapeutic Targets for Heart Failure

The importance of miRNAs as therapeutic targets for cardiovascular diseases has been deciphered mainly by investigating the expression pattern of various miRNAs in pathologic situations and measuring the consequences of genetically altering the

level of expression of selected miRNAs. The first major study illustrating a role for miRNAs in cardiac biology was the demonstration of the role of miR-1 on cardiomyocyte differentiation [14] and the investigation of miRNAs in cardiac hypertrophy and failure [15]. Van Rooij et al. [15] described the expression pattern of a number of miRNAs both in experimental hypertrophy and in samples from idiopathic end-stage failing human hearts. Since then, many studies have established the relationship between miRNAs and cardiovascular diseases.

In the study by van Rooij et al. [15], later confirmed using a larger set of miRNAs [16], an overlap is observed between the pattern of expression in the human failing hearts and the hypertrophic mouse heart suggesting a common molecular signature for cardiac remodeling. When overexpressing specific miRNAs in transgenic mice under the myosin heavy chain promoter, different phenotypes have been observed. Whereas miR-24 induced embryonic lethality, other miRNAs such as miR-124 did not induce any detectable phenotypic effect. The overexpression of miR-195 was able to recapitulate the hypertrophic phenotype observed in experimentally induced cardiac overload. Findings from this early study suggest that a specific miRNA could be responsible for the molecular genesis of diseases such as heart failure and that selecting the right miRNA could lead to promising insights into heart diseases and cardiovascular therapies. Since then, all the pathophysiologic processes involved in cardiovascular diseases including cell hypertrophy and proliferation, cell death and apoptosis, arrhythmias, and fibrosis have been shown to involve miRNAs, and computational tools have been developed to identify miRNA-target interactions and to facilitate biological discoveries [17] (also see Chap. 13).

In investigating further the role of cardiac-expressed miRNA, Olson's laboratory created the first ever miRNA knockout (KO) mice dedicated to cardiovascular research and discovered that miRNA-208, which is expressed from intron 27 of α-myosin heavy chain gene, is required for stress-induced cardiac hypertrophy. In these experiments, miR-208 KO mice exhibit blunted cardiac hypertrophy in response to pressure overload [18]. Interestingly, it was shown by Callis et al. [19] that transgenic overexpression of miR-208a is by itself sufficient to induce cardiac hypertrophy in mice. Actually, the biology related to miR-208 appears more complex than what was thought initially. While the overexpression of miR-208 is related with the occurrence of cardiac arrhythmias in mice, its genetic deletion resulted in aberrant cardiac conduction [19]. Taken together, these data demonstrate that the expression level of a single miRNA in the heart could be critical for both hypertrophic growth and the cardiac conduction system. In fact when investigating the role of systemic delivery of miR-208a inhibitors, Montgomery et al. [20] reported that systemic delivery of miR-208a inhibitors prevented the pathological myosin switch, a well-established marker of cardiac hypertrophy [21], and reduced cardiac remodeling while improving cardiac function and survival.

Interestingly, miR-208a has also been shown to regulate systemic energy homeostasis via a negative regulation of the mediator complex 13 protein expression [22]. The pharmacological inhibition of miR-208a or cardiac-specific overexpression of mediator complex 13 in mice induces resistance to high-fat diet-induced obesity and improves systemic insulin sensitivity and glucose tolerance [22]. These findings have two main consequences when considering a particular miRNA as a possible

therapeutic target for cardiovascular diseases. First, they reinforce earlier findings demonstrating the role of the heart in controlling systemic metabolism and therefore add evidence that for miRNA therapeutics for cardiovascular diseases the benefit-risk ratio for targeting a given miRNA requires preclinical and clinical investigations broader than focusing attention to cardiovascular function as it is done for each and every drug treatment. Second, these results clearly suggest that the patient population for which a miRNA therapeutic could be developed may need to be defined according to a revised more molecularly defined taxonomy. The findings also suggest that a single miRNA therapeutic may not be beneficial for all the pathogenic phenotypes identified in a particular disease such as heart failure.

Recently, the potential of miRNAs as targets to improve cardiac function in cardiovascular diseases and particularly in heart failure has been investigated using a high-throughput physiological screening approach. High-throughput physiologic screenings are methods in which a system-based, hypothesis-free approach without a predetermined molecular target is used to identify novel drug candidate that may be effective on a given phenotype [23, 24]. Among various hallmarks characterizing heart failure, a decline in cardiac function represents a well-established phenotype. Improving cardiac contractility by modulating the activity of intracellular calcium-handling proteins is currently being tested clinically using gene therapy to introduce the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), the primary protein responsible for calcium uptake by the sarcoplasmic reticulum during the excitation-contraction coupling in cardiomyocytes [25, 26] (Chap. 4).

Wahlquist et al. [27] used human embryonic kidney 293 cells co-transfected with a green fluorescent protein reporter fused to the 3′ untranslated region of SERCA2a gene to screen a whole genome collection of miRNAs and identified 144 miRNAs that are able to downregulate the calcium ATPase. Among the 82 miRNAs confirmed by testing a dose range, 15 were both evolutionarily conserved and upregulated in human heart failure. Finally, the most potent miRNA on a physiological measure of the calcium flux in cardiomyocytes was miR-25, and its effect was similar to that of short interfering RNA (siRNA) directed against SERCA2a. While the overexpression of miR-25 in the heart both decreased expression of SERCA2a and induced contractile dysfunction in mice, the administration of an antisense oligonucleotide against miR-25 reversed heart failure established in mice by chronic aortic constriction and improved survival rate in these mice [27]. This study, which reinforces the value of physiological screening strategies to identify novel therapeutic approaches [28, 29], suggests that targeting miR-25 may represent an alternative possibility to bypass the potential risks of gene therapy aimed at restoring SERCA2a expression level [30, 31]. These data show miR-25 to be part of a dynamic regulation of cardiac function by fine-tuning the complex intracellular calcium cycling mechanism. However, it requires further validation as a previous study has shown opposite results in which in vivo inhibition of miR-25 generates spontaneous contractile dysfunction in mice and sensitizes the murine myocardium to heart failure [32]. The efficacy of interacting with miR-25, whose expression is altered in various cancers, remains to be confirmed in different models and pathologic conditions to confirm or not its value as a therapeutic target for heart failure. Finally, although it is recently being

suggested that the most abundant miRNA in a cell may be the one having a prominent physiological role [33], the other miRNAs identified in the physiological screening used by Wahlquist et al. [27] might also play a role in heart failure (Chap. 13).

Alternative to mature miRNAs, passenger strands (miRNA* or miRNA star) usually released and degraded during the formation process of miRNAs could also represent valuable molecular targets for heart failure. Such a hypothesis has recently been described by Bang et al. [34] who showed that the passenger strand microRNA miR-21* behaves as a novel paracrine factor released from fibroblasts and inducing cardiac myocyte hypertrophy. In the progression from hypertrophy to failure, fibroblasts not only proliferate and favor fibrosis but also secrete extracellular matrix proteins and proinflammatory cytokines aggravating cardiac remodeling. Experimentally, cardiomyocytes cocultured with fibroblasts or in the presence of conditioned fibroblast media developed hypertrophy. Because miRNAs have been shown to be actively transported in microvesicles/exosomes in circulating fluids as well as between endothelial and cardiovascular cells [35, 36], Bang et al. [34, 37] tested the hypothesis that miRNAs could represent a paracrine cross talk between cardiac fibroblasts and cardiomyocytes. While miR-21 has been shown as a fibroblast mediator for generating hypertrophy and failure [38] and to be involved in metabolic diseases [39], the present study interestingly shows that the corresponding miR-21* is specifically packaged into and transported by exosomes from cardiac fibroblasts to cardiomyocytes and promotes cellular hypertrophy. In vivo studies demonstrated that miR-21* was detected in the pericardial fluids of mice submitted to aortic constriction-induced hypertrophy and that a systemic administration of an anti-miR-21* prevented angiotensin-induced hypertrophy [34]. Although many questions remain to be answered before considering miRNA-containing vesicles as therapeutic opportunities for cardiovascular diseases, this study clearly shows that mature miRNAs as well as miRNA* could play a significant role in the intercellular cross talks involved in pathogenesis of chronic diseases such as heart failure.

14.3 miRNA Therapeutics for the Treatment of Heart Failure

On the basis of the progress made in the antisense technologies, several options are currently being investigated for miRNAs as novel drug treatments. Today, the most advanced miRNA therapies are anti-miR-122 compounds for the treatment of hepatitis C virus infection with a miRNA inhibitor in phase 2 clinical trials [40]. In the cardiovascular disease area, no miRNA-based therapeutics have yet reached human trials, and many questions remain to be fully investigated to progress such strategies successfully.

Drug Strategies Used to Modulate miRNA Function In diseases where miRNAs could be considered as playing a beneficial rather than a pathogenic role, mimicking

the activity of endogenous miRNA appears the most rational therapeutic strategy. Double-stranded chemically modified miRNAs have therefore been developed as miRNA mimics, and miRNA mimic-related agents such as MRX34, a lipid-formulated miR-34 mimic for the treatment of cancers, are reaching the clinical stage [4, 41]. As far as inhibiting miRNA activity is concerned, several strategies have been studied including vector-based approaches or miRNA sponges, small molecules, and antisense oligonucleotides.

Several reviews have recently summarized extensively the advances made in the development of miRNA inhibitors [4, 40, 42]. miRNA sponges that are large vectors require tissue-specific expression to be active and have been used mainly for in vitro experiments and for investigating small animal models. Small molecules targeting miRNAs or SMIRs that have been identified by screening compound libraries on reporter-based assays necessitating a micromolar concentration to be active are not suitable yet for drug discovery and development. Therefore, both miRNA sponges and SMIRs have limited therapeutic potential. As a consequence, most therapeutic approaches currently used to inhibit miRNA activity are based on antisense oligonucleotide technologies. To achieve efficient in vivo inhibition of the target miRNA and allow improved affinity, stability, and pharmacokinetic properties, oligonucleotides are chemically modified. Briefly, the modifications performed mainly affect the 2′ position of the sugar ring and are 2′-fluoro, 2′-O-methyl, or 2′-O-methoxyethyl modifications and locked nucleic acid (LNA) which is the creation of a bicyclic nucleic acid by connecting the 2′ oxygen to the 4′ carbon via a methylene bridge. All these modifications greatly enhance binding affinity. Another modification is the substitution of the phosphodiester bonds with phosphorothioate bonds to increase nuclease resistance. Combinations of several of these chemical modifications lead to the most efficacious results in terms of binding affinity, nuclease resistance, and miRNA-inhibitory activity. Today, strategies that combine LNA technology with other chemical modifications, and among them fully LNA-modified 8-mer phosphorothioate oligonucleotides called tiny LNAs, are the most promising approaches.

Delivery of miRNA-Based Therapeutics Besides the use of liposome formulation, cholesterol conjugation and phosphorothioate linkage have been shown to improve the pharmacokinetic properties of antisense oligonucleotides. However, delivering miRNA inhibitors for cardiovascular diseases remains a challenge, and the development of novel formulation and delivery strategies is required to overcome this challenge, as this has been the case for biopharmaceuticals products [43]. Today, the most advanced therapeutic approaches involving miRNAs as molecular targets are directed toward severe hepatic diseases such as HCV infection or liver cancers. As an example, patients receiving subcutaneous administration of miravirsen (currently in phase II clinical trial for the treatment of hepatitis C), a LNA-modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122, showed dose-dependent reduction of HCV RNA levels [44], suggesting that the systemic delivery of miRNA inhibitors represents a possible therapeutic strategy. However, because systemically administered miRNA inhibitors, both antagomirs

and anti-miRs, predominantly accumulate in the liver and the kidney, this first proof-of-concept study may not be completely relevant for cardiovascular diseases for which high doses may be required to reach targets.

Because miRNAs physiologically act on a number of mRNA targets possibly with opposing effects depending on the tissue, the high doses required for demonstrating efficacy in the cardiovascular system may be deleterious for other organs. While further developments are necessary to improve the cardiovascular targeting of miRNA inhibitors, alternative delivery strategies could also be evaluated. Recently, Hinkel et al. [45] investigated the effect of a regional administration of a LNA anti-miR-92a in a model of ischemia-reperfusion-induced injury in pigs. In this study, the authors compared the efficacy of LNA-92a administered either systemically by intravenous infusion or locally by catheter-based delivery into the left anterior descending coronary artery or the anterior interventricular vein. When the treatment activity was measured by the expression level of the targeted miRNA, systemic and regional administrations of LNA-92a exhibited a similar activity. However, only regional administration of LNA-92a reduced infarct size and improved the postischemic myocardial function [45]. Although the level of efficacy obtained in this study appears limited compared to classically adopted interventions such as ischemic preconditioning or clinically established therapies [46, 47], these results clearly demonstrate the potential of a local delivery of miRNA inhibitors for cardiac indications such as myocardial ischemia or heart failure. This study highlights the fact that defining the correct efficacy endpoint remains to be further investigated and suggests that the expression level of the targeted miRNA may not always represent a valuable surrogate for pharmacokinetics/pharmacodynamics relationships.

Characterization of the Efficacy of miRNA Therapeutics Along with absorption, distribution, metabolism, and elimination (ADME) parameters that are investigated in every drug discovery program, several issues that may be prominent with miRNA therapeutics require special consideration. The results of the study by Hinkel et al. [45] illustrate the need for better understanding of the relationship between target engagement and miRNA therapeutics in measuring efficacy in animal models and eventually in patients. While the miRNA inhibitor represses the expression of its target mRNA, the function measured on the physiological endpoint depends on the route of administration. Because a given miRNA can modulate the expression of many target mRNAs and therefore regulate the expression level of many proteins, it is usually difficult to correlate target engagement with therapeutic efficacy. Alternative strategies, compared to those classically used in drug discovery, are therefore required. Investigating the summation of relatively small effects and comparing the resulting physiology in a tissue and in another requires novel systems-based approaches. If these methodologies using computational analysis of a large amount of data are emerging, they need to be created and developed not to oversimplify the downstream effect of miRNA functions.

The second key question to be investigated refers to both the onset and the duration of action of miRNA therapeutics. In contrast to most classic pharmacological interventions targeting a membrane receptor, an intracellular enzyme or a

protein-protein interaction, the action of miRNA inhibitors appears delayed, while, on the other hand, their effect on mRNA targets is fairly immediate [20, 48]. There is no definitive explanation for such a delay in biological action and the time required for regulating the protein expression level.

Evaluation of the Safety Profile of miRNA Therapeutics As for any novel therapeutic intervention, investigating the adverse effects and the off-target effects of long-term miRNA modulation is of prime importance.

miRNA therapeutics that are designed to be perfectly complementary to their mature miRNA targets could represent very safe therapeutic opportunities. Since miRNAs can target many mRNAs, a miRNA modulator that is specific for only one seed region could affect several pathways. Therefore, unwanted systemic effects should be monitored.

Besides nonspecific hybridization-dependent effects, any chemical modification done to improve the stability, affinity, and pharmacokinetic properties of miRNA therapeutics could cause additional adverse effects such as an activation of the immune system or a liver toxicity. A similar point of vigilance has been described with most antisense oligonucleotide strategies, and chemical modification of anti-miRNA as well as shortening the sequence that targets miRNA could reduce this risk.

Liver toxicity is another risk observed with chemically modified anti-miRNAs, and this effect appears to be induced in a sequence-independent manner. Swayze et al. [49] showed that LNA-modified oligonucleotides induced hepatotoxicity as indicated by increased transaminase activity and increased organ to body weight ratio in preclinical toxicology studies. The potential hepatotoxic effect of miRNA, a risk described for many new molecular entities and a topic of many investigations [50], requires careful examination in a case-by-case basis depending on the targeted disease, the demonstrated efficacy, and the alternative treatment opportunities available.

14.4 Concluding Remarks

In cardiovascular physiology and pathology, miRNAs represent an emerging field from which several therapeutic opportunities could be derived. Genetic and pharmacologic studies of various miRNAs have shown them to be involved in the genesis and progression of many diseases such as those associated with aging [51], and the studies summarized in this chapter using heart failure as an example provide an illustration of their potential to deliver novel therapeutic modalities. Many unknowns that remain to be investigated to move miRNA biology to therapeutic reality especially when considering cardiovascular diseases represent opportunities for the development of novel technologies, methodologies, and treatments that eventually will benefit patients. Among them are the development of integrative system biology approaches to understand fully the miRNA effects at cellular, organ, and

organism levels; the identification of delivery systems to ensure that the right dose is reaching the right molecular target, in the right tissue at the right time in a disease; and a careful understanding of potential adverse effects when administered either alone or in combination with conventional therapies. In addition to small noncoding RNAs (miRNAs), studies using high-throughput sequencing screens have described many long noncoding transcripts (long noncoding RNAs), of more than 200 nucleotides long. Together, small and long noncoding RNAs are increasingly demonstrated as key players in gene regulatory networks involved in cardiovascular biology and pathophysiology [52–55]. Both small and long noncoding RNAs are therefore opening new avenues to improve the understanding and the treatment of cardiovascular diseases like heart failure.

References

1. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136:215–33.
2. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet. 2010;11:597–610.
3. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15:509–24.
4. Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov. 2013;12:847–65.
5. Millan MJ. An epigenetic framework for neurodevelopmental disorders: from pathogenesis to potential therapy. Neuropharmacology. 2013;68:2–82.
6. Krützfeldt J, Stoffel M. MicroRNAs: a new class of regulatory genes affecting metabolism. Cell Metab. 2006;4:9–12.
7. Latreille M, et al. MicroRNA-7a regulates pancreatic β cell function. J Clin Invest. 2014; 124:2722–35.
8. van Rooij E, Olson EN. MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. J Clin Invest. 2007;117:2369–76.
9. Scalbert E, Bril A. Implication of microRNAs in the cardiovascular system. Curr Opin Pharmacol. 2008;8:181–8.
10. Suárez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. Circ Res. 2009;104: 442–54.
11. Latronico MV, Condorelli G. MicroRNAs and cardiac pathology. Nat Rev Cardiol. 2009;6: 419–29.
12. Condorelli G, Latronico MV, Cavarretta E. MicroRNAs in cardiovascular diseases: current knowledge and the road ahead. J Am Coll Cardiol. 2014;63:2177–87.
13. Harada M, Luo X, Murohara T, Yang B, Dobrev D, Nattel S. MicroRNA regulation and cardiac calcium signaling: role in cardiac disease and therapeutic potential. Circ Res. 2014;114: 689–705.
14. Kwon C, Han Z, Olson EN, Srivastava D. MicroRNA1 influences cardiac differentiation in Drosophila and regulates Notch signaling. Proc Natl Acad Sci U S A. 2005;102:18986–91.
15. van Rooij E, et al. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc Natl Acad Sci U S A. 2006;103:18255–60.
16. Ikeda S, et al. Altered microRNA expression in human heart disease. Physiol Genomics. 2007;31:367–73.
17. Hausser J, Zavolan M. Identification and consequences of miRNA-target interactions – beyond repression of gene expression. Nat Rev Genet. 2014;15:599–612.
18. 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.

19. Callis TE, et al. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. J Clin Invest. 2009;119:2772–86.
20. Montgomery RL, et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation. 2011;124:1537–47.
21. Lompré AM, Schwartz K, d'Albis A, Lacombe G, Van Thiem N, Swynghedauw B. Myosin isoenzyme redistribution in chronic heart overload. Nature. 1979;282:105–7.
22. Grueter CE, van Rooij E, Johnson BA, DeLeon SM, Sutherland LB, Qi X, Gautron L, Elmquist JK, Bassel-Duby R, Olson EN. A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. Cell. 2012;149:671–83.
23. Lemons D, Maurya MR, Subramaniam S, Mercola M. Developing microRNA screening as a functional genomics tool for disease research. Front Physiol. 2013;4:223.
24. Moffat JG, Rudolph J, Bailey D. Phenotypic screening in cancer drug discovery – past, present and future. Nat Rev Drug Discov. 2014;13:588–602.
25. Kawase Y, et al. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. J Am Coll Cardiol. 2008;51:1112–9.
26. Jessup M, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, Yaroshinsky A, Zsebo KM, Dittrich H, Hajjar RJ, Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) Investigators. Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. Circulation. 2011;124:304–13.
27. Wahlquist C, et al. Inhibition of miR-25 improves cardiac contractility in the failing heart. Nature. 2014;508:531–5.
28. Swinney DC, Anthony J. How were new medicines discovered? Nat Rev Drug Discov. 2011;10:507–19.
29. Eder J, Sedrani R, Wiesmann C. The discovery of first-in-class drugs: origins and evolution. Nat Rev Drug Discov. 2014;13:577–87.
30. Mann DL. Getting pumped about heart failure. Cell Metab. 2014;19:896–7.
31. Martinez-Fernandez A. MicroRNA therapy for the failing heart. Circ Cardiovasc Genet. 2014;7:393–4.
32. Dirkx E, et al. Nfat and miR-25 cooperate to reactivate the transcription factor Hand2 in heart failure. Nat Cell Biol. 2013;15:1282–93.
33. Broderick JA, Zamore PD. Competitive endogenous RNAs cannot alter microRNA function in vivo. Mol Cell. 2014;54:711–3.
34. Bang C, et al. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest. 2014;124:2136–46.
35. Hergenreider E, et al. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol. 2012;14:249–56.
36. Tréguer K, Heydt S, Hergenreider E. Protection des vaisseaux sanguins contre l'athérosclérose - Le rôle des miARN sécrétés par l'endothélium. Med Sci (Paris). 2012;28:584–7.
37. Indolfi C, Curcio A. Stargazing microRNA maps a new miR-21 star for cardiac hypertrophy. J Clin Invest. 2014;124:1896–8.
38. Thum T, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature. 2008;456:980–4.
39. Seeger T, Fischer A, Muhly-Reinholz M, Zeiher AM, Dimmeler S. Long-term inhibition of miR-21 leads to reduction of obesity in db/db mice. Obesity (Silver Spring). 2014. doi:10.1002/oby.20852 [Epub ahead of print].
40. van Rooij E, Kauppinen S. Development of microRNA therapeutics is coming of age. EMBO Mol Med. 2014;6:851–64.
41. Bouchie A. First microRNA mimic enters clinic. Nat Biotechnol. 2013;31:577.
42. Li Z, Rana TM. Therapeutic targeting of microRNAs: current status and future challenges. Nat Rev Drug Discov. 2014;13:622–38.
43. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. Nat Rev Drug Discov. 2014;13:655–72.

44. Janssen HL, et al. Treatment of HCV infection by targeting microRNA. N Engl J Med. 2013;368:1685–94.
45. Hinkel R, et al. Inhibition of microRNA-92a protects against ischemia/reperfusion injury in a large-animal model. Circulation. 2013;128:1066–75.
46. Bril A, Slivjak M, DiMartino MJ, Feuerstein GZ, Linee P, Poyser RH, Ruffolo Jr RR, Smith 3rd EF. Cardioprotective effects of carvedilol, a novel beta adrenoceptor antagonist with vasodilating properties, in anaesthetised minipigs: comparison with propranolol. Cardiovasc Res. 1992;26:518–25.
47. Heusch G, Skyschally A, Schulz R. The in-situ pig heart with regional ischemia/reperfusion – ready for translation. J Mol Cell Cardiol. 2011;50:951–63.
48. Olson EN. MicroRNAs as therapeutic targets and biomarkers of cardiovascular disease. Sci Transl Med. 2014;6:239ps3.
49. Swayze EE, Siwkowski AM, Wancewicz EV, Migawa MT, Wyrzykiewicz TK, Hung G, Monia BP, Bennett CF. Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. Nucleic Acids Res. 2007;35:687–700.
50. Chalhoub WM, Sliman KD, Arumuganathan M, Lewis JH. Drug-induced liver injury: what was new in 2013? Expert Opin Drug Metab Toxicol. 2014;10:959–80.
51. Thum T. Non-coding RNAs in ageing. Ageing Res Rev. 2014;17:1–2.
52. Yang KC, Yamada KA, Patel AY, Topkara VK, George I, Cheema FH, Ewald GA, Mann DL, Nerbonne JM. Deep RNA sequencing reveals dynamic regulation of myocardial noncoding RNAs in failing human heart and remodeling with mechanical circulatory support. Circulation. 2014;129:1009–21.
53. Ounzain S, et al. Genome-wide profiling of the cardiac transcriptome after myocardial infarction identifies novel heart-specific long non-coding RNAs. Eur Heart J. 2014. doi:10.1093/eurheartj/ehu180.
54. Ounzain S, et al. Functional importance of cardiac enhancer-associated noncoding RNAs in heart development and disease. J Mol Cell Cardiol. 2014;76C:55–70.
55. Han P, et al. A long noncoding RNA protects the heart from pathological hypertrophy. Nature. 2014;514:102–6.