HHS Public Access

Author manuscript

Nat Rev Drug Discov. Author manuscript; available in PMC 2019 October 25.

Published in final edited form as:

Nat Rev Drug Discov. 2012 November ; 11(11): 860–872. doi:10.1038/nrd3864.

MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles

Eva van Rooij[1], **Eric N. Olson**[2]

[1]miRagen Therapeutics, 6200 Lookout Rd, Boulder, Colorado 80301, USA

[2]Department of Molecular Biology, University of Texas Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, Texas 75390–79148, USA

Abstract

In recent years, prominent roles for microRNAs (miRNAs) have been uncovered in several cardiovascular disorders. The ability to therapeutically manipulate miRNA expression and function through systemic or local delivery of miRNA inhibitors, referred to as antimiRs, has triggered enthusiasm for miRNAs as novel therapeutic targets. Here, we focus on the pharmacokinetic and pharmacodynamic properties of current antimiR designs and their relevance to cardiovascular indications, and evaluate the opportunities and obstacles associated with this new therapeutic modality.

Cardiovascular disease remains the primary cause of morbidity and mortality worldwide. Despite the therapeutic benefits of numerous treatment options, including statins, angiotensin-converting enzyme (ACE) inhibitors, beta blockers and other drugs, the prevalence of cardiovascular disease continues to increase, underscoring the need for new therapeutic strategies.

MicroRNAs (miRNAs) are short, single-stranded RNAs that anneal with complementary sequences in mRNAs, thereby suppressing protein expression. Individual miRNAs engage numerous mRNA targets, often encoding multiple components of complex intracellular networks. Thus, the manipulation of miRNA expression or function can have a profound impact on cellular phenotypes. Depending on the abundance of a miRNA and its targets, as well as the physiological state of a cell, a miRNA can act as a fine-tuner of gene expression or an on/off switch. The functions of miRNAs are heightened under conditions of pathophysiological stress and in disease, making them attractive candidates for therapeutic manipulation.

The first report implicating miRNAs in heart disease was published in 2006 (REF. 1), in which signature patterns of miRNA expression were shown to correlate with heart failure and cardiac hypertrophy in mice and humans. Since then, numerous follow-up studies have described changes in miRNA expression in diseased human hearts[2,3] and vascular tissues[4,5],

Correspondence to E.N.O. eric.olson@utsouthwestern.edu.

Competing interests statement

The authors declare competing financial interests: see Web version for details.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

and gain- and loss-of-function studies have uncovered prominent roles for miRNAs in cardiovascular disorders including myocardial infarction, cardiac hypertrophy, heart failure, angiogenesis, vascular stenosis and fibrosis[1–3,6–12]. These actions reflect the involvement of specific miRNAs in fundamental cellular processes, such as cell survival (miR-15)[13], extracellular matrix production (miR-21 and miR-29)[9,10] and hypoxia (miR-210)[14].

The ability to therapeutically manipulate miRNA expression and function through systemic or local delivery of miRNA inhibitors or mimics, and the recent success of the first-in-human clinical trial of a miRNA therapeutic for suppression of hepatitis C virus (HCV) replication (BOX 1), have both raised possibilities for a new class of disease-modifying therapeutics based on miRNA biology. miRNA-based therapeutics have not yet reached clinical trials for cardiovascular disorders, but the promising results in numerous animal models of related diseases such as heart failure, cardiac hypertrophy, fibrosis and hyperlipidaemias, as discussed below, suggest that clinical data will soon be available.

Here, we review the mechanistic basis of miRNA functions within the context of disease modification and consider the roles of representative miRNAs in cardiovascular disease. With this perspective, we evaluate miRNAs as potential therapeutic targets for cardiovascular disorders and consider the challenges associated with this potential new therapeutic modality.

miRNA biogenesis and mechanism of action

Mature miRNAs are ~22 nucleotides in length and are transcribed by RNA polymerase II as long precursor RNAs, called primary miRNAs (pri-miRNAs), which are sequentially processed in the nucleus and cytoplasm. The RNase III Drosha associates with DiGeorge syndrome critical region 8 (DGCR8) and other cofactors to crop pri-miRNAs in the nucleus, yielding hairpin-shaped pre-miRNAs (~70 nucleotides in length) that are further shortened in the cytoplasm by Dicer to give rise to the mature miRNA[15]. In the cytoplasm, miRNAs associate with mRNAs within a multiprotein complex of Argonaute proteins, known as the RNA-induced silencing complex (RISC), which facilitates and stabilizes miRNA–mRNA interactions. miRNAs anneal via Watson–Crick base pairing, with sequences most commonly located in 3′ untranslated regions (UTRs) of mRNA[15], although there are some examples of miRNA interactions within mRNA coding regions, intron–exon junctions and 5′ UTRs[16,17]. The primary determinant of mRNA target regulation by miRNAs is perfect pairing between the sequence of nucleotides 2–7 at the 5′ end of the mature miRNA (referred to as the 'seed region') and the target mRNA, but other nucleotides also contribute to the interactions[15]. Association of a miRNA with its mRNA target results in degradation of the mRNA as well as inhibition of translation (FIG. 1). Currently, it is believed that the vast majority of the gene-regulatory effects of miRNAs occur though mRNA decay rather than translational repression[18]. Recently, pseudogenes have also been implicated in regulating miRNA activity. Pseudogene transcripts containing conserved miRNA binding sites, referred to as competing endogenous RNAs (ceRNAs), act as decoys or sponges by sequestering miRNAs and preventing them from binding to their mRNA targets[19,20].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

An especially puzzling feature of miRNA biology has been the minimal effects of miRNA-mediated loss of function. Although lin-4, the first miRNA to be discovered, was identified on the basis of its strong loss-of-function phenotype in nematodes, systematic genetic deletions of miRNAs in this organism have revealed grossly abnormal phenotypes in less than 10% of miRNA-mutant animals[21]. Similarly, genetic analyses of miRNAs in mice have revealed relatively minor functions under conditions of homeostasis in controlled laboratory settings[22]. The lack of strong loss-of-function miRNA phenotypes might be explained by compensatory mechanisms that allow for re-balancing of protein networks when the expression of individual components is subtly altered as a consequence of the absence of a miRNA.

Redundancy among homologous miRNAs within families or the targeting of individual mRNAs by several miRNAs can also diminish the consequences of the loss of individual miRNAs. However, a general theme seems to be that the actions of miRNAs become pronounced under conditions of injury or stress. Thus, the elimination of some miRNAs sensitizes cells to stress, resulting in exacerbated pathology, whereas the absence of other miRNAs can confer resistance to stress. Stress signalling impinges on numerous points in the pathway of miRNA biogenesis and function (FIG. 1). There are many examples in which miRNA abundance increases or decreases in response to stress, and changes in the expression of specific miRNAs have been shown to be diagnostic for disease progression in cardiovascular disorders[23]. The heightened function of a miRNA during stress can also be explained by changes in the abundance of its mRNA targets or differences in miRNA activity under stress[24]. For example, a stress-induced reduction in the level of expression of a miRNA can have a disease-modifying effect by increasing the levels of downstream mRNA targets, whereas a stress-induced increase in miRNA expression will result in a decrease in levels of downstream targets. Conversely, changes in the expression of mRNA targets under disease conditions can influence the effect of the miRNA on the mRNA. Although the influence of stress is likely to be miRNA- and trigger-dependent, changes in miRNA expression and functionality under disease conditions imply the existence of an active mechanism for differential miRNA activity under stress.

Therapeutic inhibition of miRNAs

Genetic gain- and loss-of-function approaches, as well as pharmacological modulation of individual miRNAs or miRNA families in animal models of disease, point to miRNAs as key regulators of many disorders, including cardiovascular disease[23]. Their pathological roles, along with their pharmacological properties, have catalysed efforts to develop miRNA therapeutics. In principle, miRNA mimics can be used to elevate the expression of beneficial miRNAs in disease settings, whereas miRNA inhibitors can be delivered to block the activity of miRNAs that drive disease progression[25]. miRNA mimicry has lagged behind the development of antimiR chemistries owing to substantial challenges in their delivery and design. Currently, the most effective means of enhancing miRNA function or replacing miRNAs that are lost or downregulated in disease is through adeno-associated viruses (AAVs). The availability of numerous different AAV serotypes allows for potential tissue enrichment as a result of the natural tropism of each individual AAV serotype towards different organs, as well as the different cellular receptors with which each AAV serotype

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

interacts[26,27]. AAV type 6 and type 9, for example, display preferential tropism for skeletal muscle and heart, respectively, when delivered systemically in rodents[28–30]. Here, we focus on the pharmacokinetic and pharmacodynamic properties of current antimiR designs.

AntimiR chemistries.

miRNAs possess several features as potential drug targets that distinguish them from other therapeutic modalities. In contrast to small-molecule inhibitors identified in cell-based screens, in which target identification can pose substantial challenges, antimiRs act through complementary base pairing with miRNAs, so their direct target sequence in mRNAs is known — at least theoretically. However, it should also be emphasized that there is substantial ambiguity in miRNA target identification, in that sequences outside the miRNA 'seed region' — as well as the secondary structure of the mRNA target and the association of mRNAs with RNA-binding proteins — can influence target engagement[31]. Thus, it is essential to experimentally document the regulation of specific targets in response to changes in miRNA abundance in order to firmly establish their contributions to miRNA functions.

Several oligonucleotide chemistries allow for high-affinity interaction of antisense oligonucleotides with miRNAs, preventing their interaction with — and repression of — target mRNAs. To achieve effective pharmacological inhibition of disease-associated miRNAs, antimiR chemistries should contain chemical modifications to enhance binding affinity, confer nuclease resistance and facilitate cellular uptake. To increase nuclease resistance, most antimiR chemistries to date harbour phosphorothioate backbone linkages (FIG. 2). In addition to increasing stability, the phosphorothioate backbone modifications promote plasma protein binding, thereby reducing clearance of the antimiRs by glomerular filtration and urinary excretion, which facilitates tissue delivery of antimiRs *in vivo*[32–34]. AntimiRs containing cholesterol, conjugated via a 2′-*O*-methyl (2′-*O*-Me) linkage, named antagomirs, are fully complementary to the mature miRNA sequence and contain several phosphorothioate moieties to increase stability (FIG. 2). Cholesterol is thought to enhance cellular uptake and *in vivo* stability[32,33], as well as promote hepatic uptake of the antimiR while lowering delivery to other tissue types.

The antagomir chemistry has been especially useful as an experimental tool; however, all antimiR chemistries currently in development use unconjugated phosphorothioate antisense molecules with various additional high-affinity 2′ sugar modifications such as 2′-*O*-methoxyethyl (2′-MOE) and 2′-fluoro (2′-F) or locked nucleic acid (LNA) and LNA-like conformationally restricted nucleotides (FIG. 2). Although all of these modifications improve nuclease resistance and increase duplex melting temperature (T_m)[35], LNAs possess the highest affinity, with an increase in T_m of +2 °C to +8 °C per introduced LNA modification against complementary RNA. A 2′-MOE-modified antimiR-122 has shown efficacy in mice[36], and an LNA-modified antimiR-122 has been shown to be efficacious in non-human primates[37]. Moreover, LNA-modified antimiR-122 was recently shown to be efficacious in humans (see the ClinicalTrials.gov website). A 2′-F- and 2′-MOE-modified antimiR-33 was recently shown to be efficacious in reducing atherosclerosis in non-human primates[38].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The high T_m of LNA-modified oligonucleotides enables efficient miRNA inhibition with truncated antimiRs. Several studies have reported efficient miRNA inhibition *in vivo*, using high-affinity 15–16-nucleotide-long LNA–DNA mixmers targeting the 5′ region of mature miRNA. The high binding affinity of seed region-targeting tiny 8-mer LNA-modified antimiRs with a complete phosphorothioate backbone allows for *in vivo* delivery and silences miRNAs without the need for additional conjugation or formulation chemistries[13,39–41] (FIG. 2). Microarray analysis indicated that 8-mers and 15-mers evoked comparable changes in miR-122 targets in the liver and induced similar physiological responses[39]. However, as many miRNAs belong to families that differ by only a few bases in the 3′ regions, antimiRs that are complementary to the full-length miRNA sequence may have greater specificity towards individual miRNA family members than shorter antimiRs that are directed against 5′ regions of miRNAs.

Potential sources of toxicity after the administration of a miRNA inhibitor include unwanted gene changes or the effects of the antimiR on off-target, non-diseased tissues; the chemistry of the antimiR is also a common source of toxicity for oligonucleotide therapeutics. Phosphorothioate oligonucleotides, for example, can inhibit the tenase complex in the intrinsic clotting cascade[42], activate the alternative pathway of the complement cascade[43] and activate innate immunity. Additionally, although LNA-containing oligonucleotides have an increased potency for reducing the levels of mRNA targets, some signs of hepatotoxicity (determined by measuring serum levels of transaminases, liver weight and body weight) have been observed with LNA-modified antisense oligonucleotides directed against mRNAs. Histopathological evaluation of tissues from LNA-treated animals confirmed the hepatocellular involvement, suggesting that although LNA-modified antisense oligonucleotides have the potential to improve potency, they potentially create a risk of developing hepatotoxicity[44]. However, contrary to this report, no hepatotoxicity was observed in mice treated with LNA-modified antimiRs (at a dose of 75 mg per kg; three doses of 25 mg per kg per day), as shown by unaltered levels of the serum transaminases alanine aminotransferase and aspartate aminotransferase 24 hours after treatment (compared to saline controls) and by the absence of histological changes in liver sections from LNA-treated animals[45]. Further toxicity studies of chemically modified miRNA inhibitors will be required to establish safety parameters for the different antimiR chemistries. Different oligonucleotide sequences are also likely to display differences in toxicity, tissue distribution and efficacy.

Pharmacokinetic properties of antimiR chemistries.

The antimiR molecules that are currently being used to target miRNAs *in vivo* are highly water-soluble and have molecular masses of several kDa. Their size and charge limit intestinal absorption, thereby preventing them from becoming good candidates for oral administration. Thus far, successful delivery of chemically modified oligonucleotide antimiRs has been dependent on parenteral administration via intravenous (i.v.), intraperitoneal (i.p.) or subcutaneous (s.c.) injection. Unlike typical (low-molecular-mass) drugs, plasma levels of antimiR chemistries are largely cleared within hours after administration as a result of their uptake into tissues[34,46], except for a low level of antimiR that remains detectable in the circulation for several weeks[13,39]. The persistent presence of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the antimiR in the circulation probably reflects its association with circulating plasma proteins and/or the gradual release of antimiRs trapped in extracellular spaces.

Studies using northern blotting, enzyme-linked immunosorbent assay (ELISA)-based detection methods or fluorescently labelled oligonucleotides have shown that antimiRs containing the typical nucleic acid ribose sugar backbone with 2′ modifications are distributed broadly to most tissues but tend to accumulate in the kidney and liver[13,32,33,45,47]. However, it is becoming apparent that oligonucleotide sequence, length and chemical modifications affect cellular distribution and functionality, thus strongly influencing the pharmacokinetic and pharmacodynamic properties of each molecule[32,33,36,39].

Currently, relatively little is known about the mechanisms of cellular uptake, storage and mode of action of the different antimiR chemistries. Once inside cells, many modified antimiRs are extremely stable and have half-lives in the order of weeks[48]. Additionally, although systemically delivered antagomirs appear to accumulate in a cytoplasmic compartment distinct from P-bodies and induce miRNA degradation by an RNA interference (RNAi)-independent pathway[32,33], LNA-modified anti-miRs seem to inhibit miRNA function by sequestering the mature miRNA[45,47], which implies that the different antimiR chemistries have different modes of action.

In the context of this Review, although it is clear that oligonucleotide chemistries directed against miRNAs are taken up by, or at least reach, cardiac and vascular cell populations, it will be interesting to determine whether some cell types are more efficiently targeted and whether stress plays a part in cellular uptake. Another question that remains unanswered is where within the cell the antimiRs reside and whether all antimiRs are actually functional. Currently used doses provide a vast excess of antimiR copies relative to miRNA, and the long-lasting duration of effects suggests that there is a cellular reservoir that — over time — enables antimiRs to inhibit newly formed miRNAs, but it is unknown how the release of antimiRs from such cellular depots is controlled. Subcellular sites of antimiR storage, as well as the kinetics of release, may vary depending on the chemical modifications to the oligonucleotide.

Efficacy of antimiRs.

Although antimiR detection studies indicate that there is a rapid uptake of antimiR chemistries in many tissues, unless the antimiR is located within the appropriate cellular compartment, these assays can be deceptive as the *in vivo* activity of an antimiR is determined by its cellular distribution. Processing of tissue during experimental analysis can potentially introduce artificial binding between the antimiR and miRNA, which does not actually occur within the intact cell, leading to an overestimation of antimiR function and binding.

The impact of a miRNA on its target depends on the relative ratio of a miRNA to its target. miRNAs display a range of intracellular concentrations, with the most abundant miRNAs being expressed at up to tens of thousands of copies per cell[49]. Strategies to inhibit miRNA function and thereby de-repress expression of their targets are based on the presumption that relatively modest increases in the expression of targets are sufficient to evoke substantial

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

therapeutic benefits. In this regard, the mRNA targets of miRNAs have been shown to display threshold effects such that the mRNA can be efficiently repressed when it is present at relatively low levels compared to the miRNA, but when the mRNA reaches such a level that the miRNA is not able to inhibit it, the biological impact of the miRNA becomes diminished[50].

In order to demonstrate the efficacy of target engagement by antimiRs *in vivo*, it is necessary to measure target de-repression. This task is burdened by the fact that target regulation in general is modest, ranging — on average — from a 20% to 50% change in mRNA expression, making it difficult to determine significant changes above naturally occurring variations in gene expression[18,51]. Additionally, proteomic studies in response to miRNA modulation have reported that the average changes in protein levels of miRNA targets are less than twofold following miRNA inhibition[52,53]. Target de-repression is often dependent on stress conditions, whereby both the severity and the type of cellular stress influence whether an mRNA is regulated by a miRNA. This is probably due to an abundance of mRNA targets and the expression of cofactors regulating the activity of miRNAs (FIG. 1).

The time course and nature of the pharmacological effects (or pharmacodynamics) of antimiRs are often complicated by the fact that the pharmacology is the summation of the de-repression of gene families and downstream events caused by the de-repression of multiple target genes controlled by that particular miRNA. Thus, the onset of the functional efficacy of antimiRs can be delayed by days or weeks following initial administration of the drug, as reported for the miR-122 inhibitor and its effects on serum cholesterol levels[9]. Delays in pharmacodynamic effects are atypical for most low-molecular-mass drugs and are more akin to so-called disease-modifying agents wherein it is established that pharmacological activity is related to the sum of pharmacological effects over time that cause a change in the disease phenotype. The delayed actions of at least some antimiRs suggest that this class of potential drugs may be better suited for chronic rather than acute disease indications. It should be noted that in these same studies it was shown that the downstream effect of antimiR treatment appeared to be reversible[45,47].

miRNA targets in cardiovascular disease

Although a long-standing dogma in the small interfering RNA (siRNA) field has been that the heart is resistant to oligonucleotide uptake, it has been well established that the heart effectively takes up exogenous DNA[54]. Indeed, several studies have demonstrated effective cardiac delivery and miRNA inhibition using antimiR chemistries and have indicated the potent effects of miRNA inhibition under disease conditions. Below, we present some recent representative examples of the therapeutic effects of antimiRs in the cardiovascular system (TABLE 1).

Control of pathological cardiac remodelling and obesity by miR-208.

Myosin heavy chain protein (encoded by *MYH* genes) is the major contractile protein of striated muscles, and myosin 6 (encoded by *MYH6*; also known as MyHC-α) is the predominant myosin isoform expressed in the adult rodent heart. An intron of this gene encodes miR-208a, which — like the host gene — is expressed specifically in the heart[55,56].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Two related miRNAs, miR-208b and miR-499, are encoded by introns of the *MYH7* (encoding myosin 7; also known as MyHC-β) and *MYH7B* genes, respectively, which are expressed specifically in cardiac and slow skeletal muscle[57]. Knockout mice lacking each of these miRNAs (referred to as myomiRs) are viable, but miR-208b-miR-499 double-knockout mice display a reduction in slow myofibres and a corresponding increase in fast myofibres, which is consistent with a role for these miRNAs in the regulation of myofibre switching[57].

Most relevant to cardiac disease is the finding that miR-208a-knockout mice display reduced fibrosis and hypertrophy in response to cardiac stress, and fail to upregulate myosin 7 expression, which is a sensitive marker of pathological cardiac remodelling[56]. These findings initially suggested that therapeutic inhibition of miR-208a might evoke similar benefits in settings of heart disease. Indeed, Montgomery *et al.*[48] showed that subcutaneous delivery of an LNA-modified oligonucleotide inhibitor directed against miR-208a induced potent and functional inhibition of the cardiomyocyte-specific miR-208a. Inhibition of miR-208a suppresses fibrosis, diminishes myosin 7 expression and improves survival in Dahl salt-sensitive rats, which are susceptible to diastolic dysfunction when maintained on a high-salt diet[48]. These findings provide proof-of-concept support for the potential therapeutic benefit of antimiR-208a agents in the setting of heart disease. This study also showed that the antimiR was detectable in the heart for up to 6 weeks following i.v., s.c. or i.p. delivery (whereas higher levels of the antimiR were detected in the kidney and liver) and that a low level of the antimiR remained detectable in the plasma for a comparable period[48]. Interestingly, the downregulation of myosin 7 expression in the heart in response to antimiR-208a was delayed[48], which mirrors the delay in cholesterol lowering after miR-122 inhibition. Whether oligonucleotide inhibition of miR-208a confers cardiac benefits in forms of heart disease beyond diastolic dysfunction remains to be determined.

Gene profiling studies have shown that the expression of a cohort of predicted mRNA targets is elevated in response to miR-208a inhibition *in vivo*, and many of these mRNA targets have unknown functions in the heart[48]. Although such genes serve as sensitive biomarkers of antimiR efficacy, their unknown functions underscore the challenges associated with establishing the mechanistic basis of the therapeutic efficacy of antimiR-208a.

Unexpectedly, mice treated with antimiR-208a display resistance to obesity, especially when on a high-fat diet[58]. This effect occurs in the absence of detectable toxicity and suggests that the heart has a role in systemic metabolic homeostasis and energy expenditure via a miR-208a-dependent mechanism. Among the strongest predicted and validated targets of miR-208a is mediator of RNA polymerase II transcription subunit 13 (MED13; also known as THRAP1), a component of the mediator complex, which interconnects RNA polymerase II at gene promoters with distal enhancers. Other mediator components have been shown to associate with numerous nuclear hormone receptors and to have key roles in metabolic control[59–61]. It is especially intriguing, in this regard, that cardiac-specific overexpression of MED13 confers resistance to obesity, as observed with miR-208a inhibition. Conversely, cardiac-specific deletion of MED13 results in obesity. These findings raise the possibility of developing antimiRs against miR-208a as therapeutic modifiers of metabolic syndrome

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(FIG. 3). In this regard, other miRNAs, including miR-103, miR-107, miR-33 and let-7, have been implicated in systemic metabolic control in mice[38,62–64].

Control of cardiac hypertrophy and fibrosis by miR-21.

Studies of miR-21 in heart disease illustrate the potential disease-modifying actions, as well as the many unknown responses, associated with therapeutic targeting of miRNAs. miR-21 is upregulated in cardiac fibroblasts in response to pathological stresses that promote cardiac fibrosis. Systemic delivery of cholesterol-modified antagomirs directed against miR-21 has been reported to prevent fibrosis and cardiac hypertrophy while preserving cardiac contractility under conditions of pressure overload. Remarkably, miR-21 antagomirs can apparently also cause regression of fibrosis and restore function in severely injured hearts. These salutary effects of miR-21 inhibition have been attributed to the regulation of a single target, Sprouty 1 (SPRY1); SPRY 1 acts as an inhibitor of mitogen-activated protein kinase (MAPK) signalling, which is a driver of cardiac dysfunction[9]. According to this model, upregulation of miR-21 in response to stress would be predicted to inhibit SPRY1 expression, thereby promoting MAPK signalling, whereas inhibition of miR-21 would have the opposite effect[9].

There is also mounting evidence for the involvement of miR-21 in fibrosis of other tissues[65–68] as well as in cancer[69]. Systemic delivery of miR-21 inhibitors, for example, has shown efficacy in suppressing extracellular matrix production in settings of muscular dystrophy, renal fibrosis and pulmonary fibrosis, suggesting that miR-21 occupies a central node in pathways of pathological fibrosis. The suppression of tumorigenesis by miR-21 inhibitors[69] appears to result from the regulation of different mRNA targets and exemplifies the ability of individual miRNAs to modulate distinct cellular processes depending on the physiological status of the cell and the abundance and availability of targets.

In contrast to these findings, mice with genetic deletion of miR-21 show no diminution in fibrosis in response to multiple cardiac stresses, indicating that miR-21 does not have an obligatory role in this pathological process[41]. Additionally, different antimiR chemistries show different functional outcomes with respect to miR-21 inhibition[41,70]. For example, a 22-mer antimiR-21 modified by cholesterol, 2′-F or 2′-MOE prevented cardiac fibrosis and loss of cardiac contractility in mice subjected to thoracic aortic banding (TAB), whereas an 8-mer tiny LNA-modified antimiR-21 oligonucleotide showed no efficacy in suppressing fibrosis or preventing cardiac dysfunction following TAB in mice, despite forming a stable heteroduplex with the miR-21 target miRNA and inducing downstream target de-repression[41,70]. The basis for the seemingly contradictory conclusions regarding the role of miR-21 in fibrosis has not been resolved, but there are several potential explanations. It is conceivable, for example, that genetic deletion of miR-21 throughout embryonic and postnatal stages allows for compensatory mechanisms that substitute for miR-21 function, and such mechanisms are not deployed under conditions of acute miR-21 inhibition with antagomirs. Alternatively, or in addition, individual antimiR-21 chemistries might have a different mode of action, hence resulting in a different outcome.

Studies by Care *et al.*[71] and Liu *et al.*72 further document the discrepancies in phenotypic outcomes resulting from oligonucleotide-based miRNA inhibition versus genetic deletion of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

a miRNA. In this case, antagomir-mediated inhibition of miR-133 induced cardiac hypertrophy in mice[71], whereas genetic deletion of miR-133 resulted in partial lethality and a dilated cardiomyopathy in the survivors[72].

Control of cardiomyocyte apoptosis and regeneration by the miR-15 family.

Members of the miR-15 family, which includes miR-15a, miR-15b, miR-16-1, miR-16-2, miR-195 and miR-497, have been implicated in cell cycle arrest and cell survival in several cell types, by regulating many anti-apoptotic and cell cycle genes[73]. Members of this miRNA family are upregulated in the heart in response to cardiac stress and myocardial infarction, which cause death of cardiomyocytes and loss of pump function[10]. Moreover, forced overexpression of miR-195 in the heart is sufficient to cause myocyte loss and heart failure, indicating that this miRNA family is a crucial component of heart disease pathogenesis[1]. The multiplicity of miR-15 family members exemplifies one of the challenges associated with miRNA inhibition as a therapeutic strategy, as sequence divergence among different members of miRNA families prevents their collective inhibition by the delivery of a single antisense oligonucleotide inhibitor.

One approach to potentially overcome such miRNA redundancy is the use of tiny 8-mer LNA-modified inhibitors, which target the conserved seed regions of miRNAs and thereby enable inhibition of co-expressed miRNA family members that may have redundant biological functions (FIG. 2). A recent report showed that an 8-mer directed against the seed region of the miR-15 family was able to target mutiple members of the miR-15 family, which all contain a similar seed region but different 3′ regions[13]. In comparison with an LNA-modified 16-mer preferentially targeting miR-15b, the 8-mer was more efficacious in eliciting downstream target de-repression, whereas both chemistries were equally distributed to cardiac tissue in mice and pigs. Mouse efficacy studies, using a model of ischaemia reperfusion, showed that inhibition of the miR-15 family reduced infarct size and improved cardiac function 2 weeks after ischaemic damage[13].

Interestingly, miR-15 family members are also upregulated in the heart during the first few days after birth[74]. Recent studies have shown that the neonatal mouse heart is capable of complete regeneration following amputation of the apex or ischaemic injury during the period before miR-15 family upregulation[75], and precocious upregulation of miR-195 in the heart suppresses this neonatal regenerative response. The inhibitory influence of miR-195 on heart muscle regeneration appears to be attributable to the inhibition of a cohort of pro-proliferative proteins[74]. Thus, antimiR-mediated inhibition of miR-15 family members represents an intriguing strategy to enhance cardiac repair following injury.

Control of stress-induced neoangiogenesis by miR-92a.

AntimiR strategies have also been effective in targeting miRNAs that are preferentially expressed in endothelial and smooth muscle cells. miR-92a, a member of the miR-17-miR-92 cluster, has been implicated in neoangiogenesis following ischaemic injury[6]. Intravenous administration of a miR-92a antagomir showed efficacious inhibition of the miRNA, and resulted in enhanced blood vessel growth as well as functional improvement of damaged tissue in models of hindlimb ischaemia and myocardial infarction[6]. The

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

neoangiogenic effect of inhibiting miR-92a *in vivo* was attributed to the de-repression of several pro-angiogenic factors, including integrin α5 — a direct target of miR-92a. Although the pro-angiogenic effect of miR-92a inhibition is currently being explored as a potential therapeutic approach in diseases such as ischaemic heart disease or peripheral artery disease, additional studies have shown that miR-92a regulates the atheroprotective transcription factors Krüppel-like factor 2 (KLF2) and KLF4 (REFS 76,77). KLF2 and KLF4 regulate gene networks that confer atheroprotective properties to the endothelium through an anti-inflammatory and anticoagulant effect[78] and, as such, antimiR-92a might exert comparable properties.

Control of extracellular matrix deposition by the miR-29 family.

A fibroblast-enriched miRNA family, miR-29, has shown strong coordinate effects by directly regulating the expression of at least 16 confirmed extracellular matrix genes[79]. The miR-29 family consists of miR-29a, miR-29b and miR-29c, which are expressed as two bicistronic clusters (miR-29a–miR-29b1 and miR-29b2–miR-29c) and are largely homologous in sequence, with several mismatches between the different members in the 3′ regions of the mature miRNA[10]. Members of the miR-29 family are downregulated in response to myocardial infarction in mice, specifically in the infarcted region, and their downregulation correlates with increased expression of extracellular matrix-related genes required for infarct healing[10]. Members of this miRNA family are also downregulated during pathological cardiac remodelling[1]. A cholesterol-conjugated miR-29 antagomir was detected in several tissues after delivery by i.v. injection in mice, which resulted in de-repression of several direct target genes[10]. The presence in the genome of four different copies of this miRNA with a comparable set of predicted targets, in combination with the fact that many of these verified targets are involved in extracellular matrix deposition, strongly supports a potent influence of miR-29 on fibrosis (FIG. 4). miR-29 mimicry or overexpression could have therapeutic benefit in different forms of tissue fibrosis by inducing a subsequent decrease in the expression of fibrosis-related genes, as has been shown for heart[10], kidney[80–82], liver[83–85], lung[86] and systemic sclerosis[87].

The obvious importance of this miRNA family was further demonstrated by recent reports on miR-29 inhibition for vascular indications. Levels of miR-29 were found to be upregulated in two animal models of aortic dilation as well as in biopsy samples of human thoracic aneurysms, which correlated with a profound downregulation of numerous extracellular matrix components[7,88]. LNA-modified oligonucleotides against miR-29 were able to abrogate aortic dilation induced by angiotensin II in mice, which suggests that miR-29 inhibition could have a role in maintaining vascular integrity during aneurysm formation[7]. These data were confirmed by studies in two additional models of aortic aneurysms, showing that antimiR-29 induced an increase in collagen expression, resulting in a significant reduction in abdominal aneurysm progression[88]. The concept of miR-29 inhibition leading to enhanced vascular integrity was further supported by data showing that miR-29 has a role in early aneurysm formation in a mouse model of Marfan syndrome[89]. Finally, inhibition of miR-29 can dramatically increase elastin expression in cells from patients with elastin haploinsufficiencies, such as Williams–Beuren syndrome, which is characterized by aortic stenosis[90]. Combined, these data support miR-29 inhibition as a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

therapeutic approach in various vascular indications such as aneurysm formation; however, even though short-term treatment with antimiR-29 does not appear to result in liver or kidney fibrosis[7], adverse effects of stimulating extracellular matrix deposition should be considered in more chronic treatment regimens.

Control of viral myocarditis by miR-155.

miR-155 is processed from an exon of a non-coding RNA transcribed from the B cell integration cluster (BIC), which is highly expressed in activated B and T cells as well as in monocytes and macrophages[91]. miR-155 levels change dynamically during both haematopoietic lineage differentiation and the course of the immune response, and to date miR-155 has been implicated in several diseases, including cancer, cardiovascular disease and viral infections[92,93]. With regard to cardiovascular disease, it was discovered that a single nucleotide polymorphism (+1166A/C) in the human angiotensin II type 1 receptor (AT1), the receptor through which angiotensin II exerts most of its actions, is associated with hypertension, cardiac hypertrophy and myocardial infarction, and disrupts a miR-155 binding site so it can no longer reduce AT1 expression[94]. Additional data suggesting that miR-155 might be involved in regulating blood pressure came from a study on homozygous twins discordant for trisomy 21. The participants of this study expressed elevated levels of miR-155 (located on chromosome 21), which correlated with a lower level of AT1 expression and low blood pressure[95].

Although it has been well established that miR-155 regulates myeloid and lymphoid immune cell function[96], it has recently been shown that miR-155 is also upregulated during the acute inflammatory phase of viral myocarditis[97]. Acute viral myocarditis is an important cause of heart failure, resulting from infection of the heart by cardiotrophic viruses. *In situ* hybridization of heart tissue suffering from acute viral myocarditis indicates that the increase in miR-155 expression is induced by haematopoietic cells and all leukocyte subtypes, including macrophages and T cells. Therapeutic treatment with an antimiR against miR-155 elicited an increase in levels of miR-155 targets, and detection of 6-carboxyfluorescein (FAM)-labelled antimiR indicated a distribution of miR-155 to cardiomyocytes, endothelial borders as well as circulating monocytes, lymphocytes and neutrophils. Moreover, antimiR-155 treatment resulted in reduced myocardial damage and increased survival in response to viral myocarditis. This effect is likely to be immune-cell-autonomous as miR-155 inhibition did not affect viral load, myocyte viability or cytokine signalling in unstressed hearts before their infiltration by immune cells[97]. Although the authors did not observe an effect of antimiR treatment on cardiac fibrosis through a potential de-repression of AT1, this will have to be taken into account when considering antimiR-155 as a therapy for viral myocarditis.

Control of angiogenesis and vascular stability by miR-23, miR-27 and miR-24.

Another miRNA cluster with prominent roles in vascular indications is the cluster composed of miR-23, miR-27 and miR-24. The human genome contains two versions of this cluster, both of which are enriched in endothelial cells[98,99]. Members of this cluster are involved in cell cycle control as well as proliferation and differentiation of various cell types, and have important functions during angiogenesis[98,99].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Choroidal neovascularization, which is characterized by abnormal vascular growth in the back of the eye, is a hallmark of age-related macular degeneration. A recent study by Zhou *et al.*99showed that miR-23 and miR-27 are upregulated during normal retinal vascular development and in response to laser-induced choroidal neovascularization. *In vivo* studies using LNA-modified antimiRs against both miR-23 and miR-27 demonstrated that inhibition of both miRNAs resulted in increased levels of SPRY2, semaphorin 6A and semaphorin 6D, which repressed neovascularization in the choroid in response to laser injury[99]. Interestingly, *in vitro* inhibition of either miR-23 or miR-27 in human umbilical vein endothelial cells caused a corresponding increase in miR-27 or miR-23 levels, respectively. *In vivo* inhibition of both miR-23 and miR-27 resulted in an increase in miR-24 levels. These effects are probably biological rather than chemical, as a scrambled oligonucleotide control with a comparable chemical composition did not induce this effect. Although this study did not present *in vivo* data after inhibition of either miR-23 or miR-27 alone, the *in vitro* data — based on target de-repression — seem to suggest that miR-27 is the major player. From a therapeutic standpoint, it would be a developmental challenge to investigate two compounds in parallel, and targeting two separate miRNAs could increase the risk of off-target effects.

The third member of the cluster, miR-24, was recently implicated in cardiac angiogenesis after myocardial infarction[100]. Cardiac ischaemia was shown to increase miR-24 levels in endothelial cells, and antagomir-based inhibition of miR-24 in endothelial cells reduced infarct size by preventing apoptosis and enhancing vascularity, probably through direct regulation of the transcription factors GATA2 and p21-activated kinase 4 (PAK4). Interestingly, in tracing the cellular fate of the antagomir by Cy labelling, the authors found that at a high dose (80 mg per kg) the antagomir was taken up by both cardiomyocytes and endothelial cells, whereas at a lower dose (5 mg per kg) the antagomir was preferentially taken up by endothelial cells[100]. This might be relevant as it was previously shown that increased levels of miR-24 might have a cardioprotective effect in myocytes during an ischaemic event by decreasing the expression of the pro-apoptotic protein BIM (BCL-2 interacting mediator of cell death)[101]. These findings further highlight the potential functional differences of miRNAs in individual cell types, which should be taken into account when considering miR-24 as a therapeutic target.

Control of pulmonary hypertension by miR-145.

Vascular smooth muscle cell (VSMC)-enriched miR-145 is expressed as part of the miR-143–miR-145 cluster[102]. Genetic deletion of this cluster showed that these miRNAs are essential for VSMC contractility[12,102,103]. Although miR-143 and miR-145 have distinct sequences, they coordinately regulate overlapping genes but also target different genes with regulatory functions in actin remodelling. Individual deletion of the miRNAs in mice indicated a prominent and distinct role for miR-145 in VSMC contractility, as this miRNA appeared to be mainly responsible for the thinning of the VSMC layer. Blood pressure analysis in these genetic models showed that removal of the cluster induced arterial hypotension at the baseline and reduced the increase in systolic pressure in response to angiotensin II[12,102].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

In lung samples of patients with both idiopathic and heritable pulmonary hypertension, miR-145 levels are upregulated in comparison with unaffected controls. Using chronic hypoxia as a model for pulmonary arterial hypertension in mice showed that antimiR-mediated inhibition of miR-145 reduced systolic right ventricular pressure[8]. Histological analysis supported these findings by showing that antimiR-145 reduced pulmonary vascular remodelling in response to hypoxia[8]. Although these initial data are encouraging and suggest that miR-145 inhibition has therapeutic potential in the setting of pulmonary arterial hypertension, genetic deletion of miR-145 resulted in a more pronounced phenotype as it reduced both blood pressure and right ventricular remodelling, which might indicate that the timing and incidence of dosing requires optimization. Additionally, as genetic deletion of the miRNA cluster indicates a more supportive function of this cluster in maintaining a normal VSMC phenotype and regulating normal contractility of arteries[12], caution should be taken when applying antimiR strategies to more chronic diseases, and local delivery options should be contemplated.

Challenges in clinical development

The preclinical studies discussed above indicate that all cardiovascular cell types can be targeted by miRNA inhibitors, and inhibition of miRNAs can have profound effects on cardiovascular function, supporting enthusiasm for further exploration of miRNAs as novel drug candidates. However, numerous challenges and questions remain in the path towards the development of miRNA-based therapeutics in general. In most of the animal studies to date, the phenotypic effects of miRNA inhibition have only been studied in the target tissue of interest, which might overlook off-target effects in additional tissues. Moreover, the doses used in most studies are unlikely to be therapeutically feasible. Follow-up preclinical studies will have to guide appropriate dosing regimens in order to establish the lowest possible efficacious doses while attempting to prevent unacceptable side effects.

The identification and validation of miRNA targets is especially relevant to the development of miRNA-based therapeutics in general. In contrast to many other therapeutic modalities, antimiR drugs are designed with the understanding that they will affect all genes that are under the control of the target miRNA. Although miRNAs often target many related genes involved in cellular processes, which are intended to be manipulated by the antimiR therapeutic (FIG. 4), a single miRNA will probably also target unrelated genes and possibly produce unexpected (and sometimes undesired) changes in gene expression. An excellent example of the diverse, and potentially unanticipated, actions of miRNAs was provided by the finding that antimiR therapeutics directed against the cardiac-specific miR-208a prevented obesity and metabolic syndrome in mice maintained on a high-fat diet[58]. Before these findings, miR-208a was thought to function primarily to modulate myosin switching in striated muscle cells[55–57]. However, mechanistic follow-up of the metabolic effects of miR-208a inhibitors revealed a fascinating function of this miRNA in the regulation of a network of transcription factors that are involved in metabolic control and energy homeostasis. The potential pleiotropy of miRNA actions contrasts with the mechanistic basis of most classical drugs, which act with maximal specificity against single cellular targets. Additional ambiguity derives from the relatively modest inhibitory effects of individual miRNAs on mRNA targets, as revealed in miRNA loss-of-function studies. It is not

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

uncommon, for example, for miRNA inhibition to result in minimal increases (<1.5-fold) in the expression of mRNA targets, suggesting that it is the cumulative impact of small changes in the expression of myriad targets — rather than pronounced changes in single targets — that mediates the biological actions of miRNAs on disease processes. Thus, it is often difficult or impossible to ascribe the effects of a miRNA to the regulation of a specific mRNA target. The multiplicity of miRNA targets also raises crucial issues with respect to possible off-target effects of miRNA inhibitors in different tissues and opposing actions of miRNAs on different phenotypic readouts. For example, if a miRNA displays broad tissue distribution, its systemic inhibition may have multiple effects in different tissues, thus confounding the interpretation of the responses to miRNA-based therapeutics. In addition, a miRNA may exert counterbalancing actions on cellular pathways such that the inhibition of a miRNA could, in principle, have both beneficial and deleterious consequences in the same or different tissues. Because miRNAs often control multiple components of complex regulatory networks, modulation of a specific miRNA — either up- or downregulation — can result in re-balancing of the network. In this respect, the multiplicity of miRNA targets can minimize bypass or desensitization mechanisms that can diminish the efficacy of certain drugs directed against single targets.

Despite these potential challenges, antimiR therapeutics have recently become a reality, following the successful Phase I and Phase II clinical trials of Santaris Pharma's antimiR against miR-122, miravirsen, which is currently in development for the treatment of HCV (BOX 1). It is hoped that these promising findings will accelerate the development of miRNA-targeting therapeutics into additional disease areas such as cardiovascular disease.

miRNAs have also been found in plasma and blood samples and have emerged as potential biomarkers for disease. However, many questions remain regarding the basis for the altered expression of such biomarkers, their relationship to disease progression and their power for predicting disease progression and clinical outcome.

In conclusion, the obvious relevance and importance of miRNAs during disease, in addition to the success of the first clinical data showing the safety and efficacy of an antimiR, support enthusiasm for the continued exploration of miRNAs as a new class of drug targets.

Acknowledgements

We gratefully acknowledge J. Cabrera for graphics.

Glossary

microRNAs

(miRNAs). Short, non-coding RNAs that suppress protein expression by most commonly binding to complementary sequences located within 3′ untranslated regions of target mRNAs.

Hepatitis C virus

(HCV). An infectious disease primarily affecting the liver.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

RNA-induced silencing complex

(RISC). A multiprotein complex that incorporates a microRNA to recognize complementary sequences in mRNAs and block protein expression.

Pseudogenes

Non-coding transcripts that are often conserved across species and contain conserved microRNA binding sites that can act as decoys to interfere with microRNA activity.

AntimiRs

Antisense oligonucleotides designed to target specific microRNAs.

Pharmacokinetic

In the context of antimiR (microRNA inhibitor) designs, this refers to the potency of an antimiR in binding to and inhibiting a microRNA *in vivo*

Pharmacodynamic

In the context of antimiR (microRNA inhibitor) designs, this refers to mRNA de-repression of direct microRNA targets in response to treatment with an antimiR.

Phosphorothioate

Backbone linkage in which a sulphur atom replaces one of the non-bridging oxygen atoms in the phosphate group of oligonucleotides to increase nuclease resistance.

Antagomir

A cholesterol-conjugated antimiR (microRNA inhibitor) chemistry that is complementary to the full-length sequence of the target microRNA, consisting of 2′-*O*-methyl (2′-*O*-Me) linkages and containing several phosphorothioate moieties to increase stability.

2′ sugar modifications

High-affinity 2′ sugar modifications such as 2′-*O*-methyl (2′-*O*-Me), 2′-*O*-methoxyethyl (2′-MOE), 2′-fluoro (2′-F) or locked nucleic acid; used in oligonucleotide chemistries to improve nuclease resistance and increase duplex melting temperature (T_m).

Locked nucleic acid

(LNA). A 2′ sugar modification in which the ribose is locked in a C3′-endo conformation by the introduction of a 2′-*O*-,4′-C methylene bridge that strongly increases the affinity for complementary RNA and increases the duplex melting temperature (T_m) by + 2° C to + 8° C per introduced LNA modification.

Duplex melting temperature

(T_m). The temperature at which half of a particular duplex dissociates and becomes single-stranded; in this case the temperature at which an antimiR (a microRNA inhibitor) will dissociate from complementary RNA.

Tiny

A locked nucleic acid (LNA)-containing 8-mer antimiR (microRNA inhibitor) with a complete phosphorothioate backbone targeting the seed region of a microRNA or microRNA family.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

P-bodies

Cytoplasmic sites of mRNA turnover. Also referred to as stress granules.

MyomiRs

The collection of microRNAs co-expressed with three different myosin heavy chain (*MYH*) genes: *MYH6* (miR-208a), *MYH7* (miR-208b) and *MYH7B* (miR-499).

MED13

Mediator of RNA polymerase II transcription subunit 13 (also known as THRAP1); a component of the mediator complex that can associate with numerous nuclear hormone receptors and have key roles in metabolic control. MED 13 is regulated by the microRNA miR-208.

Aneurysm

An abnormal widening or ballooning of a portion of an artery resulting from weakness in the wall of the blood vessel.

Marfan syndrome

A systemic connective tissue disorder in which aortic aneurysm formation is the leading cause of death.

Angiotensin II type 1 receptor

(AT1). The receptor through which angiotensin II exerts most of its cardiac actions. This receptor has been associated with hypertension, cardiac hypertrophy and myocardial infarction, and is a direct target of the microRNA miR-155.

Viral myocarditis

Inflammation of the heart that can lead to cardiomyopathy.

Miravirsen

Pharmacological name for antimiR-122 (a microRNA inhibitor targeting miR-122), which has shown therapeutic efficacy in clinical trials by providing long-lasting suppression of viraemia.

References

1. van Rooij E et al. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc. Natl Acad. Sci. USA 103, 18255–18260 (2006). [PubMed: 17108080] This is the first description of changes in miRNA levels in murine and human heart disorders, and provides evidence for the relevance of miRNAs in heart disease.
2. Ikeda S et al. Altered microRNA expression in human heart disease. Physiol. Genomics 31, 367–373 (2007). [PubMed: 17712037] This study describes unique miRNA signature patterns for different forms of human heart disease.
3. Thum T et al. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. Circulation 116, 258–267 (2007). [PubMed: 17606841] This study suggests a relationship between changes in miRNAs and the expression of fetal genes in human heart disease.
4. Bonauer A, Boon RA & Dimmeler S Vascular microRNAs. Curr. Drug Targets 11, 943–949 (2010). [PubMed: 20415654]
5. Zampetaki A & Mayr M MicroRNAs in vascular and metabolic disease. Circ. Res 110, 508–522 (2012). [PubMed: 22302757]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

6. Bonauer A et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 324, 1710–1713 (2009). [PubMed: 19460962]
7. Boon RA et al. MicroRNA-29 in aortic dilation: implications for aneurysm formation. Circ. Res 109, 1115–1119 (2011). [PubMed: 21903938]
8. Caruso P et al. A role for miR-145 in pulmonary arterial hypertension. Circ. Res 111, 290–300 (2012). [PubMed: 22715469]
9. Thum T et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 456, 980–984 (2008). [PubMed: 19043405]
10. van Rooij E et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc. Natl Acad. Sci. USA 105, 13027–13032 (2008). [PubMed: 18723672]
11. Wang S et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell 15, 261–271 (2008). [PubMed: 18694565]
12. Xin M et al. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev. 23, 2166–2178 (2009). [PubMed: 19720868]
13. Hullinger TG et al. Inhibition of miR-15 protects against cardiac ischemic injury. Circ. Res 110, 71–81 (2012). [PubMed: 22052914]
14. Mutharasan RK, Nagpal V, Ichikawa Y & Ardehali H microRNA-210 is upregulated in hypoxic cardiomyocytes through Akt- and p53-dependent pathways and exerts cytoprotective effects. Am. J. Physiol. Heart Circ. Physiol 301, H1519–H1530 (2011). [PubMed: 21841015]
15. Bartel DP MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233 (2009). [PubMed: 19167326] This is a key review explaining miRNA biogenesis and function.
16. Orom UA, Nielsen FC & Lund AH MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. Mol. Cell 30, 460–471 (2008). [PubMed: 18498749]
17. Tay Y, Zhang J, Thomson AM, Lim B & Rigoutsos I MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455, 1124–1128 (2008). [PubMed: 18806776]
18. Guo H, Ingolia NT, Weissman JS & Bartel D P Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466, 835–840 (2010). [PubMed: 20703300]
19. Salmena L, Poliseno L, Tay Y, Kats L & Pandolfi PP A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146, 353–358 (2011). [PubMed: 21802130]
20. Tay Y et al. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 147, 344–357 (2011). [PubMed: 22000013]
21. Miska EA et al. Most *Caenorhabditis elegans* microRNAs are individually not essential for development or viability. PLoS Genet. 3, e215 (2007). [PubMed: 18085825]
22. Park CY, Choi YS & McManus MT Analysis of microRNA knockouts in mice. Hum. Mol. Genet 19, R169–R175 (2010). [PubMed: 20805106]
23. Small EM, Frost RJ & Olson EN MicroRNAs add a new dimension to cardiovascular disease. Circulation 121, 1022–1032 (2010). [PubMed: 20194875]
24. Leung AK & Sharp PA MicroRNA functions in stress responses. Mol. Cell40, 205–215 (2010). [PubMed: 20965416]
25. Stenvang J, Petri A, Lindow M, Obad S & Kauppinen S Inhibition of microRNA function by antimiR oligonucleotides. Silence 3, 1 (2012). [PubMed: 22230293]
26. Kota J et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell 137, 1005–1017 (2009). [PubMed: 19524505]
27. Miyazaki Y et al. Viral delivery of miR-196a ameliorates the SBMA phenotype via the silencing of CELF2. Nature Med. 18, 1136–1141 (2012). [PubMed: 22660636]
28. Bish LT et al. Adeno-associated virus (AAV) serotype 9 provides global cardiac gene transfer superior to AAV1, AAV6, AAV7, and AAV8 in the mouse and rat. Hum. Gene Ther 19, 1359–1368 (2008). [PubMed: 18795839]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

29. Gao G et al. Adenovirus-based vaccines generate cytotoxic T lymphocytes to epitopes of NS1 from dengue virus that are present in all major serotypes. Hum. Gene Ther 19, 927–936 (2008). [PubMed: 18788905]
30. Zincarelli C, Soltys S, Rengo G & Rabinowitz JE Analysis of AAV serotypes 1–9 mediated gene expression and tropism in mice after systemic injection. Mol. Ther 16, 1073–1080 (2008). [PubMed: 18414476]
31. Pasquinelli AE MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. Nature Rev. Genet 13, 271–282 (2012). [PubMed: 22411466] This paper reviews the different influences of stress on miRNA function.
32. Krutzfeldt J et al. Specificity, duplex degradation and subcellular localization of antagomirs. Nucleic Acids Res. 35, 2885–2892 (2007). [PubMed: 17439965]
33. Krutzfeldt J et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature 438, 685–689 (2005). [PubMed: 16258535] This is the first study to show that antimiRs can be used to silence miRNAs *in vivo* using antagomirs.
34. Levin AA A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. Biochim. Biophys. Acta 1489, 69–84 (1999). [PubMed: 10806998]
35. van Rooij E, Purcell AL & Levin AA Developing microRNA therapeutics. Circ. Res 110, 496–507 (2012). [PubMed: 22302756]
36. Esau C et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab. 3, 87–98 (2006). [PubMed: 16459310]
37. Lanford RE et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 327, 198–201 (2010). [PubMed: 19965718] This paper describes the therapeutic efficacy of antimiR-based inhibition of miR-122 in primates infected with HCV.
38. Rayner KJ et al. Inhibition of miR-33a/b in nonhuman primates raises plasma HDL and lowers VLDL triglycerides. Nature 478, 404–407 (2011). [PubMed: 22012398] This study shows the therapeutic benefit of antimiR-mediated inhibition of miR-33 in non-human primates in increasing plasma levels of high-density lipoprotein (HDL) and lowering very-low-density lipoprotein (VLDL) triglycerides.
39. Obad S et al. Silencing of microRNA families by seed-targeting tiny LNAs. Nature Genet. 43, 371–378 (2011). [PubMed: 21423181]
40. Garchow BG et al. Silencing of microRNA-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. EMBO Mol. Med 3, 605–615 (2011). [PubMed: 21882343]
41. Patrick DM et al. Stress-dependent cardiac remodeling occurs in the absence of microRNA-21 in mice. J. Clin. Invest 120, 3912–3916 (2010). [PubMed: 20978354]
42. Sheehan JP & Phan TM Phosphorothioate oligonucleotides inhibit the intrinsic tenase complex by an allosteric mechanism. Biochemistry 40, 4980–4989 (2001). [PubMed: 11305914]
43. Henry SP et al. Complement activation is responsible for acute toxicities in rhesus monkeys treated with a phosphorothioate oligodeoxynucleotide. Int. Immunopharmacol 2, 1657–1666 (2002). [PubMed: 12469940]
44. Swayze EE et al. Antisense oligonucleotides containing locked nucleic acid improve potency but cause significant hepatotoxicity in animals. Nucleic Acids Res. 35, 687–700 (2007). [PubMed: 17182632]
45. Elmen J et al. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res. 36, 1153–1162 (2008). [PubMed: 18158304]
46. Geary RS, Yu RZ & Levin AA Pharmacokinetics of phosphorothioate antisense oligodeoxynucleotides. Curr. Opin. Investig. Drugs 2, 562–573 (2001).
47. Elmen J et al. LNA-mediated microRNA silencing in non-human primates. Nature 452, 896–899 (2008). [PubMed: 18368051]
48. Montgomery RL et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. Circulation 124, 1537–1547 (2011). [PubMed: 21900086]
49. Chang J et al. miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. RNA Biol. 1, 106–113 (2004). [PubMed: 17179747]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

50. Mukherji S et al. MicroRNAs can generate thresholds in target gene expression. Nature Genet. 43, 854–859 (2011). [PubMed: 21857679]
51. Hendrickson DG et al. Concordant regulation of translation and mRNA abundance for hundreds of targets of a human microRNA. PLoS Biol. 7, e1000238 (2009). [PubMed: 19901979]
52. Baek D et al. The impact of microRNAs on protein output. Nature 455, 64–71 (2008). [PubMed: 18668037]
53. Selbach M et al. Widespread changes in protein synthesis induced by microRNAs. Nature 455, 58–63 (2008). [PubMed: 18668040] References 52 and 53 show that a single miRNA can repress the production of hundreds of proteins but this repression is typically relatively mild. Furthermore, although direct repression of translation occurs, mRNA destabilization is the major driver of gene repression.
54. Wolff JA & Budker V The mechanism of naked DNA uptake and expression. Adv. Genet 54, 3–20 (2005). [PubMed: 16096005]
55. Callis TH et al. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. J. Clin. Invest 119, 2772–2786 (2009). [PubMed: 19726871]
56. van Rooij E et al. Control of stress-dependent cardiac growth and gene expression by a microRNA. Science 316, 575–579 (2007). [PubMed: 17379774] This study describes the first genetic deletion by a miRNA, miR-208, which is a cardiomyocyte-specific miRNA involved in cardiac remodelling during stress.
57. van Rooij E et al. A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Dev. Cell 17, 662–673 (2009). [PubMed: 19922871]
58. Grueter CH et al. A cardiac microRNA governs systemic energy homeostasis by regulation of MED 13. Cell 149, 671–683 (2012). [PubMed: 22541436] This paper describes how inhibition of a cardiac-specific miRNA, miR-208, can have an effect on total body metabolism by regulation of downstream cardiac targets.
59. Taubert S, Van Gilst MR, Hansen M & Yamamoto KR A mediator subunit, MDT-15, integrates regulation of fatty acid metabolism by NHR-49-dependent and -independent pathways in C. elegans. Genes Dev. 20, 1137–1149 (2006). [PubMed: 16651656]
60. Wang W et al. Mediator MED23 links insulin signaling to the adipogenesis transcription cascade. Dev. Cell 16, 764–771 (2009). [PubMed: 19460352]
61. Yang F et al. An ARC/mediator subunit required for SREBP control of cholesterol and lipid homeostasis. Nature442, 700–704 (2006). [PubMed: 16799563]
62. Frost RJ & Olson EN Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. Proc. Natl Acad. Sci. USA 108, 21075–21080 (2011). [PubMed: 22160727]
63. Trajkovski M et al. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 474, 649–653 (2011). [PubMed: 21654750]
64. Zhu H et al. The Lin28/let-7 axis regulates glucose metabolism. Cell 147, 81–94 (2011). [PubMed: 21962509]
65. Ardite E et al. PAI-1-regulated miR-21 defines a novel age-associated fibrogenic pathway in muscular dystrophy. J. Cell Biol 196, 163–175 (2012). [PubMed: 22213800]
66. Liu G et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. J. Exp. Med 207, 1589–1597 (2010). [PubMed: 20643828]
67. Zarjou A, Yang S, Abraham E, Agarwal A & Liu G Identification of a microRNA signature in renal fibrosis: role of miR-21. Am. J. Physiol. Renal Physiol 301, F793–F801 (2011). [PubMed: 21775484]
68. Zhong X, Chung AC, Chen HY, Meng XM & Lan HY Smad3-mediated upregulation of miR-21 promotes renal fibrosis. J. Am. Soc. Nephrol 22, 1668–1681 (2011). [PubMed: 21852586]
69. Hatley ME et al. Modulation of K-Ras-dependent lung tumorigenesis by microRNA-21. Cancer Cell 18, 282–293 (2010). [PubMed: 20832755]
70. Thum T et al. Comparison of different miR-21 inhibitor chemistries in a cardiac disease model. J. Clin. Invest 121, 461–462; author reply 462–463 (2011). [PubMed: 21285516]
71. Care A et al. MicroRNA-133 controls cardiac hypertrophy. Nature Med. 13, 613–618 (2007). [PubMed: 17468766]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

72. Liu N et al. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev. 22, 3242–3254 (2008). [PubMed: 19015276]
73. He JF, Luo YM, Wan XH & Jiang D Biogenesis of miRNA-195 and its role in biogenesis, the cell cycle, and apoptosis. J. Biochem. Mol. Toxicol 25, 404–408 (2011). [PubMed: 22190509]
74. Porrello ER et al. MiR-15 family regulates postnatal mitotic arrest of cardiomyocytes. Circ. Res 109, 670–679 (2011). [PubMed: 21778430]
75. Porrello ER et al. Transient regenerative potential of the neonatal mouse heart. Science 331, 1078–1080 (2011). [PubMed: 21350179]
76. Fang Y & Davies PF Site-specific microRNA-92a regulation of Kruppel-like factors 4 and 2 in atherosusceptible endothelium. Arterioscler. Thrornb. Vase. Biol 32, 979–987 (2012).
77. Wu W et al. Flow-dependent regulation of Kruppel-like factor 2 is mediated by microRNA-92a. Circulation 124, 633–641 (2011). [PubMed: 21768538]
78. Parmar KM et al. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. J. Clin. Invest 116, 49–58 (2006). [PubMed: 16341264]
79. Kriegel AJ, Liu Y, Fang Y, Ding X & Liang M The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. Physiol. Genom 44, 237–244 (2012).
80. Qin W et al. TGF-β/Smad3 signaling promotes renal fibrosis by inhibiting miR-29. J. Am. Soc. Nephrol 22, 1462–1474 (2011). [PubMed: 21784902]
81. Wang B et al. Suppression of microRNA-29 expression by TGF-β1 promotes collagen expression and renal fibrosis. J. Am. Soc. Nephrol 23, 252–265 (2012). [PubMed: 22095944]
82. Xiao J et al. miR-29 inhibits bleomycin-induced pulmonary fibrosis in mice. Mol. Then 20, 1251–1260 (2012).
83. Roderburg C et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology 53, 209–218 (2011). [PubMed: 20890893]
84. Sekiya Y, Ogawa T, Yoshizato K, Ikeda K & Kawada N Suppression of hepatic stellate cell activation by microRNA-29b. Biochem. Biophys. Res. Commun 412, 74–79 (2011). [PubMed: 21798245]
85. Zhang Y et al. Protective role of estrogen-induced miRNA-29 expression in carbon tetrachloride-induced mouse liver injury. J. Biol. Chem 287, 14851–14862 (2012). [PubMed: 22393047]
86. Cushing L et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol 45, 287–294 (2011). [PubMed: 20971881]
87. Maurer B et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. Arthritis Rheum. 62, 1733–1743 (2010). [PubMed: 20201077]
88. Maegdefessel L et al. Inhibition of microRNA-29b reduces murine abdominal aortic aneurysm development. J. Clin. Invest 122, 497–506 (2012). [PubMed: 22269326]
89. Merk DR et al. miR-29b participates in early aneurysm development in Marfan syndrome. Circ. Res 110, 312–324 (2012). [PubMed: 22116819]
90. Zhang P et al. Inhibition of microRNA-29 enhances elastin levels in cells haploinsufficient for elastin and in bioengineered vessels — brief report. Aiterioscler. Thrornb. Vase. Biol 32, 756–759 (2012).
91. Turner M & Vigorito E Regulation of B- and T-cell differentiation by a single microRNA. Biochem. Soc. Trans 36, 531–533 (2008). [PubMed: 18481999]
92. Faraoni I, Antonetti FR, Cardone J & Bonmassar E miR-155 gene: a typical multifunctional microRNA. Biochim. Biophys. Acta 1792, 497–505 (2009). [PubMed: 19268705]
93. Tili E, Croce CM & Michaille JJ miR-155: on the crosstalk between inflammation and cancer. Int. Rev. Immunol 28, 264–284 (2009). [PubMed: 19811312]
94. Martin MM et al. The human angiotensin II type 1 receptor + 1166 A/C polymorphism attenuates microRNA-155 binding. J. Biol. Chem 282, 24262–24269 (2007). [PubMed: 17588946]
95. Sethupathy P et al. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3′ untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. Am. J. Hum. Genet 81, 405–413 (2007). [PubMed: 17668390]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

96. Thai TH et al. Regulation of the germinal center response by microRNA-155. Science 316, 604–608 (2007). [PubMed: 17463289]
97. Corsten MF et al. MicroRNA profiling identifies microRNA-155 as an adverse mediator of cardiac injury and dysfunction during acute viral myocarditis. Circ. Res 111, 415–425 (2012). [PubMed: 22715471]
98. Bang C, Fiedler J & Thum T Cardiovascular importance of the microRNA-23/27/24 family. Microcirculation 19, 208–214 (2012). [PubMed: 22136461]
99. Zhou Q et al. Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23 ~ 27 ~ 24 clusters. Proc. Natl Acad. Sci. USA 108, 8287–8292 (2011). [PubMed: 21536891]
100. Fiedler J et al. MicroRNA-24 regulates vascularity after myocardial infarction. Circulation 124, 720–730 (2011). [PubMed: 21788589]
101. Qian L et al. miR-24 inhibits apoptosis and represses Bim in mouse cardiomyocytes. J. Exp. Med 208, 549–560 (2011). [PubMed: 21383058]
102. Boettger T et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the mir 143/145 gene cluster. J. Clin. Invest 119, 2634–2647 (2009). [PubMed: 19690389]
103. Elia L et al. The knockout of miR-143 and -145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. Cell Death Differ. 16, 1590–1598 (2009). [PubMed: 19816508]
104. Marquart TJ, Allen RM, Ory DS & Baldan A miR-33 links S REBP-2 induction to repression of sterol transporters. Proc. Natl Acad. Sci. USA 107, 12228–12232 (2010). [PubMed: 20566875]
105. Najafi-Shoushtari SH et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. Science 328, 1566–1569 (2010). [PubMed: 20466882]
106. da Costa Martins PA et al. MicroRNA-199b targets the nuclear kinase Dyrk1a in an auto-amplification loop promoting calcineurin/NFAT signalling. Nature Cell Biol. 12, 1220–1227 (2010). [PubMed: 21102440]
107. Ren XP et al. MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20. Circulation 119, 2357–2366(2009). [PubMed: 19380620]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Box 1 |

Clinical trials of antimiR-122 in the treatment of HCV

AntimiR therapeutics (inhibitors of microRNAs) recently became a reality when Santaris Pharma reported both the safety and efficacy of its antimiR against the microRNA miR-122, miravirsen, in humans. Data from a Phase I trial showed that either a single dose (up to 12 mg per kg) or multiple ascending doses (up to five doses of 5 mg per kg) of miravirsen are well tolerated in healthy volunteers. These studies indicated that miravirsen has an attractive pharmacokinetic profile and a clear dose-dependent pharmacology, without having dose-limiting toxicities at the doses used in these studies.

A follow-up Phase 11 a trial (see the 3 October 2011 press release on the Santaris Pharma website) indicated that miravirsen was well tolerated and provided continuous and prolonged antiviral activity that extended well beyond the end of active therapy in patients infected with hepatitis C virus (HCV). In this multiple-ascending-dose study, patients were enrolled seguentially to one of three cohorts (nine active: three placebo per cohort) and miravirsen was administered at doses of 3, 5 or 7 mg per kg with a total of five weekly subcutaneous injections over 29 days. Treatment with miravirsen provided robust, dose-dependent antiviral activity that was maintained for more than 4 weeks after the last dose. Notably, in four out of nine patients treated at the highest dose (7 mg per kg), HCV RNA became undetectable during the study. No serious adverse events were observed and only mild and infreguent adverse events such as headaches, coryza and diarrhoea were reported. Furthermore, there were no clinically significant changes in safety tests, vital signs or electrocardiograms. Taken together, these data indicate that miravirsen, given as a 4-week monotherapy to patients with HCV, provides long-lasting suppression of viraemia and provides a strong barrierto viral infection.

Additional Phase II studies of miravirsen are planned in combination with direct-acting antivirals and as a single agent.

Author Manuscript

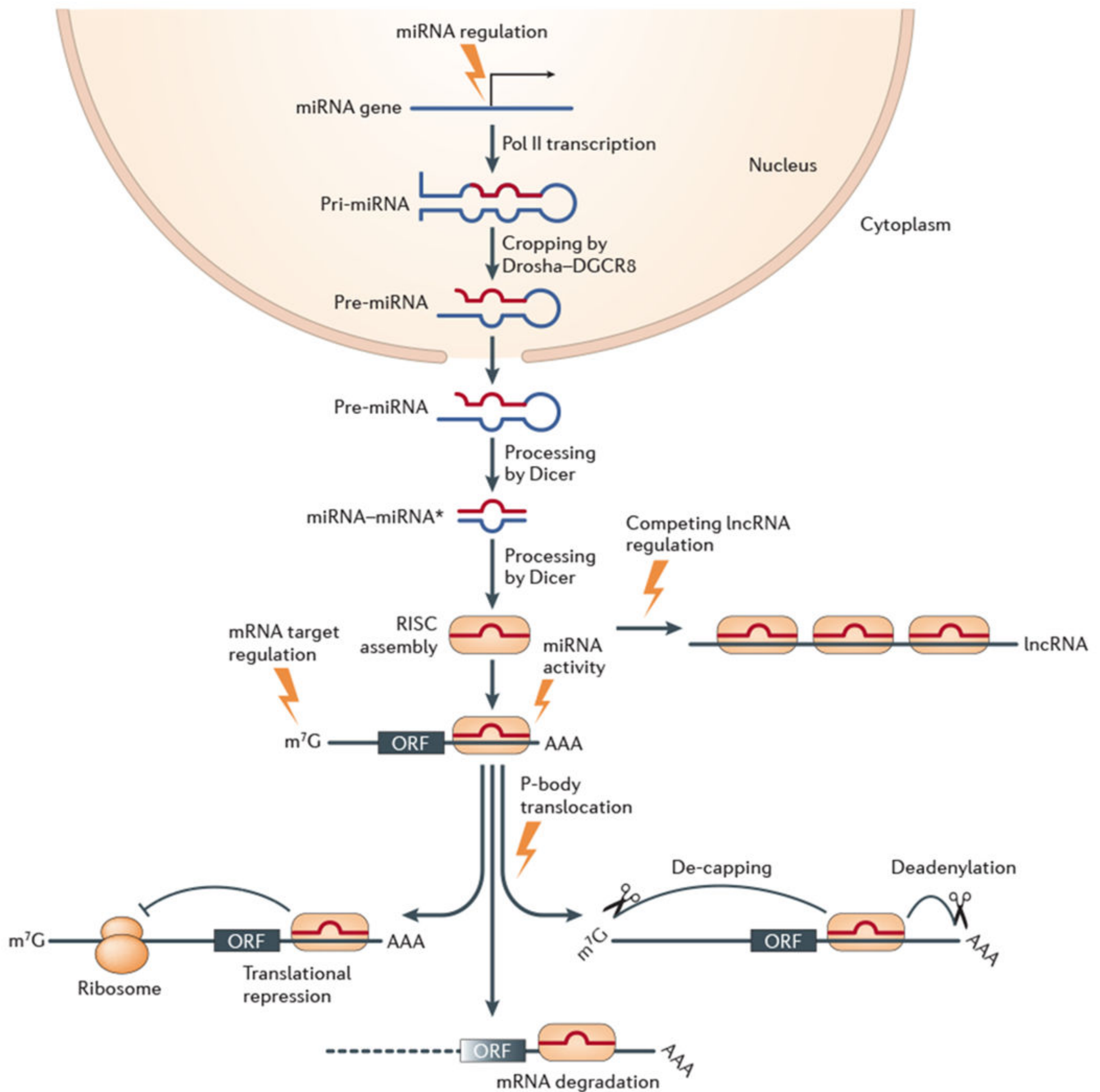

Figure 1 |. MicroRNA biogenesis and mechanism of action.

MicroRNA (miRNA) genes are usually transcribed by RNA polymerase II (Pol II) to form a capped and polyadenylated transcript. The miRNA precursor, termed primary miRNA (pri-miRNA), forms a hairpin-shaped loop structure that is cleaved by the RNase III Drosha and DiGeorge syndrome critical region 8 (DGCR8), yielding a hairpin-shaped precursor miRNA (pre-miRNA)that is ~70 nucleotides in length. The pre-miRNA is exported from the nucleus into the cytoplasm, where it is further cleaved by the RNase III enzyme Dicer, yielding an imperfect miRNA–miRNA* duplex that is about 22 nucleotides in length. Although either

strand of the duplex may potentially act as a functional miRNA, only one strand is usually incorporated into the RNA-induced silencing complex (RISC). miRNAs incorporated in the RISC often recognize their targets — nucleotides 2–7 of miRNA (known as the 'seed region'). Association of a miRNA with its mRNA target results in degradation of the mRNA as well astranslational inhibition. Recently, pseudogenes have also been implicated in regulating miRNA activity. Pseudogene transcripts are often conserved across species and many contain conserved miRNA binding sites, referred to as competing endogenous RNAs (ceRNAs), which act as decoys or sponges by seguestering miRNAs and preventing them from binding to their mRNA targets. Stress conditions can influence miRNA biogenesis at multiple levels (indicated on the figure by lightening bolts). IncRNA, long non-coding RNA; m^7G, 7-methylguanosine (a modified form of guanosine attached to the 5′ ends of mRNAs); ORF, open reading frame.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

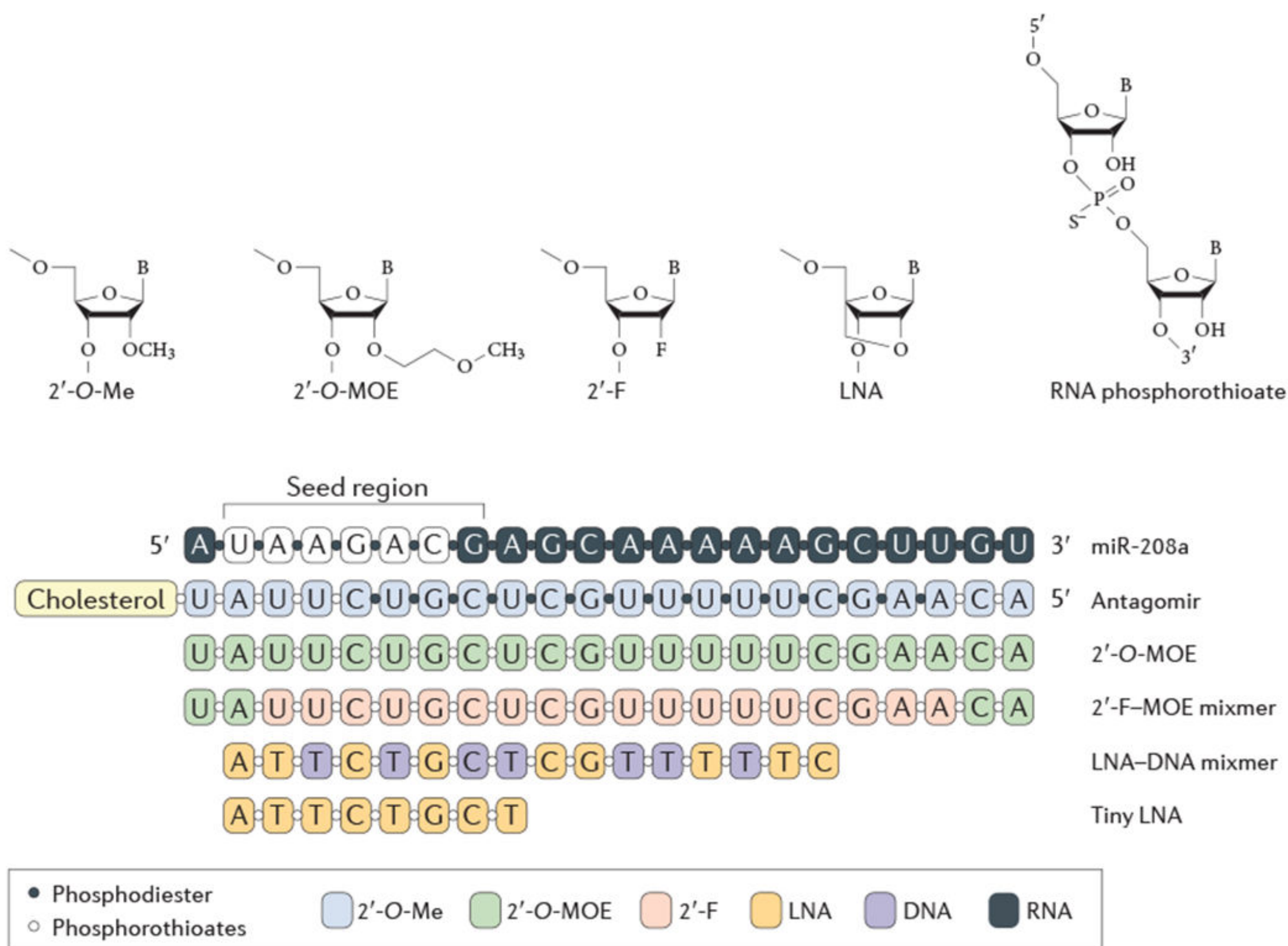

Figure 2 |. AntimiR chemistries.

AntimiR (inhibitor of microRNA (miRNA)) chemistries currently use various high-affinity 2′ sugar modifications such as conformationally restricted nucleotides with 2′-*O*-methyl (2′-*O*-Me), 2′-*O*-methoxyethyl (2′-MOE), 2′-fluoro (2′-F) or locked nucleic acid (LNA) modifications. To increase nuclease resistance, most antimiR chemistriesto date harbour phosphorothioate backbone linkages, whereby a sulphur atom replaces one of the non-bridging oxygen atoms in the phosphate group. AntimiRs containing cholesterol, conjugated via a 2′-*O*-Me linkage, named antagomirs, are fully complementary to the mature miRNA sequence and contain several phosphorothioate moieties to increase their stability. Several unconjugated phosphorothioate antisense molecules with various high-affinity 2′sugar modifications (such as 2′-MOE, 2′-F or LNA) are currently also being used. Although all of these modifications improve nuclease resistance and increase duplex melting temperature, the high duplex melting temperature of LNA-modified oligonucleotides enables efficient miRNA inhibition with shorter antimiRs.

Author Manuscript

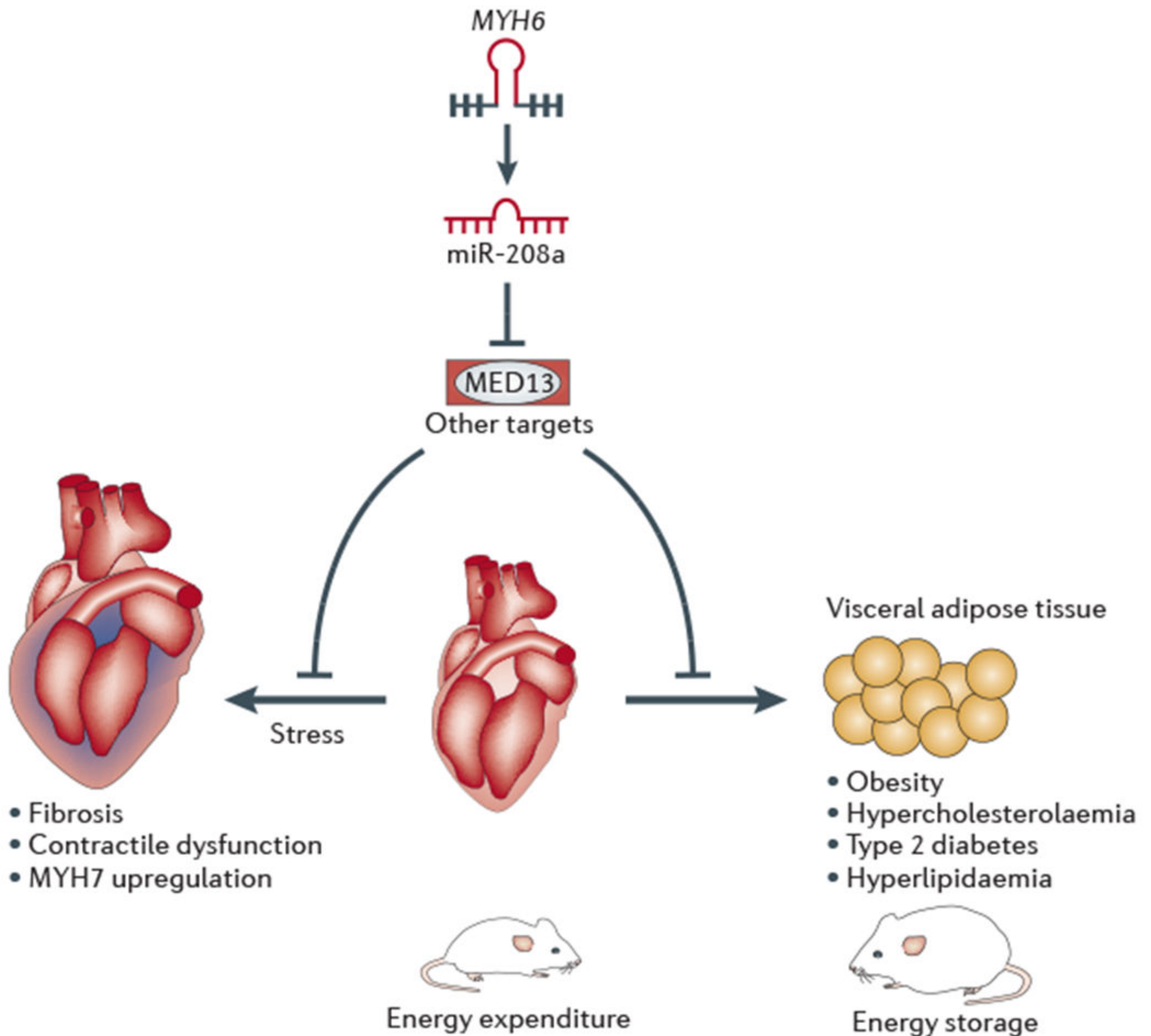

Figure 3 |. Multiple functions of miR-208 in the heart.

The microRNA miR-208a is encoded by an intron of the gene encoding myosin 6 (*MYH6*). miR-208a is required for the upregulation of myosin 7 (*MYH7*) and cardiac fibrosis in response to stress. miR-208a inhibits mediator of RNA polymerase II transcription subunit 13 (MED13), a component of the mediator complex, which regulates metabolic genes and additional targets. Inhibition of miR-208a with an antimiR (a miRNA inhibitor) inhibits cardiac remodelling and enhances systemic energy metabolism, resulting in beneficial effects in the settings of obesity and diabetes.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

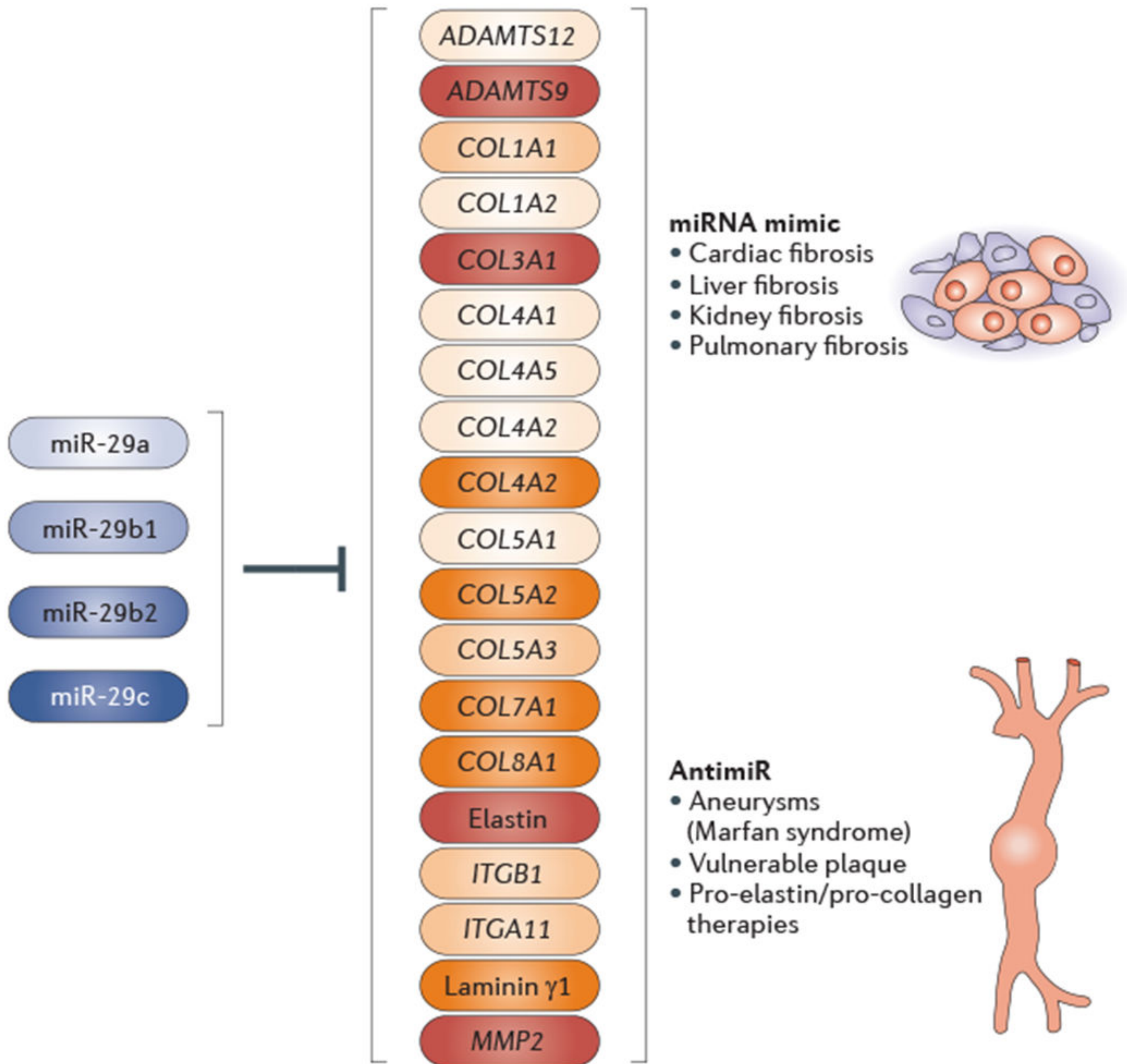

Figure 4 |. MicroRNAs often regulate related target genes.

Members of the miR-29 family exemplify the influence of microRNAs (miRNAs) on targets involved in common cellular processes. In this case, miR-29 coordinately inhibits the expression of numerous extracellular matrix proteins, such that the downregulation of this miRNA family under conditions of cardiovascular stress leads to vascular remodelling and fibrosis. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; COL1A1, collagen type 1 α1; ITGA11, integrin α11; MMP2, matrix metalloproteinase 2.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1 |

Therapeutic targeting of cardiovascular miRNAs*

miRNA	Indication	Chemistry	Therapeutic effect	Refs
miR-15 family	Post-MI remodelling	Tiny LNA	Reduces infarct size by increasing the number of viable myocytes after ischaemic injury, resulting in improved cardiac function	13
miR-15 family	Cardiac regeneration	LNA-DNA mixmer	Increases the number of mitotic cardiomyocytes	74
miR-21	Cardiac fibrosis	Antagomir	Inhibits and reverses cardiac fibrosis, leading to enhanced cardiac function in response to pressure overload	9
miR-23 and miR-27	Retinal angiogenesis	LNA-DNA mixmer	Represses neovascularization in the choroid in response to laser injury	99
miR-24	Post-MI remodelling	Antagomir	Reduces infarct size by increasing capillary density, resulting in improved cardiac function after MI	100
miR-33	Atherosclerosis	LNA-DNA mixmer	Increases plasma HDL in mice	104,105
miR-33	Atherosclerosis	2′-F-2′-MOE mixmer	Increases plasma HDL and decreases VLDL in non-human primates on a high-fat diet	38
miR-29	Aneurysms	LNA-DNA mixmer	Increases collagen expression, resulting in a significant reduction in vascular dilation and aneurysm progression	7,88
miR-92a	Neoangiogenesis	Antagomir	Enhances blood vessel growth and functional improvement of damaged tissue in models of hindlimb ischaemia and MI	6
miR-145	Pulmonary hypertension	LNA-DNA mixmer	Reduces systolic right ventricular pressure during pulmonary hypertension	8
miR-155	Viral myocarditis	LNA-DNA mixmer	Lowers myocardial damage and increases survival in response to viral myocarditis	97
miR-199b	Cardiac hypertrophy	Antagomir	Inhibits cardiomyocyte hypertrophy and fibrosis, resulting in improved cardiac function in different models of heart disease	106
miR-208a	Pathological cardiac remodelling	LNA-DNA mixmer	Blocks cardiac remodelling and improves cardiac function and survival in hypertension-induced heart failure	48
miR-208a	Metabolic disease	LNA-DNA mixmer	Reduces weight gain and improves glucose handling and plasma lipid levels in response to a high-fat diet	58
miR-320	Post-MI remodelling	Antagomir	Reduces infarct size in response to ischaemic injury	107

2′-F, 2′-fluoro; 2′-MOE, 2′-*O*-methoxyethyl; antimiR, microRNA inhibitor; HDL, high-density lipoprotein; LNA, locked nucleic acid; MI, myocardial infarction; miRNA, microRNA; VLDL, very-low-density lipoprotein.

*Preclinical rodent and large animal studies have shown that antimiR-mediated inhibition of specific miRNAs has therapeutic potential in different aspects of cardiovascular disease.