

MicroRNA therapeutics for cardiovascular disease

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Abstract

MicroRNAs (miRNAs) are short, single-stranded, noncoding RNAs that exert their function through annealing with complementary sequences in specific messenger RNAs (mRNAs) to inhibit their translation into proteins. Even though it was only a decade ago that the first human miRNA was discovered, therapeutics to regulate miRNA function are already entering the clinical space. The main reasons for this rapid transition into the clinic are their prominent roles during disease, their known and conserved sequences, and their heightened functions under conditions of disease. Based on lessons learned from antisense technologies, the opportunity to potently regulate miRNAs *in vivo* already exists and rapidly opened up the potential for miRNAs to become a new class of drugs, which is underscored by the wealth of recent animal and even human efficacy data indicating the therapeutic benefit of miRNA modulation. Also, for cardiac disease, important miRNA functions have been uncovered and preclinical efficacy studies using oligonucleotide chemistries to modulate miRNA levels have proven to be effective in targeting pathological miRNAs, resulting in therapeutic benefit. ■ *Heart Metab.* 2014;65:4-8

Keywords: anti-miR; cardiovascular disease; microRNA; miRNA mimic; therapeutics

MicroRNAs (miRNAs) are short RNA molecules that act by inhibiting the expression of specific proteins encoded by messenger RNAs (mRNAs).¹ The primary transcripts of miRNAs are transcribed as long precursor RNAs, called pri-miRNAs. Pri-miRNAs are sequentially processed in the nucleus and cytoplasm into 20 to 22 nucleotide strands of RNA, called mature miRNA. The mature miRNA associates with mRNAs within a multiprotein complex of Argonaute proteins, known as the RNA-induced silencing complex (RISC), to facilitate the interaction with target mRNAs.¹ Through complementary base pairing, they anneal with protein coding mRNAs and inhibit their protein translation through mRNA decay or translational repression (Figure 1).² It is now thought that the human genome

contains approximately 1500 miRNAs, of which roughly 300 are detected in the heart.³

An interesting aspect about miRNA activity is that while miRNAs are often moderate regulators under homeostatic conditions, their function becomes more pronounced under conditions of injury or stress.⁴ The heightened function of a miRNA during stress can be explained by the fact that stress, or “disease” signals, influences numerous aspects of miRNA biogenesis and function, such as the abundance of the miRNA, changes in expression of the mRNA targets, or differences in proteins regulating miRNA activity under stress.⁵ While the influence of stress is likely miRNA- and trigger-dependent, changes in miRNA expression and functionality under disease conditions imply

Abbreviations

MI: myocardial infarction; **miRNA:** microRNA; **RISC:** RNA-induced silencing complex

the existence of an active mechanism for differential miRNA activity under stress.

MicroRNA-modulating drugs

In contrast to a classic drug approach, anti-miR drugs are designed knowing that they will affect all genes that are under the control of the target miRNA. The

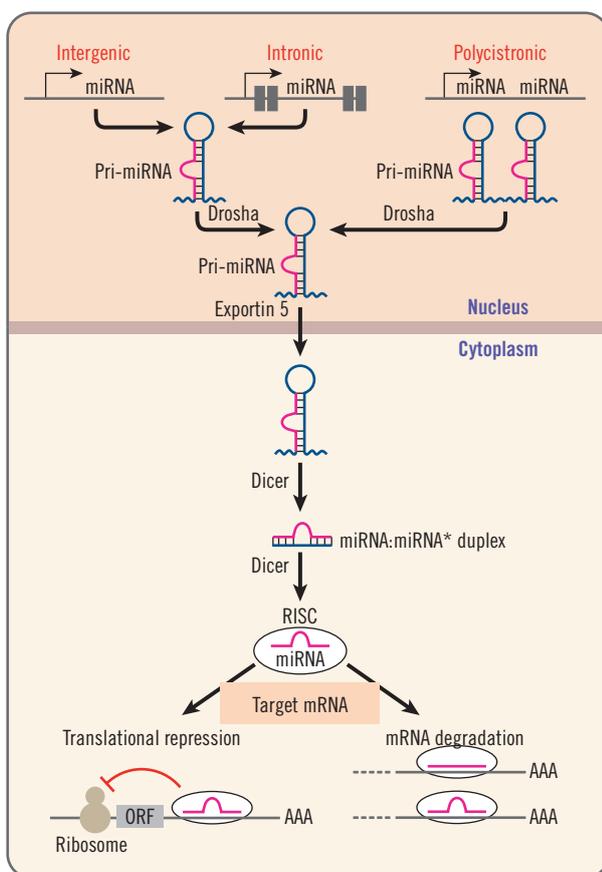


Fig. 1 miRNA biogenesis and function. miRNA genes are usually transcribed by RNA polymerase II to form a miRNA precursor, termed a pri-miRNA, that forms a hairpin-shaped loop structure, called pre-miRNA, which is cleaved by an enzyme called Drosha. The pre-miRNA is exported from the nucleus into the cytoplasm, where it is further cleaved by the RNase III enzyme Dicer, yielding an imperfect miRNA:miRNA* duplex about 22 nucleotides in length. Only one strand is usually incorporated into the RISC to associate with mRNA targets. Association of a miRNA with its mRNA target results in degradation of the mRNA, as well as translational inhibition. Stress conditions can influence miRNA biogenesis on multiple levels. **Abbreviations:** AAA, ATPases Associated with diverse cellular Activities; mRNA, messenger RNA; miRNA, microRNA; ORF, open reading frame; pri-miRNA, primary microRNA; RISC, RNA-induced silencing complex; RNase, ribonuclease.

From: van Rooij. *The art of microRNA research*. Circ Res. 2011;108(2):219-234. © 2011, Wolters Kluwer Health.

regulation of a single miRNA can have a profound impact on cellular phenotypes because individual miRNAs often target numerous related mRNA genes that encode multiple components of complex intracellular networks.⁶ Pharmacological modulation of individual miRNAs can be achieved by modified antisense oligonucleotides, referred to as anti-miRs, to inhibit a miRNA or by a miRNA mimic to replace a miRNA (Figure 2).⁷ Of these two approaches, the anti-miRs are currently the most advanced.

The first in vivo mammalian study using modified cholesterol-conjugated oligonucleotides complementary to inhibit miR-122, a liver-specific miRNA, was published in 2005.⁸ Many follow-up pharmacokinetic studies have taught us that these compounds can be delivered subcutaneously and distributed to all organs, including the heart, with a preferential delivery to the kidney and liver, and that the in vivo half-life of anti-miRs is in the order of weeks.⁹ For anti-miRs to achieve effective pharmacological inhibition of disease-associated miRNAs, they have to show a high level of in vivo stability, specificity, and binding affinity to the miRNA of interest. Currently, several chemical modifications are used to increase

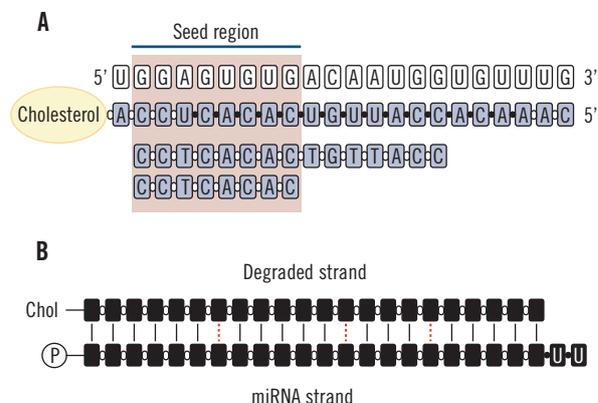


Fig. 2 miRNA-based therapeutics. Pharmacological modulation of individual miRNAs can be achieved by modified antisense oligonucleotides, referred to as anti-miRs, to inhibit a miRNA or as a miRNA mimic to replace a miRNA. **A.** An anti-miR can either be fully complementary to the mature miRNA sequence or harbor a truncated sequence targeting the 5' region of the mature miRNA. Systemic delivery will allow the anti-miR to find and bind to the target miRNA and prevent it from functioning. **B.** Restoring the function of lost or downregulated miRNAs can be achieved by therapeutic mimicry or reexpression of a miRNA by using synthetic RNA duplexes designed to mimic the endogenous functions of the miRNA of interest. These miRNA mimics harbor chemical modifications that improve their stability and cellular uptake without interfering with its miRNA function. An example of a synthetic double-stranded miRNA mimic is shown.

Abbreviations: Chol, cholesterol; miRNA, microRNA.

From: van Rooij. *The art of microRNA research*. Circ Res. 2011;108(2):219-234. © 2011, Wolters Kluwer Health.

nuclease resistance, facilitate cellular uptake, and reduce clearance by glomerular filtration and urinary excretion.^{8,10,11} The first anti-miR drug has now entered the clinical arena as Santaris Pharma recently reported on both the safety and efficacy of their anti-miR against miR-122, miravirsin, in humans. The data indicated that miravirsin given as a 4-week monotherapy to hepatitis C (HCV) patients provided long-lasting suppression of viremia and a high barrier to viral infection.¹²

Restoring the function of lost or downregulated miRNAs can be achieved by therapeutic mimicry or reexpression of a miRNA by using synthetic RNA duplexes designed to mimic the endogenous functions of the miRNA of interest. These miRNA mimics harbor chemical modifications that improve their stability and cellular uptake without interfering with its miRNA function.⁷ The first phase 1 study of the liposome-formulated miR-34 mimic-based drug was recently announced to commence in patients with primary liver cancer or metastatic cancer with liver involvement. This is the first miRNA mimic to advance into the clinic and, thus, is an important milestone for the development of miRNA-based replacement therapeutics.¹³

These advancements in miRNA therapeutics toward the clinic support the enthusiasm for exploring opportunities in additional disease areas.

Cardiac microRNA therapeutics

For heart disease, miRNAs have been shown to be major players. The first evidence suggesting the involvement of miRNAs in heart disease came from studies demonstrating changes in expression of specific miRNAs in diseased hearts from mice and humans.¹⁴ By now, numerous miRNAs have been shown to influence cardiomyocyte hypertrophy, cardiomyocyte survival, changes in cardiac metabolism, and other processes associated with the progression of heart disease.¹⁵ The rapidly growing knowledge on the functional relevance of miRNAs during cardiac disease, the shortage of effective therapies, and the ability to potently and specifically regulate miRNAs in vivo has catalyzed efforts to explore pharmacological manipulation of miRNAs for treating heart disease. Many preclinical rodent studies have shown effective cardiac delivery and miRNA inhibition after subcutaneous delivery of anti-miRs and have indicated the

potent effects of inhibition under disease conditions,⁴ of which a few relevant examples are outlined below.

An especially intriguing miRNA for the heart is the cardiac-specific miRNA, miR-208a.^{14,16} This miRNA is located within an intronic region of the gene encoding for α -myosin heavy chain, the major contractile protein of the heart. In response to cardiac stress, the adult heart changes from the α - to the β -myosin heavy chain isoform, which is thought to contribute to a diminution of cardiac contractility. Both genetic deletion or anti-miR-based inhibition of miR-208a in rodents prevents myosin switching and reduces pathological remodeling of the heart under disease conditions.^{14,17} Its cardiac specificity and its role in contractility and remodeling make miR-208a an attractive therapeutic candidate.

Adult cardiomyocytes have lost their ability to divide, which prevents the heart from being able to repair itself following injury. Identifying the key regulators of cardiomyocyte proliferation and therapeutic manipulation of this process represents one of the central challenges in cardiovascular medicine today. Recent studies showed that miRNAs can regulate the process of heart regeneration in mice. One miRNA, called miR-15, is upregulated in response to cardiac stress and myocardial infarction (MI), which causes death of cardiomyocytes and loss of pump function.^{18,19} Inhibition of miR-15 with an anti-miR protects the cardiomyocyte against ischemic damage and allows cardiomyocyte proliferation, which enhances the regeneration ability of the adult heart following injury.^{20,21} These findings can potentially provide a powerful new means of promoting heart repair by preventing myocyte loss through miRNA modulation.

For vascular indications, anti-miR strategies have been effective in targeting miRNAs that are preferentially expressed in endothelial and smooth muscle cells. miR-92a, a member of the miR-17-92 cluster, has been implicated in neoangiogenesis following ischemic injury.²² Intravenous administration of anti-miR-92a showed efficacious inhibition of the miRNA, which resulted in enhanced blood vessel growth as well as functional improvement in damaged tissue in models of hind limb ischemia and myocardial infarction, as a result of the derepression of multiple pro-angiogenic factors.²² Recent data in a porcine model of ischemic injury showed a potential advantage of localized delivery of anti-miR-92a to the heart, since

catheter-based delivery, but not systemic delivery, of anti-miR-92a showed a reduction in infarct size, which correlated with an improved ejection fraction and left ventricular end-diastolic pressure, indicating relevance of more directed cardiac delivery approaches.²³

Although, so far, no miRNA-based therapeutics for cardiovascular disorders have reached human trials, the wealth of positive preclinical data in numerous animal models of diseases such as heart failure, cardiac hypertrophy, fibrosis, and hyperlipidemia suggest that human data will soon be forthcoming.

Looking to the future

Their prominent roles during disease, their known and conserved sequences, and their heightened functions under conditions of disease make miRNAs attractive candidates for therapeutic manipulation. The realization that miRNAs might be viable therapeutic targets for many serious health conditions triggered many companies to focus on translating these exciting scientific discoveries into real-world, commercial uses. However, while we are making important steps forward in developing anti-miR chemistries as a novel therapy, numerous hurdles and questions remain in the path toward the development of miRNA-based therapeutics in general.

While some miRNAs have a very cell- or tissue-specific expression pattern, many miRNAs are broadly expressed and may have multiple effects in different tissues. Potential sources of toxicity after administration of a miRNA inhibitor can result not only from toxicities induced by the chemistry or unwanted gene changes, but can also arise from effects of the anti-miR on off-target, nondiseased tissues. For more chronic treatment regimens, localized delivery strategies, such as cardiac catheters or injectable hydrogel techniques, should be contemplated to prevent unwanted side effects.

Currently, there are still many unknowns surrounding the mode of action, cellular uptake, and distribution of the different anti-miRs. Their long half-life and activity (up to weeks or even months) indicates a high degree of stability, but also suggests that the cell has a reservoir of anti-miRs that, during the course of time, slowly releases the anti-miRs into the cytoplasm to inhibit the newly formed miRNA copies. More in-depth biochemistry will be required to gain the appropriate insights.

Despite the hurdles that still need to be overcome, the excitement surrounding miRNAs as a novel class of drugs is tremendous and the anticipated success of the early forerunners that are already in the clinic will likely trigger the search for additional therapeutic miRNA targets. Further optimization of the inhibitors and mimics as well as the search for techniques for efficient and safe delivery in vivo will trigger advancement of miRNA therapeutics. While we await more efficacy data in human subjects, the next few years promise to provide important insights into miRNA and anti-miR biology, and hopefully even further strengthen the enthusiasm for this new therapeutic approach. ■

Eva van Rooij is cofounder and SAB member of miRagen Therapeutics.

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