

MicroRNAs: A New Paradigm in the Treatment and Diagnosis of Heart Failure?

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Abstract

MicroRNAs (miRNAs) are a group of newly discovered small RNAs, non-coding, which represent one of the most exciting areas of modern medical science as they modulate a huge and complex regulatory network of gene expression. Lines of evidence have recently suggested that miRNAs play a key role in the pathogenesis of heart failure. Some miRNAs highly expressed in the heart, such as miR-1, miR-133 and miR-208, are strongly associated with the development of cardiac hypertrophy, while the exact role of miR-21 in the cardiovascular system remains controversial. Serum levels of circulating miRNAs such as miR-423-5p are being evaluated as potential biomarkers in the diagnosis and prognosis of heart failure. On the other hand, the manipulation of levels of miRNAs using techniques such as mimicking the miRNAs (miRmimics) and antagonistic miRNAs (antagomiRs) is making increasingly evident the enormous potential of miRNAs as promising therapeutic strategies in heart failure.

Introduction

The syndrome of heart failure (HF) is considered the final common pathway of every heart disease and a major cause of death^{1,2}. This syndrome has an alarming mortality rate of approximately 50% in five years, which can overcome many types of cancer³. In Brazil, HF represents a major cause of hospitalization for cardiovascular disease, and when considering all causes of death, it represents a mortality rate of 6.3%^{4,5}.

The recent discovery of microRNAs (miRNAs) has placed them among the most exciting areas of modern medical science. MiRNAs are a group of small RNAs, non protein encoders, with approximately 19-25 nucleotides of extension. Differing from the wide range of RNAs encoded by the human

genome, this type of RNA has been noted for its unique ability to modulate an enormous and complex regulatory network of gene expression⁶.

The biological role of miRNAs in the cardiovascular system of mammals has recently become a research field of rapid evolution. Several studies have demonstrated the crucial role of miRNAs not only in embryonic cardiovascular development, but also in cardiovascular disease.

Biology of microRNAs

The human genome encodes a broad range of types of RNAs, in which the function of most of these molecules has been only partially elucidated and remains unknown. Along with other more common types of RNA such as mRNA (messenger RNA or protein-coding RNA) and those with structural functions, such as tRNA (transfer RNA) and rRNA (ribosomal RNA) are the non-protein-coding RNAs, including the miRNAs.

It is known that miRNAs are usually synthesized from miRNA-specific genes or specific genetic regions that are not associated with the production of proteins (introns)⁷. The maturation of miRNAs involves a complex metabolic pathway that begins in the nucleus and extends to the cell cytoplasm (Fig. 1)⁶.

MiRNAs exert their regulatory effects by binding their nucleotides to those of the target messenger RNA (mRNA) in a process called pairing. This binding prevents the ribosomes from translating the genetic information contained in the mRNA, resulting in decreased protein synthesis of the target gene without impacting the corresponding levels of RNAm⁶.

The miRNA-mRNA interaction, however, does not necessarily need to be perfect, i.e., all miRNA nucleotides bound to the mRNA. In mammals, this binding is usually imperfect. Therefore, lack of necessity for a complete interaction coupled with the fact that miRNAs have small sequences, a single miRNA can regulate hundreds of target genes, and cooperate in control of a single target gene^{8,9}.

The involvement of miRNAs in regulatory control of gene expression and association with different functions make it clear that miRNAs can alter the progression of several diseases.

MicroRNAs and the cardiovascular system

Although the biological functions of miRNAs are not fully understood, studies have shown that some miRNAs are specifically present in certain tissues or cell types, including

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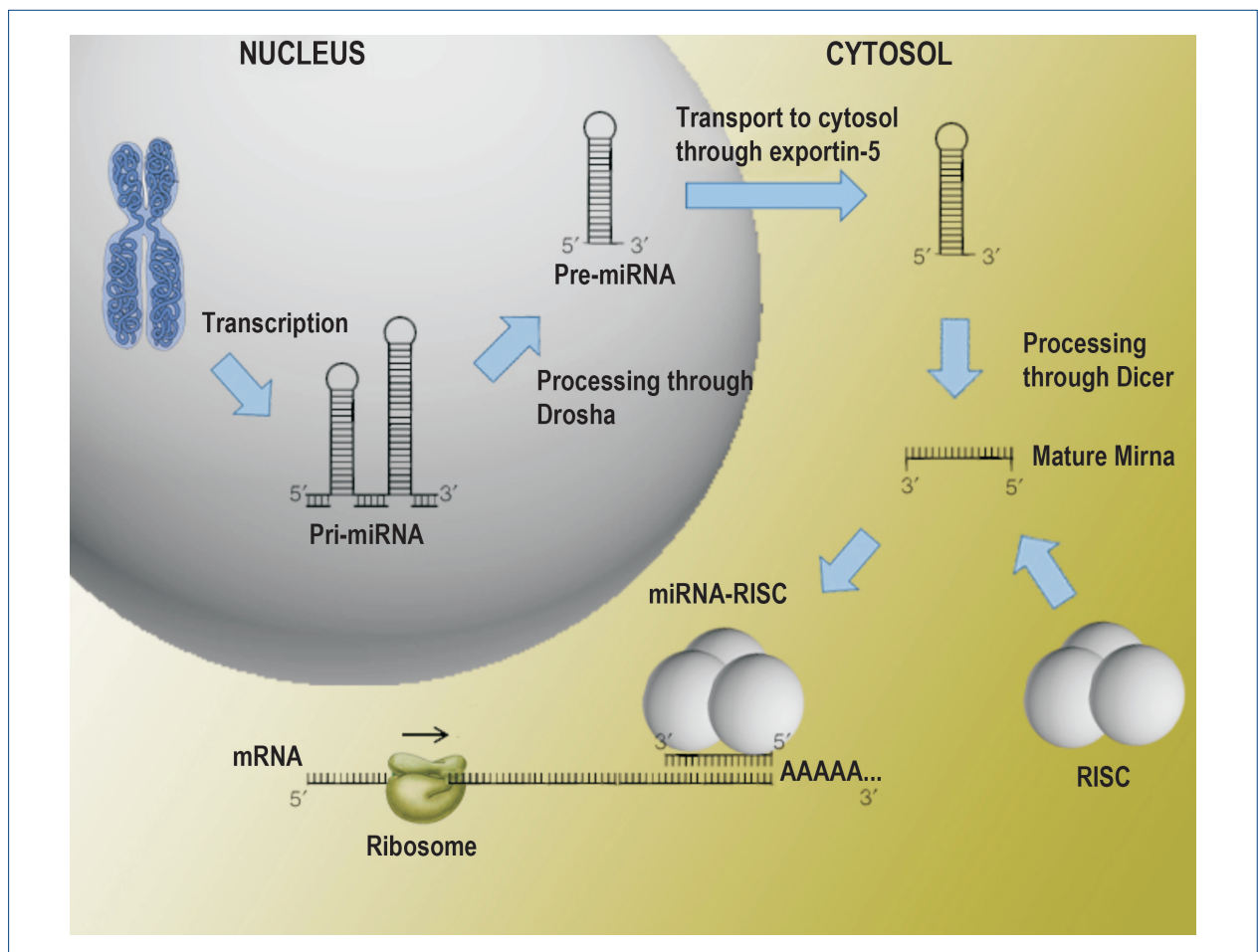


Figure 1 — Biogenesis and mechanism of action of microRNAs in mammalian cells. At the nucleus, the first step in the maturation of the miRNA is given by the enzyme RNA polymerase II in transcribing a long strand of primary miRNA (pri-miRNA) from a given gene⁴⁵. The resulting pri-miRNA is quite long and can contain one to six precursor regions (pre-miRNAs) that will form distinct miRNAs⁴⁶. The enzyme complex called Drosha will cleave the pri-miRNA releasing pre-miRNAs in the cell nucleus which are then exported to the cytoplasm^{47,48}. In the cytoplasm, the pre-miRNA is cleaved by the enzyme Dicer^{49,50}. As a result, a double-stranded RNA is formed with approximately 22 nucleotides. The two strands are then separated. However, only one of them will potentially act as a functional miRNA, while the other is usually degraded⁵¹. The miRNA strand, now mature, is incorporated into an enzyme set called RISC (RNA-induced silencing complex). The miRNA-RISC complex binds to the target mRNA in a sequence-specific manner, inducing cleavage or preventing the binding of ribosomes⁵². This process is known as gene silencing and leads to depletion of protein levels.

the heart^{10,11}. On the other hand, miRNAs whose expression is not restricted to the heart can have an important heart-specific role¹². Therefore, new miRNAs are being discovered in other cells that make up the cardiovascular system, such as fibroblasts, endothelial cells and smooth muscle cells, which cannot be ignored when studying the physiology of the cardiovascular system or its response to stress.

So far, there seems to be about 150-200 miRNAs expressed in the cardiovascular system. Many of these miRNAs are dynamically regulated in response to acute cardiac stress and in some cases during long-term compensatory response of the heart to a chronic injury or hemodynamic overload^{13,14}. Therefore, there is growing evidence that the expression of miRNAs is an important part of the mechanism of response to acute stress of the heart, contributing both to cardiac homeostasis and to the heart disease.

Some miRNAs such as miR-128, miR-302, miR-367 and miR-499 are potentially heart-specific, but more studies are needed for confirmation. Only the miRNAmiR-208 is known as heart-specific and plays an important role in the maintenance of heart development and function^{15,16}. However, recent studies have shown that in long events such as heart injuries, this miRNA can flow into the bloodstream and be detected in peripheral blood. Thus, its levels of expression may be linked to the diagnosis and prognosis of diseases^{17,18}.

In the skeletal muscle, mir-1, mir-133A, miR-133b and miR-206, together, account for approximately 25% of the expression of miRNAs and are often referred to as miomiRs¹⁹. The miRNAs miR-1, miR-133a and miR-133b are highly expressed in the skeletal muscle and in the heart, while the miR-206 is specifically expressed in skeletal muscle. All four muscle miRNAs are induced during muscle differentiation and play a critical role in the regulation of this process²⁰.

MicroRNAs in cardiac hypertrophy and heart failure

Cardiac hypertrophy is also accompanied by an exchange of the genetic program that leads to the reactivation of cardiac genes normally expressed in fetal heart during embryonic development²¹. In 2007, a striking similarity was found between the expression pattern of miRNAs in hearts of adult individuals with heart failure and hearts of fetuses at 12-14 weeks of gestation. About 80% of miRNAs analyzed were found similarly regulated in both hearts. The most significant changes were associated with increased expression of miR-21, miR-29b, miR-129, miR-210, miR-211, miR-212, miR-423 miRNAs, and reduced expression of miR-30, miR-182 e miR-526²².

Deregulation of other miRNAs, however, has also been associated with heart failure (table 1). In an experimental mouse model in pressure overload has been applied to the heart, was one of the first changes observed was reduced expression of miR-1. This change in miR-1 expression level preceded the increase in cardiac mass and contractile dysfunction¹³. This result suggests that the reduction in the level of miR-1 expression may be a cause rather than an effect of the underlying pathogenesis. Therefore, both *in vitro*²³ and *in vivo*²⁴ data suggest that reduced expression of miR-1 is required for increased cell mass.

In addition to miR-1, another muscle-specific miRNA, the miR-133, also has reduced expression during cardiac hypertrophy²⁴⁻²⁶. Mice with reduced expression of miR-133 showed cardiomyopathy, heart failure and an abnormal proliferation of cardiomyocytes²⁷. In a recent study, the expression of miR-133 was induced in a rat model subjected to acute hypertrophic stimulus. Although the weight of the heart has not been standardized, other aspects of hypertrophy, such as apoptosis and fibrosis were restored to basal levels¹⁴.

The miR-21 is one of the few miRNAs that show a regular pattern of over-expression in heart failure. Likewise, the miR-21 is also highly expressed in many cancers and cell lines, suggesting that this miRNA has a common behavior in response to stress and pathological growth of cells. However, the exact role of miR-21 in the development of cardiac hypertrophy remains controversial²⁸.

Although the expression patterns of some miRNAs are already known and associated with heart failure, certain miRNAs may be differentially expressed in certain types of disease. A study by Ikeda et al²⁹ analyzed the expression patterns of miRNAs in myocardial samples of patients with ischemic cardiomyopathy, idiopathic cardiomyopathy and aortic stenosis. Interestingly, their results show that subsets of miRNAs are differentially regulated in each etiology²⁹. Similar results were also found by Sucharov et al³⁰. These data show that differences in expression patterns of miRNAs may be clinically important if used for the purposes of diagnosis and/or prognosis.

On the other hand, not only the subsets of miRNAs have influence on the phenotype: some specific miRNAs appear to be key regulators. In 2006, van Rooij et al²⁶ showed that the increased expression of miR-195 in the myocardium of mice was sufficient to induce a pathological cardiac growth and heart failure within several weeks after birth. Moreover, while no phenotype was obtained by increasing expression of

miR-214, miR-24 resulted in embryonic lethality. This study indicates that some specific miRNAs may play determining roles in cardiac hypertrophy²⁶.

The drugs prescribed to the patient must also be taken into account. By using the zebrafish as a model, Simon-Sanchez et al³¹ demonstrated that morphine regulates the differentiation of dopaminergic neurons through the reduction of expression levels of miR-133b.³¹ Although the zebrafish is evolutionarily distant from man, this data indicates that the drugs may influence the expression of miRNAs.

MicroRNAs in diagnostic testing and prognosis of heart failure

Because many miRNAs are tissue specific, most clinical studies have been based on the measurement of expression levels of miRNAs in origin tissue samples. However, some miRNAs were recently found in the bloodstream and are referred to as circulating or c-miRNAs. The mechanisms involved in the release of miRNAs in the blood are not well understood. The fact that these c-miRNAs can be detected in peripheral blood make them potentially useful for fast and easy tests, assisting the diagnosis or guiding therapy.

The first study in mice showed that the plasma level of miR-208 (heart-specific miRNA) is related to myocardial injury and is detectable after induction of this injury¹⁸. In humans, the miRNAs miR-1^{32,33}, miR-133³⁴, miR-208a³⁵ and miR-499³⁶ have been proposed as good biomarkers of acute myocardial infarction, showing significantly higher plasma levels compared to patients without this condition.

Cheng et al³² reported that the profiles of miRNAs are differentially expressed in the myocardium based on the etiology of heart failure, suggesting that each form of etiology is characterized by a distinct expression profile of miRNA³². However, the need for an invasive procedure to obtain samples from the myocardium makes the clinical application of this approach very limited. However, recent evidence has shown that the c-miRNA miR-423-5p shows an increased expression during heart failure and can be used as a biomarker³⁷.

In 2009, Matkovich et al. evaluated the expression profile of miRNAs in heart failure patients before and after treatment with left ventricular assistance devices. Interestingly, 71.4% of miRNAs differentially regulated in heart failure were normalized after treatment³⁸. These results suggest that miRNAs may serve as markers of myocardial recovery in patients with advanced heart failure.

MicroRNAs in the treatment of heart failure

Two therapeutic strategies involving the knowledge of miRNAs have been recently studied: the use of antagomirs and miR-mimics. These strategies are based on the normalization of tissue level of specific miRNAs, silencing those who have over-expressed or replacing those that have a deficit in expression in pathological processes (Fig. 2).

In a pathological condition in which certain miRNAs are over expressed, the first thing one think is how to intervene in the effect caused by the excessive increase in the expression of these miRNAs. For this purpose, a class of antimiRNAs called antagomirs was developed.

Table 1 — Profile of expression of miRNAs in human heart failure and their functions in the cardiovascular system (HF)

miRNA	Expression in HF	Function in the cardiovascular system	References
1	Reduced	Development and function of cardiac and skeletal muscle	13,23,24
10a,b	Reduced	Involved in vascular inflammation	29,30,53
15a,b	Increased	Induction of apoptosis; regulates the suppression of postnatal mitosis of cardiomyocytes.	12,26,29,30,54,55
16	Increased	Induction of apoptosis; regulates the suppression of postnatal mitosis of cardiomyocytes.	12,26,30,43,54,55
19a,b	Reduced	--	12
21	Increased	Induced in endothelial cells by shear stress; modulates apoptosis and the activity of eNOS; regulates the function of vascular smooth muscle cells	22,43,56,57
23a,b	Increased	Restricts the formation of the heart valve; involved in the regulation of cardiac hypertrophy	12,30,26,43,58,59
24	Increased	Regulates vascularization after myocardial infarction; inhibits apoptosis in cardiomyocytes	12,26,43,60
27a,b	Increased	Regulates gene expression of beta myosin; induces cardiac hypertrophy and dysfunction in mice	12,43,61,62
34a,b	Increased	Induces senescence of endothelial progenitor cells and prevents angiogenesis	22,63
92	Reduced	Angiogenesis inhibitor	30,64
100	Increased	Involved in the regulation of beta-adrenergic receptors	12,30
101a,b	Reduced	The decreased expression of miR-101 in endothelial cells promotes angiogenesis	12,65
103	Increased	Induced in response to hypoxia	12,43,66
125a,b	Increased	Regulates the expression of endothelin-1 in endothelial cells	12,22,29,30,26,43,67
130a	Increased	Translational Control of FOG-2 expression in cardiomyocytes	22,43,68
132	Increased	Involved in the angiogenesis program	22,69
133	Reduced	Development and function of cardiac and skeletal muscle; regulation of beta-adrenergic receptors	24,30
139	Reduced	--	30
143	Increased	Promote differentiation and suppress the proliferation of smooth muscle cells	43,70
145	Increased	Necessary for the reprogramming of adult fibroblasts and smooth muscle cells to induce differentiation of multipotent stem cells in vascular smooth muscle.	12,29,70
150	Reduced	Crucial for the differentiation of endothelial cells	30,60
181	Increased	Regulation of T cell sensitivity to antigens	12,29,30,71
195	Increased	Involved in myocyte hypertrophy and dilated cardiomyopathy	12,30,26,43
199a	Increased	Essential for maintaining the size of cardiomyocytes	30,26,44,72
214	Increased	--	12,26,29
221	Reduced	Regulates endothelial dysfunction; involved in angiogenesis; proliferation of vascular smooth muscle cells and hyperplasia; involved in vascular inflammation	30,73-76
222	Reduced	Involved in angiogenesis; proliferation of vascular smooth muscle cells and hyperplasia; involved in vascular inflammation	12,30,74,75,76
320	Increased	Involved in the regulation of cardiac ischemic injury	12,22,77
330	Increased	--	22
342	Increased	--	12,29,30
365	Increased	--	22
	Reduced	--	12,30
423	Increased	--	22
424	Increased	--	12,26,30,43
451	Reduced	MiR-144/451 together confer protection against death of cardiomyocytes induced by ischemia / reperfusion	12,78
483	Reduced	--	30
486	Reduced	--	30
497	Increased	--	12,26,30,43
638	Increased	--	43

Antagomirs are small antagonistic nucleotide sequences, of single strands, artificially synthesized to be perfectly complementary to a specific mature miRNA. When injected systemically or locally, antagomirs interact with miRNAs in the cytoplasm and hybridize specifically to the target mature miRNA making it difficult to bind the miRNA with its respective mRNA. Thus, the antagomirs act as competitive inhibitors of miRNA and lead to a decrease in the effect caused by excessive increase in the expression of certain miRNAs³⁹.

Far from being utopian, this therapeutic strategy has already been studied by many researchers. In a pioneering study, Thum et al⁴⁰ induced mice to cardiac hypertrophy through pressure overload. After three weeks, the mice received antagomir designed to functionally inhibit miR-21 (miRNAs over-expressed in cardiac fibroblasts during hypertrophy). As a result, it was observed that the mice showed a significant regression of cardiac hypertrophy and fibrosis, as well as attenuation of impairment of cardiac function⁴⁰. Another successful approach was published in 2011 by Montgomery et al⁴¹, in which the anti-miR-208a was systemically administered during hypertension induced by heart failure in hypertensive rats leading to a potent silencing of miR-208a in the heart. The therapeutic inhibition of miR-208a avoided the pathological change of myosin and cardiac remodeling, improving cardiac function and survival⁴¹.

These results demonstrate that the use of antagomirs can be useful in preventing and/or reversing cardiac hypertrophy. However, most studies to date have focused on "silencing" only isolated miRNAs. However, considering that more than one miRNA may be involved in the pathological process, probably several miRNAs must be silenced to obtain an effective therapy.

Just as the increased expression of some miRNAs may be related to the outbreak of pathogenic processes, decreased expression of specific miRNAs can also lead to a pathological state.

The intervention to be made to normalize the level of expression of these miRNAs, however, is based on the administration of molecules that will functionally mimic natural miRNAs.

The miRmimics are short double-strand artificial nucleotide sequences that resemble miRNA precursors (pre-miRNA). When introduced into cells, the miR-mimics are recognized by the miRNA biogenesis machinery and processed by the enzyme Dicer, and then incorporated into the RISC enzyme complex. Thus, the mimics will work as a replacement of little expressed miRNAs by regulating the target mRNA as endogenous miRNAs⁴².

The replacement of miRNAs, however, is subject to an additional obstacle: specificity. The miR-mimics should act only on the target tissue. Otherwise, as if administered systemically, they could result in one or more miRNAs exercising regulatory function in tissues where these miRNAs are not normally expressed. This erroneous regulation would likely lead to triggering side effects.

To overcome this obstacle, more complex and accurate management systems are required. To this end, the use of viral vectors has proven to be promising. These vectors are produced by bioengineering from non-pathogenic viruses belonging to the Parvovirida family and have a high affinity with the myocardium⁴³.

Like antagomirs, the therapeutic effectiveness of miR-mimics is also being studied. In a study by Suckau et al., a viral vector

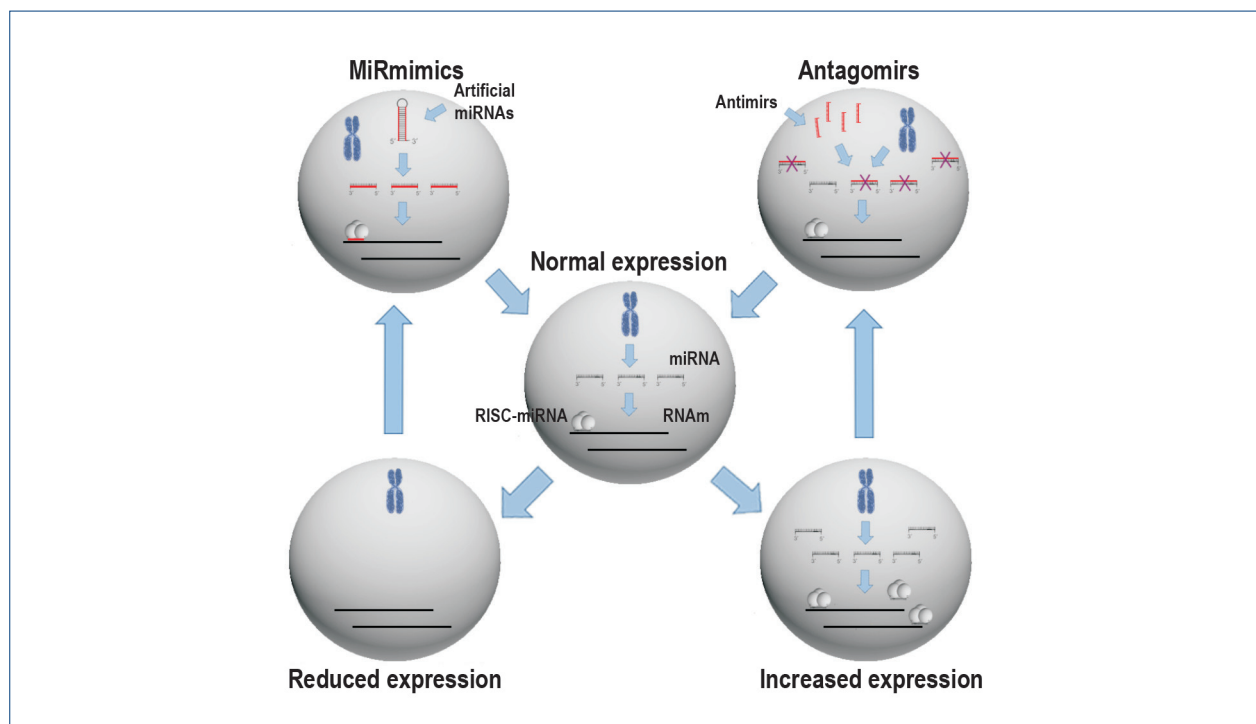


Figure 2 – Schematic overview of two treatment strategies (antagomirs and RNAi) involving the knowledge of miRNAs.

optimized with mimics has been successfully used in rats with pressure overload. As a result, the authors observed that there was a normalization of cardiac dilation and a significant reduction of cardiac hypertrophy, cardiomyocyte diameter and cardiac fibrosis⁴⁴.

Conclusion

Understanding the biology of miRNAs and their role in pathogenic processes is an exciting new milestone in cardiovascular medicine. The potential of miRNAs is increasingly evident as new tools in the diagnosis and prognosis, as well as promising therapeutic strategies in many sub-areas of cardiology, including heart failure. However, before becoming a reality, many studies are still needed. Overcoming obstacles, miRNA-

based therapies can become part of the arsenal of cardiologists in the treatment, diagnosis and prognosis of heart failure.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any post-graduation program.

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