

## MicroRNAs in cardiovascular disease

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### Abstract

Rapid and accurate diagnosis of heart attacks—and the assessment of damage—are critical for improving coronary care. Mature microRNAs (miRNAs) are abundant, easily measured, and relatively stable in blood plasma. If they prove indicative of disease states, miRNAs measured from peripheral blood may be a particularly attractive source for routine clinical assessments.

### Introduction

MicroRNAs (miRNAs) were discovered almost twenty years ago, but during the past 5 years, research has exploded in this field (e.g., approximately 9,000 manuscripts have been published since 2006) (see miRNA reviews [1,2]). The major reason for this flood of interest has resulted from the demonstration that mature miRNAs (i.e., single-stranded RNA molecules comprising 21–23 nucleotides in length) can play a critical role in the control of most biological processes, including cell differentiation and proliferation, migration, development, and apoptosis. Importantly, recent investigations have established that aberrant regulation of mature miRNA expression is closely associated with the onset and progression of many diseases, including cardiovascular disease. It is now clear that epigenetic modifications such as DNA methylation or histone acetylation can affect miRNA expression, and potentially be responsible for the aberrant miRNA regulation observed in cardiovascular disease. Moreover, a specific group of miRNAs defined as epigenetic miRNAs can directly target effectors of the epigenetic machinery and indirectly affect the expression of genes involved in cardiovascular disease. Given that the dysregulation of miRNA expression appears to play a significant role in mediating the pathology of diseased hearts, miRNAs may represent unique diagnostic and therapeutic targets.

### miRNAs as diagnostic tools for cardiovascular disease

The ideal biomarker for any disease must be accessible using noninvasive techniques, economical to quantify, specific to the disease of interest, and a reliable early indicator of disease before clinical symptoms appear. Given that mature miRNAs are abundant, easily measurable, and relatively stable in the plasma/serum, peripheral blood may be a particularly attractive source for the routine clinical measurement of these molecules in disease settings [3]. In support of this premise, several independent studies have demonstrated that, in plasma samples isolated from patients with acute myocardial infarction, cardiac myocyte-associated mature miR-208b and -499 were greatly elevated when compared with controls [4,5]. Another study demonstrated that mature miR-1 levels were significantly higher in plasma from acute myocardial infarction patients compared with subjects without acute myocardial infarction, and the level returned to normal upon discharge following medication [6]. Importantly, these investigators also established that plasma mature miRNA levels were not affected by a wide range of clinical confounders, including age, gender, body mass index, kidney function, systolic blood pressure, and white blood cell count. Taken

**Table 1. miRNA nomenclature as adopted by the miRNA Registry [20]**

miRNA	Nomenclature	Example
miRNA	The prefix "mir" is followed by a dash and a number, which is attributed sequentially <sup>a</sup>	mir-123 was likely discovered before mir-423
Pre-miRNA	Indicated by the uncapitalized prefix "mir"	mir-155
Mature form of miRNA	Indicated by the capitalized prefix "miR"	miR-155
miRNAs with nearly identical sequences (i.e., one or two nucleotides different)	Denoted with an additional lowercase letter	miR-125a miR-125b
Pre-miRNAs that are located at distinct genomic loci but that lead to identical mature miRNA	Denoted with an additional dash-number suffix	mir-125b-1 mir-125b-2
Two mature miRNAs that have originated from opposite arms of the same pre-miRNA, when relative expression levels are known	The predominant miRNA is named as described above and the other miRNA expressed at lower levels is followed by an asterisk	miR-155 miR-155*
When the relative expression levels of the two mature miRNAs originating from opposite arms of the same pre-miRNA are not known	The suffix -5p is added to the miRNA originating from the 5' arm, and -3p is added to the miRNA from the 3' arm	miR-423-5p miR-423-3p
miRNA that originates from different species	The species of origin is designated by a three-letter prefix	hsa-miR-155 ( <i>Homo sapiens</i> ) mmu-miR-155 ( <i>Mus musculus</i> )

<sup>a</sup>Before receiving a number designation, the guidelines require that the new miRNA gene is experimentally verified by cloning or with evidence of expression and processing.

together, these studies suggest that following acute myocardial infarction, mature miR-1, -208, and -499 are released into the plasma as a result of damaged cardiac cells and may therefore be useful biomarkers in myocardial infarction patients to evaluate the efficacy of treatment or for risk stratification in patients with chronic coronary artery disease.

Although it appears that mature plasma miRNAs may be useful indicators of myocardial injury, can plasma miRNA signatures also be utilized to diagnose other forms of cardiovascular disease? To begin to address this question, Tijssen et al. [7] assayed mature plasma miRNAs isolated from 12 healthy controls and 12 stable, chronic heart failure patients. These investigators excluded subjects with recent cardiac ischemia or infarction, so their results were less likely to be influenced by major cardiac cell loss. Importantly, they did not find increased mature miR-1, -208, or -499 in the plasma of heart failure patients. However, they did identify six miRNAs that were elevated in the plasma of patients with heart failure, among which mature miR-423-5p was the most strongly related to the clinical diagnosis of heart failure. While miR-423-5p may be an attractive novel plasma miRNA biomarker specific for heart failure, several important questions remain unanswered. What cell type(s) produce miR-423-5p? Do increased levels of plasma mature miR-423-5p reflect the existence of a specific miRNA secretory/shedding pathway or does the miRNA release result from cell death and subsequent emptying of cellular contents into the extracellular space?

Interestingly, it has been suggested that extracellular mature miRNAs in plasma and other body fluid types may participate in some form of cell-to-cell communication. It is further speculated that these mature miRNAs convey specific information, and therefore only some cellular mature miRNAs would be exported or released from cells in response to biological stimuli [8]. However, others have argued that these molecules are not biologically active given that it is unlikely that these secreted mature miRNAs can be incorporated into miRNA-induced ribonucleoprotein silencing complexes (RISCs) in target cells [9]. It will be extremely intriguing to monitor how this controversy is resolved, and what the implications will be for miRNAs in this setting.

### miRNAs and cardiac regeneration

Three manifestations of heart disease—cardiac hypertrophy, heart failure, and myocardial infarction—are each associated with distinct miRNA expression patterns. In instances where attenuation of a particular miRNA appears pathogenic, therapeutically increasing miRNA levels could be beneficial. Myocardial regeneration provides one of the most appealing therapeutic options to restore myocardial function to an injured or diseased heart, and there is some evidence that miRNAs could facilitate the process.

Mesenchymal stem cells derived from adult bone marrow have emerged as one of the most promising stem cell types for treating cardiovascular disease [10]. In a fascinating study, Lai et al. [11] demonstrated that mesenchymal stem cells shed/secreted phospholipid

vesicles consisting of cholesterol, sphingomyelin, and phosphatidylcholine and that these purified particles reduced tissue damage in a mouse model of myocardial ischemia/reperfusion injury. Additionally, this laboratory also demonstrated that mesenchymal stem cells shed/secreted phospholipid vesicles that were enriched with pre-miRNAs (i.e., ~70-nucleotide hairpin structures that are incorporated into the RISC where they are cleaved by Dicer to give rise to mature functional miRNAs), which, unlike single-stranded, mature miRNA, can be readily taken up by other cell types [9]. In contrast to secreted mature miRNAs described above, pre-miRNAs can become biologically active outside the cell, and therefore may provide some benefit against ischemic injury.

Several other surprising aspects of miRNA biology also seem to play a role in stem cell therapy and cardiac regeneration. For example, cardiomyocyte progenitor cells can be easily expanded and efficiently differentiated into beating cardiomyocytes, and alteration of miR-1 and -499 expression levels enhances the cardiomyogenic differentiation [12]. Additionally, Suzuki et al. [13] demonstrated that diazoxide, which has been widely demonstrated to suppress cell apoptosis and promote cell survival, potentiates mesenchymal stem cell survival via nuclear factor kappa-B (NF- $\kappa$ B)-dependent miR-146a expression by targeting Fas. Finally, Kim et al. [14] demonstrated that ischemic preconditioning (brief, deliberately induced episodes of ischemia) reduced apoptosis in mesenchymal stem cells via translocation of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and the subsequent induction of miR-210 expression. They also demonstrated that the cytoprotection afforded by ischemic preconditioning was regulated, in part, by miR-210-mediated repression of FLICE-associated huge protein (FLASH)/caspase-8-associated protein-2 (Casp8ap2). Interestingly, their in-vivo studies in a rat model of acute myocardial infarction demonstrated that not only did miR-210 play a role in improved transplanted stem cell survival after engraftment but that the surrounding heart tissue also expressed higher levels of miR-210. It is tempting to speculate that the engrafted mesenchymal stem cells may be able to serve as a source of miR-210 by shed/secreted phospholipid vesicles for delivery to the host cardiomyocytes.

Taken together, these studies suggest mesenchymal stem cells can potentially exert miRNA-mediated biological effects on cardiomyocytes, endothelial cells, and vascular smooth muscle cells through the secretion of pre-miRNA in phospholipid vesicles, which in turn may provide benefit against ischemic injury. Therefore, the engineering of mesenchymal stem cells to deliver specific miRNAs

may facilitate the healing process. Furthermore, these studies suggest that experimental approaches that augment or attenuate miRNA levels may also be utilized to enhance cardiomyogenic differentiation and accelerate stem cell viability of engraftment in the infarcted myocardium.

### miRNA therapeutics

Recent studies have identified miRNA expression signature patterns that are associated with pathological cardiac hypertrophy, heart failure, and myocardial infarction in humans. Therefore, when miRNA attenuation plays a pathological role in cardiovascular disease, an in-vivo therapy that leads to increased levels of a specific mature miRNA, or miRNAs, would be desirable. Thus, to increase the effective concentration of a depleted endogenous miRNA and, in turn, more potently block targeted gene expression, the delivery of a partially double-stranded stem-looped RNA (pre-miRNA) that mimics the Dicer cleavage product (i.e., mature, functional miRNA) is required. Remember that the double-stranded structure is necessary for the appropriate processing and incorporation into the RISC, after which the miRNA mimic can function like the endogenous mature miRNA. In a ground-breaking in-vivo study, Wang et al. [15] demonstrated that infusion of miR-9 mimics reduced isoproterenol-induced cardiac hypertrophy and improved cardiac function, in part, through the regulation of the miR-9 target, myocardin. While this therapeutic intervention would supplement the mature miRNA levels that were decreased during disease progression, it may also result in potential off-target effects given that the infused mimic would also be taken up by tissues that do not normally express the miRNA of interest. It may be possible to circumvent this problem by delivering miRNA mimics in a cardiac-specific manner by utilizing serotype-specific adeno-associated viruses (i.e., AAV9) genetically modified to express a given miRNA mimic with a cardiac-specific promoter [16]. While systemic viral delivery of miRNAs to the heart during disease has yet to be performed, AAV9 has been successfully utilized to deliver RNA interference to cardiac tissue and effectively restore cardiac function in rodents with heart failure [17].

In contrast, when miRNA overexpression plays a pathogenic role in cardiovascular disease, an in-vivo therapy that leads to decreased levels of a specific mature miRNA, or miRNAs, would be desirable. Therefore, to reduce the effective concentration of an overexpressed endogenous miRNA and, in turn, increase targeted gene expression, the delivery of either cholesterol-conjugated antisense miRNA inhibitors (antagomirs) or miRNA sponges (i.e., RNA containing 4–10

binding sites for the miRNA of interest) is required. Importantly, the pioneering “proof of concept” studies for utilizing antagomirs and sponges in a cardiovascular setting by Carè et al. [18] demonstrated that the in-vivo inhibition of miR-133 by infusion of an antagomir or a sponge, delivered inside the cell by adenoviral infection, caused marked and sustained cardiac hypertrophy in mice, associated with a re-induction of fetal gene expression. In support of this first study, Thum et al. [19] demonstrated that in-vivo silencing of miR-21 by a specific antagomir in a mouse pressure-overload-induced disease model reduced cardiac ERK-MAP kinase (extracellular signal-regulated kinase–mitogen-activated protein kinase) activity, inhibited interstitial fibrosis, and attenuated cardiac dysfunction. Taken together, these studies provide evidence validating the efficacy of utilizing antagomirs and sponges to silence miRNA function in vivo.

### Conclusions: opportunities and limitations

Breakthroughs in our understanding of heart pathophysiology that can then be translated to the clinical setting through the innovation of novel diagnostics and therapeutics are urgently needed. Recent studies in humans and mice have discovered profound and unexpected functions for miRNAs in numerous facets of cardiac biology, including the control of myocyte growth, contractility, fibrosis, and angiogenesis. The apparent importance of miRNAs and the ability to manipulate them in vivo provides a unique opportunity to exploit miRNAs therapeutically. Furthermore, miRNA-based diagnostic markers may be superior to the currently available markers.

That being said, miRNA-based therapeutics also pose a unique set of challenges from those associated with classic drugs given that miRNAs have numerous molecular targets, which increases the probability that the targeting of a miRNA may perturb multiple cellular functions, some pathological and others beneficial. Therefore, in advance of therapeutic targeting of specific miRNAs, a detailed knowledge of their gene targets, functions, and tissue distribution is required. Given that miRNA research is still in an early phase, much of this information is incomplete. For example, the identification of authentic miRNA/mRNA targets remains problematic because mammalian miRNAs bind to mRNA with imperfect complementarity, and currently we only have a partial understanding of how binding sites are recognized. Thus, some of the bioinformatically predicted targets turn out to be false while others are entirely overlooked. Additionally, issues concerning pharmacokinetics, biodistribution, and cell penetration also represent likely obstacles to miRNA therapeutic

strategies. Finally, novel strategies and techniques must be developed to deliver miRNA therapeutics in a tissue-specific manner (i.e., heart and/or vasculature for treatment of cardiovascular disease). In conclusion, as our knowledge regarding miRNA/mRNA target recognition, miRNA function, and miRNA therapeutic delivery improve, this information may result in a new wave of medicinal treatments.

### Abbreviations

miRNA, microRNA; RISC, RNA-induced silencing complex.

### Competing interests

The authors declare that they have no competing interests.

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