

MicroRNAs as therapeutics for heart disease

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Abstract

MicroRNAs (miRNAs) are small, noncoding RNA molecules that have been demonstrated to play critical roles in multiple aspects of whole body physiology by repressing downstream messenger RNA (mRNA) target transcription. With reference to the cardiovascular system, a number of miRNAs undergo deregulated expression in response to numerous stresses including ischemia, heart failure, and pulmonary hypertension, which led to the discovery of novel targets for treating these various cardiovascular diseases, including miRNA-214, miRNA-155, and miRNA-204. As our understanding of miRNA biology and the regulation of their downstream mRNA target genes improves, it should lead to the discovery of novel targets for the treatment of various types of cardiovascular disease. ■ *Heart Metab.* 2014;65:37-39

Keywords: cardiovascular disease; heart; heart failure; ischemic heart disease; microRNA; pulmonary arterial hypertension

Cardiovascular disease, consisting primarily of ischemic heart disease and heart failure, is a primary cause of death and disability in the world today.^{1,2} Fortunately, epidemiological studies and randomized clinical trials have provided compelling evidence that cardiovascular disease is highly manageable.³ Current treatment regimens, which are comprised of either percutaneous or surgical techniques to enhance myocardial blood supply, or pharmacotherapy to reduce myocardial oxygen demand, have greatly improved the overall prognosis of patients living with cardiovascular disease. Nevertheless, a large number of patients remain that prove to be either ineligible for, or refractory to, conventional treatment, and percutaneous or surgical revascularization is associated with a distinct set of risks. Thus, new approaches to treat such patients are necessary. One such potential novel therapy

involves the targeting of microRNAs (miRNAs), which will be the focus of this article.

miRNAs are small, noncoding RNA molecules (~22 nucleotides in length) that have been demonstrated to play critical roles in multiple aspects of cardiac function via repression of target gene messenger RNA (mRNA) transcription. miRNAs are able to repress gene function by binding to sequences in the 3' untranslated regions of their associated target mRNAs, which exhibit complementarity to nucleotides 2 to 8 of the associated miRNA, referred to as the seed region.⁴ miRNAs are divided primarily into 3 classes depending on the genomic location of their miRNA-encoding sequences; intergenic miRNAs are under the transcriptional control of distinct promoters, while both intronic and exonic miRNAs exhibit transcriptional control through their host-gene promoters.⁴ The generation of mature miRNAs and sub-

Abbreviations

LV: left ventricular; **miRNA:** microRNA; **mRNA:** messenger RNA; **PAH:** pulmonary arterial hypertension; **TAC:** transverse aortic constriction

sequent repression of gene expression first involves primary miRNAs (\approx hundreds to thousands of nucleotides in length), which fold imperfectly into hairpin-shaped precursor miRNAs (\approx 70 to 100 nucleotides in length), and these precursor miRNAs are transported into the cytoplasm via exportin 5, and are further processed into a short, mature miRNA duplex via the endonuclease Dicer. This mature miRNA duplex is subsequently integrated into the RNA-induced silencing complex, resulting in translation inhibition, mRNA degradation, and the posttranscriptional repression of gene expression.

The remainder of this article will primarily illustrate novel findings supporting miRNA-targeted therapy as a novel approach for various forms of cardiovascular diseases including ischemia/reperfusion injury, cardiac hypertrophy, heart failure, and pulmonary arterial hypertension (Figure 1). For more detailed information on the complex regulation of miRNAs and their contribution to cardiac development and disease, please refer to the following in-depth reviews.^{4,5}

In the setting of ischemic heart disease, miRNA expression is significantly altered and associated with the progression of disease pathogenesis, including perturbed angiogenesis, enhanced apoptosis, and contractile dysfunction.⁵⁻⁷ Elegant studies from Aurora et al demonstrated a significant role for miRNA-214 as an adaptive protective mechanism against ischemia/reperfusion injury via regulation of Ca^{2+} homeostasis and signaling.⁸ Indeed, they demonstrated a significant upregulation of miRNA-214 expression in wild type mice at both 24 hours and 7 days following a temporary left anterior descending (LAD) coronary artery occlusion. However, in whole heart lysates from mice deficient for miRNA-214 (miRNA-214^{-/-}), there was a marked upregulation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger-1 protein (NCX1) at both 24 hours and 7 days following temporary LAD coronary artery occlusion, which contributes to ischemia/reperfusion injury via reverse mode Ca^{2+} exchange.⁸ Furthermore, other Ca^{2+} -sensitive factors altered in miRNA-214^{-/-} hearts at 7 days following temporary LAD coronary artery occlusion included calmodulin-dependent protein kinase II α (CamKII α)

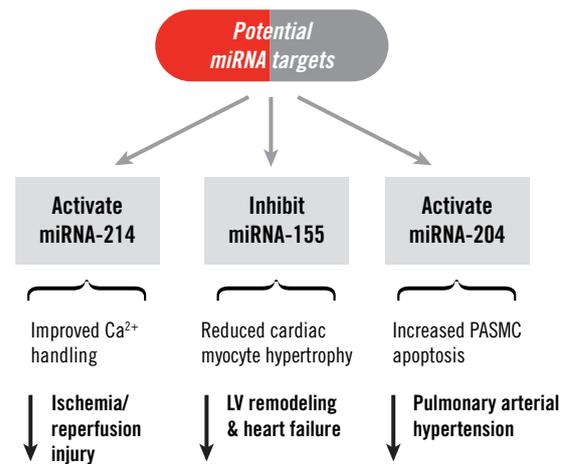


Fig. 1 Effects of potential microRNA targets.

Abbreviations: LV, left ventricular; miRNA, microRNA; PASM, pulmonary arterial smooth muscle cell.

and cyclophilin D (CypD). Importantly, these alterations in NCX1/CamKII α /CypD were associated with an exacerbation of left ventricular (LV) remodeling and dysfunction at 7 days post-LAD coronary artery occlusion and were recapitulated in neonatal cardiomyocytes (NCMs) treated with anti-miRNA-214 (miRNA-214 antagonist) exposed to hypoxia/reoxygenation, which increased apoptosis.

During heart failure progression, Seok et al demonstrated that miRNA-155 expression is significantly reduced in heart extracts at 3 days, 2 weeks, and 4 weeks following transverse aortic constriction (TAC).⁹ Despite this reduction in miRNA-155 expression, the authors also observed, in mice deficient for miRNA-155, a marked improvement in LV function and adverse LV remodeling at 4 weeks post-TAC surgery, which was associated with a significant reduction in mRNA expression of two heart failure markers; brain natriuretic peptide and myosin heavy chain β .⁹ Similarly, genetic elimination of miRNA-155 in cardiac-specific calcineurin-overexpressing mice also improved LV function and adverse LV remodeling in this mouse model predisposed to early heart failure. Furthermore, inhibition of miRNA-155 in NCMs prevented phenylephrine-induced cellular hypertrophy.

miRNA-based targeting may also have a role in the setting of pulmonary arterial hypertension (PAH) because lung miRNA profiles from rats treated with monocrotaline (MCT) or subjected to chronic hypoxia demonstrate that the expression of a number of miRNAs are altered.¹⁰ Studies from Courboulin et al demonstrated that activation of signal trans-

ducer and activator of transcription 3 (STAT3) is responsible for miRNA-204 downregulation in primary cultured pulmonary arterial smooth muscle cells (PASMCs) from PAH patients, and that miRNA-204 expression negatively correlates with PAH severity in mice, rats, and humans.¹¹ Moreover, treatment with a miRNA-204 mimic inhibited proliferation and increased apoptosis of PASMCs cultured from PAH patients, whereas nebulized delivery of this miRNA-204 mimic decreased mean pulmonary arterial pressure and pulmonary arterial wall thickness in rats with MCT-induced PAH.¹¹ Analysis of these rats also demonstrated a significant reduction in proliferation and increased apoptosis in the distal pulmonary arteries of rats with MCT-induced PAH that were treated with the miRNA-204 mimic.

Conclusions

Taken together, these examples provide exciting evidence supporting miRNAs as novel targets for many facets of cardiovascular disease. At this stage, all miRNAs in development for cardiovascular disease are currently in preclinical stages, with the first phase 1 trial for a miRNA-based target, MRX34 (miRNA-34 mimic), currently in phase 1 trials to assess safety in the treatment of patients with primary liver cancer.^{12,13} Undoubtedly, the next decade of research in the field should provide valuable information regarding the true potential that miRNA-based therapies may have for the treatment of cardiovascular disease. ■

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