

MicroRNA therapy of heart failure

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Abstract

MicroRNAs (miRNAs) are short, noncoding RNAs that have revolutionized the way we interpret genetic regulation. A component of what was once considered junk DNA, miRNAs regulate virtually every protein-coding gene in a highly dynamic fashion. Growing evidence has indicated that miRNAs are centrally involved in the pathogenesis of heart failure and strategies to modulate their activity therapeutically are under intense investigation. miRNA expression can be modified using several different approaches by either upregulating or blocking function. This article will describe these promising strategies and the challenges that must be overcome to develop meaningful therapies for heart failure. ■ *Heart Metab.* 2014;65:31-33

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MicroRNA biology

MicroRNAs (miRNAs) are short, noncoding RNAs (≈ 22 nucleotides) that modify gene expression by downregulating genes at the post-transcriptional level during various developmental or disease processes.¹ These ≈ 22 nucleotide RNAs bind to their targets in the 3' untranslated region (UTR) of messenger RNAs (mRNAs) to inhibit translation or evoke degradation of the mRNA. With miRNAs highly integrated into every cellular event, the potential impact on cardiac function is profound when considering the ramifications of misregulation of processes such as fibrosis,^{2,3} angiogenesis,⁴ cell growth,⁵⁻⁷ apoptosis,⁸ and electrophysiology.⁹ This convergence has led to the investigation of miRNAs as sources of pathophysiology and as biomarkers of heart failure (HF) in addition to therapeutic targets.

MicroRNA changes in heart failure

Although numerous miRNAs are expressed in the heart;

miR-1, miR-133, miR-208, and miR-499 are among the most highly expressed. While miR-1 and miR-133 are also present in skeletal muscle, miR-208 and miR-499 appear to be more cardiac specific. Referred to as the myomiRs, miR-1, miR-133a/b, and miR-206 play a key role in myogenic differentiation including cardiomyocyte differentiation.¹⁰ Accordingly, as heart failure progresses, remodeling occurs, inducing an embryonic developmental miRNA genetic signature. A seminal 2007 study by Thum et al¹¹ impressively demonstrated that adult hearts in HF were strikingly similar to fetal hearts with approximately 80% of miRNAs regulated analogously.^{1,12} One of the changes noted was increased expression of miR-21, which has now become one of the most consistent findings in HF (*Table I*). Inconsistencies between studies analyzing miRNA signatures in HF have complicated therapeutic development. The discrepancies are primarily the result of the type and stage of HF that patients or animal models are undergoing. Although these differences do not impede the use of miRNAs as therapeutics, it does warrant caution for selection.

Abbreviations

c-miRNA: circulating microRNA; **miRNA:** microRNA; **mRNA:** messenger RNA

MicroRNAs as biomarkers of heart failure

Another advantage of miRNAs is that, in addition to tissue specificity, many miRNAs can be found in the serum and are collectively known as circulating miRNAs (c-miRNAs). Easily detected in peripheral blood, c-miRNAs enable tracking of HF progression and can serve as biomarkers of efficacy in therapeutic trials (Table I). Serum levels of miR-208a/b, miR-499, miR-133a/b, miR1, and miR-423-5p have all been tested as biomarkers and compared with the current standard biomarker for HF, troponin.^{13,14} All showed good correlation with miR208a, demonstrating the highest level of sensitivity (~90%).¹⁵ c-miRNA cardiac signatures were further validated by a study that assessed c-miRNA profiles before and after placement of ventricular assist devices. The study showed normalization of >70% of miRNAs tested, demonstrating feasibility for a noninvasive therapeutic assessment.¹²

Anti-microRNA oligonucleotides as therapeutics

There are primarily two mechanisms to regulate miRNAs therapeutically. For those miRNAs that are upregulated in HF, anti-miR oligonucleotides (AMOs) can be designed and synthesized to be complementary to mature miRNAs and inhibit binding to their 3'UTR targets. AMOs that are further modified to optimize uptake in vivo and minimize off-target effects are termed antagomirs. They include incorporation of a 2'-O-methyl group to resist nucleases and a phosphorothiate linkage (PS) to improve distribution in tissues. There are numerous examples of successful

preclinical studies using antagomirs in vivo.¹² An antagomir designed to sequester miR-133 was shown to reverse cardiac hypertrophy when delivered systemically in mice,¹⁶ while delivery of a miR-21 antagomir suppressed fibrosis and hypertrophy.¹⁷

Encouraging results in preclinical studies have led to the integration of miRNAs into clinical trials. The majority of this inclusion has focused on miRNA expression signatures as biomarkers for disease progression and surrogate markers for therapeutic efficacy following pharmacological therapies.¹⁸ Challenges for translation of antagomir therapy for HF include: (i) efficient delivery to the heart; (ii) repeated dosing; and (iii) potential off-target effects. One antagomir has reached clinical trial—miravirsen. Miravirsen, a potential treatment for hepatitis C virus (HCV) infection, is an antagomir of miR-122, a hepatic-specific miRNA that directly targets HCV.¹⁹ Dose-dependent efficacy was achieved in chronic HCV-infected patients with miravirsen without adverse events.¹⁹ This trial, along with impending trials using miRNA-based therapies for cancer,¹⁸ provide valuable safety data that can be applied to future trials for HF.

MicroRNA mimics

In disease states where miRNA expression has been downregulated, miRNA mimics can be delivered to normalize expression. The mimic must be recognized by the miRNA biogenesis machinery as a double-stranded oligonucleotide in order to be processed and expressed appropriately. Off-target effects pose even greater risks and thus encapsulation into viral vectors with specific tissue tropisms have come to the forefront as the preferred method for delivery. Adeno-associated virus (AAV) is the most widely used vector for miRNA delivery. It is a small virus (<4.7 kb) that does not cause disease and persists as an episome for years to decades in tissues. There

miRNA	Expression in HF	Therapeutic or biomarker	Expected outcome	References
miR-21	Upregulated	Therapeutic	Reverse hypertrophy/fibrosis	12,17
miR-208	Upregulated	Both	Normalization of contractility	13
miR-423-5p	Upregulated	Biomarker	Serum biomarker of heart failure	12
miR-499	Downregulated	Both	Cardioprotection	1,14
miR-1, miR-133	Downregulated	Therapeutic	Prevention/reversal of cardiomyopathy	6,7,12
miR-29	Downregulated	Both	Reversal of fibrosis	3

Table I MicroRNA (miRNA) therapeutic targets for heart failure.

are multiple serotypes, including AAV-8 and AAV-9, that lead to efficient transduction of cardiomyocytes.²⁰ AAV-9 delivery of several miRs (miR-122, miR-29, and miR21) in preclinical murine studies has demonstrated a strong proof-of-principle for this approach (Table I). Delivery of AAV vectors carrying miR mimics directly to the heart rather than through the systemic circulation provides additional benefits including dose reduction, minimizing off-target gene expression, and maximizing transduction levels in the heart. Challenges for direct delivery to the heart still pose some translational hurdles due to the protected internal location; however intracoronary delivery has now been shown to be safe in a gene therapy trial with patients with advanced heart failure.²¹ This phase 2 study tested safety and efficacy of intracoronary infusion of the sarcoplasmic reticulum Ca²⁺-ATPase (SERCA) delivered using AAV-1. AAV-1–SERCA intracoronary delivery was shown to be safe and well tolerated in the 1-year study with a trend toward clinical significance in functional capacity of patients in the high-dose group.²¹ This sentinel study has laid the foundation for treatment of HF using AAV-mediated intracoronary delivery.

Conclusions

The therapeutic potential of miRNA mimics and antagomirs for HF is irrefutable. Preclinical studies have shown virtually complete reversal of HF in animal models and antagomirs are now transitioning into the clinic for other diseases such as HCV. Caution is warranted, particularly with the use of miRNA mimics, as the long-term effects of overexpression are not known yet. miRNAs have evolved to tightly regulate gene expression in a highly dynamic and titrated manner. Upregulation of miRNAs may be beneficial for normalizing disease states; however, overexpression may tilt the balance in the wrong direction. As clinical safety and efficacy data accumulate in favor of a stepwise approach with targeted delivery of miRNA-based therapies, the risk of complications will be lowered. To summarize, miRNA therapy for heart failure is a very promising approach to treatment. As miRNA signatures become more defined, it will be feasible to identify early indicators of heart failure prior to clinical manifestations. Early intervention to normalize miRNA expression in the heart has the potential to revolutionize the way heart disease is managed in the next decade. ■

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