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## MicroRNAs and Heart Failure Diagnosis MiR-acle or MiR-age?

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microRNA; miR; heart failure; cardiomyopathy

Heart disease is the greatest noninfectious health hazard ever to confront the human race. It is the leading cause of death in industrialized nations, and, accordingly, it is responsible for a huge economic burden to society. It is estimated that 5 million Americans have heart failure (HF), a syndrome with mortality of approximately 50% at 5 years.<sup>1</sup> Now, evidence indicates that the epidemic of HF is extending to the developing world.

The road to advances in the treatment of HF is littered with failures, and many new means of diagnosing and tracking disease progression have disappointed. Therefore, there is an urgent need to identify novel mechanisms, markers, and therapeutic targets in HF pathogenesis.

MicroRNAs (miRNAs or miRs) are among the most exciting areas in medicine and science today. MicroRNAs are endogenous, highly conserved,  $\approx 22$ -nucleotide-long noncoding RNAs that regulate the stability and subsequent translation of nascent mRNA transcripts. Generally, miRs inhibit protein translation and/or promote mRNA degradation by base-pairing with 3' untranslated regions within a transcript. MicroRNAs were first discovered in 1993 in *Caenorhabditis elegans* and are known to be critical to early vertebrate development and in an increasing number of basic molecular processes. Over the last few years, miRs have emerged as important mechanisms in human disease, including cardiovascular disease, diabetes, and cancer.<sup>2–6</sup>

Now, there is burgeoning interest in testing whether miRs could serve as markers for disease progression or prognostication. Indeed, in the cancer literature, strategies are emerging to use miR profiles for diagnosis and risk stratification. Currently, miR profiling is being evaluated in prostate cancer, head and neck squamous cell carcinomas, breast cancer, acute lymphoblastic leukemia, malignant mesothelioma, and other tumors.<sup>2,7–10</sup> Most clinical studies to date on miRs have relied on tissue-based measurement of miR abundances, but miRs are also released into the bloodstream and can be measured in human plasma and serum. These circulating miRs may potentially provide for easy and rapid testing in clinical populations, assisting in diagnosis or guiding therapy.

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### Disclosures

None.

Data on the role of miRs in cardiovascular disease are emerging rapidly. In one of the initial and seminal studies of miRs and the heart, stress-induced regulation of a group of miRs was reported in an animal model of cardiac hypertrophy; various miRs were either up- or downregulated after transverse aortic banding.<sup>11</sup> These authors also reported that cardiomyocyte overexpression of miR-195, one of the miRs that was upregulated in hypertrophy, was sufficient to trigger overt pathological hypertrophy and HF.<sup>11</sup> Similar expression patterns were observed in aortic-banded mice and in samples from human HF patients.

miR-208, a cardiac-specific miR encoded within an intron of the  $\alpha$ -MHC ( $\alpha$ -myosin heavy chain) gene, plays a prominent role in cardiac hypertrophy, fibrosis, and  $\beta$ -MHC expression.<sup>12</sup> Mice with a homozygous miR-208 deletion manifest a blunted stress-induced cardiac remodeling response. MiR-133a has also been implicated in similar pathological roles; mice deficient for miR-133a manifest cardiomyopathy, clinical HF, and abnormal cardiomyocyte proliferation via dysregulation of serum response factor and cyclin D2-dependent pathways.<sup>13</sup> Other work has demonstrated that miR-18b, -21, -23a participate in cardiomyocyte plasticity.<sup>14</sup> With regard to tissue injury, some data from animal models point to miR-208 as a potential biomarker of myocardial injury when compared to serial measurements of circulating cardiac troponin I.<sup>15</sup> In aggregate, these data implicate several miRs in events fundamental to cardiac development, plasticity, and injury.

With regard to clinical investigation, a few studies have now been published. Initial work has focused on miR expression in human tissue samples. One group examined miR expression using quantitative RT-PCR analyses of explanted human hearts.<sup>16</sup> Analysis of patients with ischemic cardiomyopathy, dilated cardiomyopathy, and aortic stenosis showed that 43 miRs were differentially expressed in at least one study group. Also, cluster analysis uncovered distinct miR expression profiles between each group and compared to controls. Two studies have examined miR expression in HF patients treated with mechanical support. In one study, 28 different miRs were upregulated >2.0-fold in the HF group, and there was near normalization in the posttreatment (left ventricular assist device) group.<sup>17</sup> Another study of myocardial tissue in 17 patients undergoing left ventricular assist device explantation showed a decrease in miR-1, -133a, and -133b in dilated cardiomyopathy patients but an increase in ischemic cardiomyopathy patients.<sup>18</sup> Together, these data lend credence to the notion that disease-specific miR expression profiles exist for cardiomyopathies of diverse etiology. In an interesting study comparing human cardiac tissue from patients with HF, normal human hearts, and fetal human hearts, similarities in miR expression profiles were reported in the fetal heart and in HF relative to control, reminiscent of the well-established, disease-associated reawakening of a fetal gene program.<sup>19</sup>

MiRs may also serve as biomarkers in patients with acute coronary syndromes. For example, levels of circulating miR-208 are increased in the plasma of patients with acute myocardial infarction.<sup>20</sup> Three other miRs were also increased (ie, miR-1, -133a, and -499). These data suggest that miRs may emerge as useful biomarkers in myocardial infarction patients or for risk stratification in patients with chronic coronary artery disease.

In this issue of *Circulation Research*, Tijssen et al build on this previous work.<sup>21</sup> The authors studied 12 healthy controls and 12 stable, chronic HF patients. Of note, the study analyzed circulating miR expression profiles from plasma. This is an advance relative to previous studies, because “easy-access” peripheral blood miR assessment is potentially practical for routine clinical use. Compared to control cases, defined as patients presenting with dyspnea but without HF, 16 circulating miRs afforded good discrimination for the diagnosis of HF. Next, a validation group was chosen to further test this group of miRs. In these analyses,

miR-423-5p was found to have impressive discriminative capacity with an area under the curve of 0.91 for distinguishing HF cases from healthy controls. This miR also had good discriminating capacity for differentiating the HF cases from the non-HF cases in a dyspnea registry (area under the curve, 0.83). MiR-423-5p also significantly correlated with circulating BNP levels and left ventricular ejection fraction. Other miRs were found to be increased in HF, but they were not as specific as miR-423-5p.

As with all good studies, this elegant work raises important new questions. Among them, in the HF group, more than one-third had an ejection fraction of  $\geq 45\%$  (11 of 30 subjects), suggesting strongly that their HF occurred in the context of preserved systolic function (HFpEF). Because HFpEF is both prevalent and associated with significant morbidity, it will be important moving forward to analyze these patients as a separate category from HF with systolic dysfunction. (Preliminary evidence presented here suggests that miR423-5p levels were elevated similarly in both patient groups.) Also, it will be interesting to characterize circulating miR-423-5p alongside other miRs implicated in previous basic and clinical studies. Future studies should involve larger HF cohorts stratified by other clinical features, such as ischemic versus nonischemic etiology, functional class, and disease phenotype (eg, restrictive, infiltrative, congenital). It will also be important to determine whether circulating miRs are informative in predicting responsiveness to pharmacological or device-based therapies.

The work reported here heightens interest in elucidating the basic biology of miR-423-5p. What is its cellular source? What are its targets? How is its expression regulated? Also, the presence of miRs in the circulation raises a number of interesting questions. Which cells produce them, and what is the mechanism for their release from the cell? Does this reflect the existence of a specific miR secretory pathway? If so, does this pathway discriminate between different miRs, or does miR export simply reflect the spectrum of miRs in a particular cell type? Does miR release result from cell death and consequent emptying of cellular contents into the extracellular space? How are miRs transported through the circulation? Do they reside in specific particles? What is their half-life? Do they participate in communication between tissues? If so, how are they taken up by the target tissue? Do they use the same internalization pathways used by exogenous oligonucleotides? Do they require lipoprotein receptors for uptake? Is the SID-1 channel involved?

The biology of miRs is an exciting new frontier in cardiovascular medicine. We see enormous potential in diagnostics, risk stratification, and prognostication, as well as for guiding therapy across a wide spectrum of cardiovascular disorders. Pharmacological targeting of miRs is developing rapidly to either block (antagomirs) or amplify (miR mimics) miR actions. Indeed, a miR-126 antagomir is capable of blunting ischemia-induced angiogenesis.<sup>22</sup> In cardiovascular medicine, we have grown accustomed to the all-too-frequent failure of strategies that target novel molecular and cellular cascades. Although a great deal of additional research is needed, miR biology is much more likely to emerge as a “miR-acle” than a “miR-age.”

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## Non-standard Abbreviations and Acronyms

<b>HF</b>	heart failure
<b>miR</b>	microRNA

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