MicroRNAs as Novel Myocardial Biomarkers
Kenneth B. Margulies*

The search for new biomarkers of cardiovascular disease remains a large and growing enterprise. Biomarkers are used to identify the risk, presence, and/or severity of disease; to guide diagnostic and therapeutic interventions; and to provide clues to disease mechanisms and pathophysiological distinctions within clinically similar populations. Among cardiovascular biomarkers, those used for identifying the presence or severity of myocardial injury have the most extensive history. Beginning with the first report 55 years ago that circulating transaminases are increased in acute myocardial infarction (1), the search for new and better biomarkers of myocardial injury has continuously evolved. As recently reviewed (2), cardiac troponin has emerged after decades of clinical and laboratory investigation as a highly diagnostically sensitive and specific biomarker of myocardial injury, with a currently indispensable role in the accurate and timely diagnosis of patients with suspected acute coronary syndromes (ACSs). 2 Central to this preeminence is the tissue specificity of cardiac troponin isoforms, increases in assay sensitivity, and an extensive body of evidence validating the diagnostic, prognostic, and therapy-guiding utility of cardiac troponins in diverse clinical settings. Because the benefits of ACS treatments are favorably influenced by the speed with which they are applied, the ideal myocardial injury biomarker must also permit rapid measurement; cardiac troponin assays have also proved satisfactory in this regard.

Despite the established strengths of cardiac troponins for prompt and reliable detection of myocardial injury, the ability to reliably detect ischemia in the absence of myocyte necrosis and the ability to distinguish alternative causes and mechanisms of myocyte damage (inflammation, oxidative stress, apoptosis) represent unmet needs and opportunities for new biomarkers, which have been highlighted in recent reviews (2, 3). Resistance to inaccuracy caused by confounding factors such as renal failure is another favorable attribute that is not ideally achieved with cardiac troponin assays.

Although most testing of clinically applied biomarkers has involved measurement of circulating peptides or proteins with biochemical or immunoassay techniques, advances in molecular biology and technology have fueled an increasing interest in nucleotide-based biomarkers that might address unmet needs and/or enhance diagnostic or therapeutic effectiveness. For example, additional valuable insight might be derived from biomarkers that identify patient-specific factors, such as DNA polymorphisms, that modulate a patient’s risk and responses to treatment. Alternatively, mRNA in peripheral blood cells (PBCs) may provide unique insights into the interplay between the myocardium and the circulation, and PBC mRNA has recently been used to noninvasively detect rejection of cardiac allografts. For that application, early studies identified 40 mRNA transcripts derived from PBCs with a favorable dynamic range, differential expression in patients with and without allograft rejection, and regression of pathologic expression in association with histologic regression (4). Further development of this approach led to the commercialization of a clinical diagnostic tool (5) and identified the potential to achieve greater diagnostic clarity in areas in which standard histologic techniques have difficulty discriminating rejection from other biological responses (6). Although RNA profiling of PBCs in transplant medicine continues to evolve, it is clear that the early parallel sampling of myocardial and blood samples propelled the identification and validation of less invasive biomarkers.

In this issue of Clinical Chemistry, Ji et al. applied an analogous experimental approach to identify circulating microRNAs (miRNAs) that might accurately reflect myocardial injury in vivo (7). In these studies, a systematic search used standard real-time reverse-transcription PCR (RT-PCR) techniques to identify miR-208, a cardiac-specific miRNA detectable in circulating plasma after myocardial injury. Moreover, miR-208 was detectable in the plasma of rats with isoproterenol-induced cardiac injury but not in healthy control rats, after renal infarction, after left ventricular hypertrophy, or after bilateral nephrectomy. Importantly, increases in plasma miR-208 after isoproterenol administration were correlated with increases in circulating concentrations of cardiac troponin. Together, these findings indicate that plasma miR-

1 Cardiovascular Institute, University of Pennsylvania School of Medicine, Philadelphia, PA.
* Address correspondence to the author at: University of Pennsylvania School of Medicine, Rm. 608 BRR II/III, 421 Curie Blvd., Philadelphia, PA 19104.
Received July 29, 2009; accepted August 4, 2009.
Previously published online at DOI: 10.1373/clinchem.2009.131649
2 Nonstandard abbreviations: ACS, acute coronary syndrome; PBC, peripheral blood cell; miRNA, microRNA; RT-PCR, reverse-transcription PCR; LVAD, left ventricular assist device.
miRNAs comprise 21–23 nucleotides that regulate (usually negatively) the expression of many mRNA targets. Because individual miRNAs may affect the expression of multiple genes, they are capable of coordinated and potent effects on cell function. In fact, miRNAs have been implicated in developmental processes, cell proliferation, differentiation, stress responses, apoptosis, and oncogenesis. In mouse models of left ventricular pressure overload and genetic cardiomyopathies, 3 separate studies have demonstrated altered regulation of miRNAs that appear to be necessary for the development of cardiac hypertrophy in response to pathophysiological stress (9–11). In 2 of the studies, manipulation of a single miRNA was able to produce or suppress cardiac myocyte hypertrophy (9, 11). In the third study, the same miRNA studied by Ji et al. (miR-208) was found to be a central mediator of the elegant balancing of α- and β-myosin heavy chain isoforms within the healthy and diseased myocardium (10).

Recent data from other fields suggest that peripheral blood miRNAs could reflect and/or mediate myocardial adaptations to disease. For example, miR-184 from cell-free plasma has been reported as a biomarker for squamous carcinoma of the tongue in humans (12). Ten of 56 miRNAs differentially produced after ischemia-reperfusion injury in the rat brain were coordinately altered in the peripheral blood (13). Other studies have indicated that circulating miRNAs and miRNA packages within small (50–90 nm) vesicles, termed “exosomes,” that offer protection against degradation, can serve as a means of intercellular communication and regulation between distant cells (14). With respect to the myocardium, a recent study found that 21 miRNAs were dysregulated in failing hearts (compared with nonfailing hearts) exhibited partial or complete normalization after sustained myocardial unloading via a left ventricular assist device (LVAD) (15). These findings with miRNAs contrast with those of studies demonstrating that LVAD-associated improvements in myocardial phenotype were rarely associated with regression of pathologic mRNA abnormalities in human hearts (16). In this context, the study of Ji et al. is the first to examine circulating miRNAs as potential biomarkers of myocardial disease; however, these recent studies indicate that the most exciting potential applications of profiling myocardial and circulating miRNAs are likely to go beyond the detection of myocardial injury (for which cardiac troponin assays are already excellent and fast) to the detection of other processes for which alternative biomarkers are lacking and the imperative for a rapid turnaround time is less pressing.

Authors’ Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.
Employment or Leadership: None declared.
Consultant or Advisory Role: K.B. Margulies, Ortho Clinical Diagnostics.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: K.B. Margulies, Ortho Clinical Diagnostics.
Expert Testimony: None declared.
Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

8. Boyd SD. Everything you wanted to know about small RNA but were afraid to ask. Lab Invest 2008;88:569–78.