MicroRNAs as Novel Myocardial Biomarkers

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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, the search for new and better biomarkers of myocardial injury has continuously evolved. As recently reviewed, 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). 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. 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. Further development of this approach led to the commercialization of a clinical diagnostic tool and identified the potential to achieve greater diagnostic clarity in areas in which standard histologic techniques have difficulty discriminating rejection from other biological responses.

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. 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-

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Nonstandard abbreviations: ACS, acute coronary syndrome; PBC, peripheral blood cell; miRNA, microRNA; RT-PCR, reverse-transcription PCR; LVAD, left ventricular assist device.
208 is a cardiac-specific biomarker that reflects the extent of cardiac myocyte injury but that is not substantially influenced by antecedent cardiac hypertrophy, renal function, or cellular injury involving other organs. Although these findings are novel, the validity and utility of circulating miR-208 as an injury biomarker must be viewed as preliminary. In particular, the performance of miR-208 as a biomarker must be evaluated in humans, both in response to cardiac ischemia and injury from more typical causes such as coronary occlusion and over longer time frames to better characterize the release and clearance of miR-208 from the circulation.

The report of an injury biomarker derived from RNA also begs the question of whether miR-208 adds appreciably to the information provided by cardiac troponin. Although Ji et al. note that RT-PCR permits great analytical sensitivity, the analytical sensitivities of cardiac troponin assays have already progressed to the point that values above the detection threshold are observed in settings not previously recognized as producing cardiomyocyte necrosis, such as in nonischemic heart failure, pulmonary embolism, and sepsis. This experience suggests that insufficient analytical sensitivity is not a compelling argument for developing new markers that identify cardiac myocyte injury. Another consideration is whether miR-208 provides new insights into myocardial biology during ACSs that complement the information provided by cardiac troponins. In this regard, the fact that myocardial hypertrophy alone did not produce detectable increases in miR-208 suggests that this biomarker is truly specific for myocyte disruption, but this finding also tends to compromise the assertion that it provides complementary insights into pathobiology. Finally, it is also worth considering whether the assessment of miRNA or mRNA via quantitative RT-PCR will be able to achieve the turnaround time required for use in the emergency room settings most often confronting patients with ACSs. Given the currently available technology and the other clinical applications of RT-PCR, this goal seems unlikely.

Although miR-208 is unlikely to displace cardiac troponins as the preeminent biomarker of myocardial injury, the studies by Ji et al. do suggest that miRNAs offer other promising opportunities for enhanced diagnostic, prognostic, and mechanistic insights into cardiovascular disease. miRNAs comprise 21–23 nucleotides that regulate (usually negatively) the expression of target mRNAs. Because individual miRNAs may affect the expression of many mRNA targets (8), 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 miRNAs packaged 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 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.

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