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Circulating p53-Responsive MicroRNAs as Predictive Biomarkers in Heart Failure Following Acute Myocardial Infarction: The Long and Arduous Road from Scientific Discovery to Clinical Utility

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The past decade has witnessed an exponential proliferation of new biomarkers that are useful in evaluating patients with heart failure (reviewed in¹). Indeed 2 biomarkers, BNP and NT-BNP are FDA approved for the diagnosis of patients with heart failure, whereas BNP, NT-BNP, galectin-3 and soluble ST-2 are FDA approved for determining the prognosis of patients with heart failure. In the present issue of *Circulation Research*, Matusmoto and colleagues report that several p53-responsive microRNAs, including miR-192, miR-194 and miR-34a, were elevated in the sera of selected patients enrolled in the Osaka Acute Coronary Insufficiency study (n= 8603 patients) who developed heart failure one year after acute myocardial infarction.² Given that p53 has been implicated in the development of heart failure in mice,³ these findings are of potential interest both from a mechanistic standpoint, as well as clinically from a diagnostic standpoint. In the current study the authors examined a panel of 377 microRNAs in registry patients who developed heart failure (n=7 patients), and then confirmed their findings in a validation cohort of 21 patients. The authors reported previously that miR-192 was significantly (p = 0.04) elevated in selected registry patients.⁴ Noting that miR-192 was responsive to p53, in the present study the authors rescreened the sera of heart failure patients and identified significant increases in the levels of 2 additional p53-responsive microRNAs, namely miR-194(increased ~ 6-fold) and miR-34a (increased ~ a 3-fold). The presence or absence of heart failure in the registry was determined from a retrospective chart review; however, the criteria that were used to diagnose heart failure were not specified. Remarkably, the levels of miR-192, miR-194 and miR-34a were highly enriched in the exosome fraction of the heart failure patients when compared to control subjects, whereas the levels of miR-192, miR-194 and miR-34a in the non-exosome supernatant fraction were not different between heart failure and control subjects, suggesting that the circulating levels of these p53-responsive microRNAs might in some way be involved in the pathogenesis of heart failure. The authors were careful to demonstrate that forced expression of p53 was sufficient to increase the expression levels of miR-192, miR-194 and miR-34a in cultured rat myoblasts (H9c2 cells), and that knockdown of all 3 these microRNAs (but not single miRs) enhanced cell survival following treatment with doxorubicin, whereas transfer of all 3 these microRNAs (but not single miRs) via the culture media resulted in decreased cell viability.

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Further, miR-194 and miR-34a were weakly but significantly correlated ($r = 0.33$ [$p=0.01$] and $p = 0.38$ [$p = 0.003$], respectively) with left ventricular end-diastolic dimension one year after myocardial infarction. Matsumoto and colleagues concluded that p53-responsive microRNAs may be useful for stratifying the risk of future events in patients with ischemic-induced heart failure, as well as cardiac remodeling in post-infarct patients. Before addressing the significance of this study, it is instructive to first review what little is known with regard to microRNAs as cardiovascular biomarkers.

MicroRNAs as Biomarkers in Heart Failure

Recent studies have identified profound roles for a family of tiny regulatory non-coding RNAs, known as microRNAs (miRs) in the control of diverse aspects of cardiac structure and function.^{5,6} Multiple lines of evidence now suggest that microRNAs play an important role in the pathogenesis of heart failure through their ability to negatively regulate expression levels of genes that govern the process of adaptive and maladaptive cardiac remodeling. Moreover, the extant literature suggests that specific microRNAs are differentially regulated in the failing heart.^{7,8} Recently, stable levels of circulating microRNAs have been detected in the peripheral blood, raising the intriguing possibility that elevated levels of microRNAs may represent a new class of biologically active biomarkers. Indeed, some of the innate properties of microRNAs make them highly attractive as potential biomarkers. For example microRNAs can be readily detected in small sample volumes using specific and sensitive quantitative real-time PCR (qRT-PCR) methodology. MicroRNAs are quite stable, and can be isolated from most body fluids, including serum, plasma, urine, and saliva. Further, microRNAs are highly conserved between species, which permits their analyses in pre-clinical animal models of disease.

Although it is clear that microRNAs function as a mechanism for post-transcriptional regulation, what remains uncertain is whether the presence of microRNAs in the circulation is secondary to the passive release by necrotic and/or degrading cells, or whether they are actively secreted by cells. This distinction is important, insofar as the former possibility suggests that the release of microRNAs is accidental, and may lack biological function, whereas the latter possibility suggests that the elaboration of circulating microRNAs is regulated, and may therefore have important, but as yet unknown, biological functions. A growing body of evidence, including the current report by Matsumoto et al in this issue of the *Journal*, supports the hypothesis that microRNAs are actively secreted. There are currently three known methods by which miRNAs are packaged and secreted: (1) in lipid vesicles, such as exosomes or microvesicles and apoptotic bodies; (2) bound by RNA-binding proteins, such as nucleophosmin-1 and Argonaute-2; and (3) associated with high-density lipoproteins. Exosomes are small (50–90 nm) vesicles that are released into the extracellular environment following the fusion of the multivesicular bodies with the plasma membrane, whereas microvesicles are larger (up to 1 μm) particles that are shed from the cell membrane. MicroRNAs that are located in exosomes or microvesicles are extremely resistant to RNAase-dependent degradation because they are protected by lipid bilayers. MicroRNAs can also be stabilized in the circulation when localized in the apoptotic bodies (“apobodies”), which are small sealed membrane vesicles that are produced from cells undergoing apoptotic cell death. However, the great majority of microRNAs (~ 90%) circulating in the blood appear to be bound to protein complexes such as Argonaute-2 or nucleophosmin-1, which protect microRNAs from nucleases. Of note, the structurally diverse extracellular packing of microRNAs may also permit identifying tissue-specific microRNAs by selecting for protein surface markers, which could potentially improve the diagnostic sensitivity and specificity of disease-specific microRNAs.

Although microRNAs are theoretically attractive as biomarkers, it has been challenging to measure microRNAs with the precision and reproducibility that is required for clinical diagnostic work. Some of the main issues relate to specimen collection, factors influencing RNA extraction efficiency, and the technical issues involved in accurate qRT-PCR, including amplification, normalization, and contamination.⁹ Quantitating the absolute amount of total microRNA in body fluid specimens is not possible because of the extremely low concentrations. In addition, there is no consensus with respect to small RNA reference genes that can be used as internal controls to control for biological variability. Lastly, quantification of valid microRNAs in the serum can be complicated by contamination by microRNAs that are leaked from cellular blood components during the collection process, either through phlebotomy-induced hemolysis or cell separation and/or sample processing. However, the cardiac studies published to date suggest that microRNAs have sufficient fidelity to serve as prognostic markers in acute myocardial infarction, with receiver operator characteristics demonstrating areas under the curve ranging from 0.77 – 0.98, which is suitable for clinical work.¹⁰

Circulating p53-Responsive MicroRNAs as Predictive Biomarkers for the Development of Heart Failure Following Acute Myocardial Infarction

In the current issue of *Circulation Research*, Matsumoto and properly conclude with the statement that “further investigations with the increased number of samples and/or in other cohorts are required to confirm the present findings and future clinical applications of miR-192, miR-194 and miR-34a as predictive indicators of heart failure.” Based on the aforementioned problems with respect to using microRNAs as biomarkers, it will be important going forward to expand the validation sample to databases that contain several hundreds of patients with diverse racial backgrounds, in whom the definition of heart failure is clearly established using accepted criteria. Moreover, given that upregulation of a single pathway (e.g. p53) at a single point in time is unlikely to explain the complex pathogenesis of heart failure, and given that the correlation between the expression levels of miR-194 and miR-34a and the degree of left ventricular remodeling only explained 10–15% of the variability in left ventricular dimension in the current study, it would also be informative to follow circulating levels of miR-192, miR-194 and miR-34a serially as a function of time post-infarction, in order to determine whether serial measurements are more predictive of left ventricular remodeling and clinical outcomes. Lastly, since there are a number of inexpensive, well-characterized biomarkers that accurately predict the development of heart failure post-infarction, including: BNP, NT-BNP, Mid-regional pro-Atrial Natriuretic Peptide, soluble ST2, C-terminal pro-endothelin-1, Mid-regional pro-Adrenomedullin and co-peptin,¹¹ it will be important to determine whether the addition of microRNAs to single and/or multi-marker biomarker panels is of added value beyond what is currently available commercially. Accordingly, the path forward for developing microRNAs as biomarkers that predict the development of heart failure following acute myocardial infarction will likely not be a simple one. This statement notwithstanding, we believe that studies such as the current study by Matsumoto and colleagues are important because they may provide novel “bioinformatic windows” into the pathogenesis of heart failure. For example, although Matsumoto and colleagues did not identify potential miR-192, miR-194 and miR-34a downstream gene targets in their current manuscript, a provisional search of possible downstream gene targets obtained from Targetscan (<http://www.targetscan.org/>) and MiRDB (<http://mirdb.org>), followed by a KEGG (Kyoto Encyclopedia of Genes and Genomes) functional pathway analysis, disclosed significant changes in gene expression in pathways implicated in TGF-beta and WNT signaling, as well as hypertrophic and dilated cardiomyopathy, any or all of which may be important with respect to the development of post-infarction cardiac remodeling. Thus, the potential benefit for analyzing circulating

microRNAs in the peripheral circulation of heart failure patients may extend well beyond the canonical role that has been established for our current panoply of biomarkers, by allowing investigators to obtain time-sensitive mechanistic insights into the pathogenesis of heart failure.

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REFERENCES

1. van Kimmenade RR, Januzzi JL Jr. Emerging biomarkers in heart failure. *Clin Chem*. 2012; 58:127–138. [PubMed: 22086968]
2. Matsumoto S, Sakata Y, Nakatani, Suma S, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y, Komuro I. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. *Circ Res*. 2013; 112
3. Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkentin JD, Zou Y, Komuro I. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature*. 2007; 446:444–448. [PubMed: 17334357]
4. Matsumoto S, Sakata Y, Nakatani D, Suna S, Mizuno H, Shimizu M, Usami M, Sasaki T, Sato H, Kawahara Y, Hamasaki T, Nanto S, Hori M, Komuro I. A subset of circulating microRNAs are predictive for cardiac death after discharge for acute myocardial infarction. *Biochem Biophys Res Commun*. 2012; 427:280–284. [PubMed: 22995291]
5. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G, Wang Z. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med*. 2007; 13:486–491. [PubMed: 17401374]
6. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell*. 2007; 129:303–317. [PubMed: 17397913]
7. Divakaran V, Mann DL. The emerging role of microRNAs in cardiac remodeling and heart failure. *Circ Res*. 2008; 103:1072–1083. [PubMed: 18988904]
8. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. *Nature*. 2011; 469:336–342. [PubMed: 21248840]
9. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods*. 2010; 50:298–301. [PubMed: 20146939]
10. Salic K, De Windt LJ. MicroRNAs as biomarkers for myocardial infarction. *Curr Atheroscler Rep*. 2012; 14:193–200. [PubMed: 22392328]
11. Chan D, Ng LL. Biomarkers in acute myocardial infarction. *BMC Med*. 2010; 8:34. [PubMed: 20529285]