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TRANSLATIONAL PERSPECTIVE

MicroRNA-Based Diagnostics in Heart Diseases

Current Limitations and Future Perspectives

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The majority of the human genome does not code for proteins. For a long time, researchers have focused almost exclusively on genes whose transcription results in protein-coding messenger RNAs. This has changed over the past years, and the non-protein-coding genome has become of increasing interest to scientists and clinicians alike. Among the noncoding RNA transcripts, microRNAs (miRs) with a typical length of 17 to 25 nucleotides have been identified as important modulators of post-transcriptional gene expression (1). By altering the levels of messenger RNAs, miRs are involved in the regulation of several intracellular (patho)physiological pathways (1). Furthermore, as a response to different stimuli, miRs are exported from the cellular compartment, enter the circulation, and thus can participate in intercellular communication (1). Circulating miRs are found bound to RNA-binding proteins (eg, argonaute) and high-density lipoproteins or transported in extracellular vesicles that, because of their high stability, can be reliably measured and quantified in bodily fluids, such as blood plasma (1). Evidence suggests that the expression of circulating miRs is altered in several cardiovascular diseases such as atherosclerosis or heart failure (1,2). Moreover, after years of research in this field, cardiac tissue-specific miRs, such as mir-208a, which is found to be upregulated in patients with coronary artery disease, have been identified (1). Another potentially important aspect of circulating miRs is their use in predicting prognoses. For example, expression of circulating miR-126 is reported to be down-regulated in patients with coronary artery disease and might be used for risk assessment in these patients (1). Therefore, circulating miRs have the potential to be used as cardiologic biomarkers.

This is highlighted by a recently published study investigating miRs as biomarkers in acute myocarditis (3). Using a novel approach, Blanco-Domínguez et al (3) focused on elevated levels of type 17 helper cells (Th17), which were shown to be increased during the acute phase of myocarditis, in contrast to myocardial injury, after myocardial infarction (MI) (3). They found that myocarditis is associated with increased circulating levels of Th17-derived mmu-miR-721, or its human homologue hsa-miR-Chr8:96, across different murine models of myocarditis (including from infectious and autoimmune origins) and in human myocarditis patients (3). Patients with myocardial injury caused by MI and MI with nonobstructive coronary arteries showed lower levels of hsa-miR-Chr8:96 (3). The variability in the expression of hsa-miR-Chr8:96 was pronounced, and it was not evaluated in patients with dilated cardiomyopathy, which is a key differential diagnosis from myocarditis whose pathophysiology is also influenced by Th17 cells (3). Despite these restrictions, the evidence for the value of hsa-miR-Chr8:96 as a biomarker for myocarditis appears substantial. As there are important drawbacks to the existing diagnostic tools for myocarditis, cardiac magnetic resonance and

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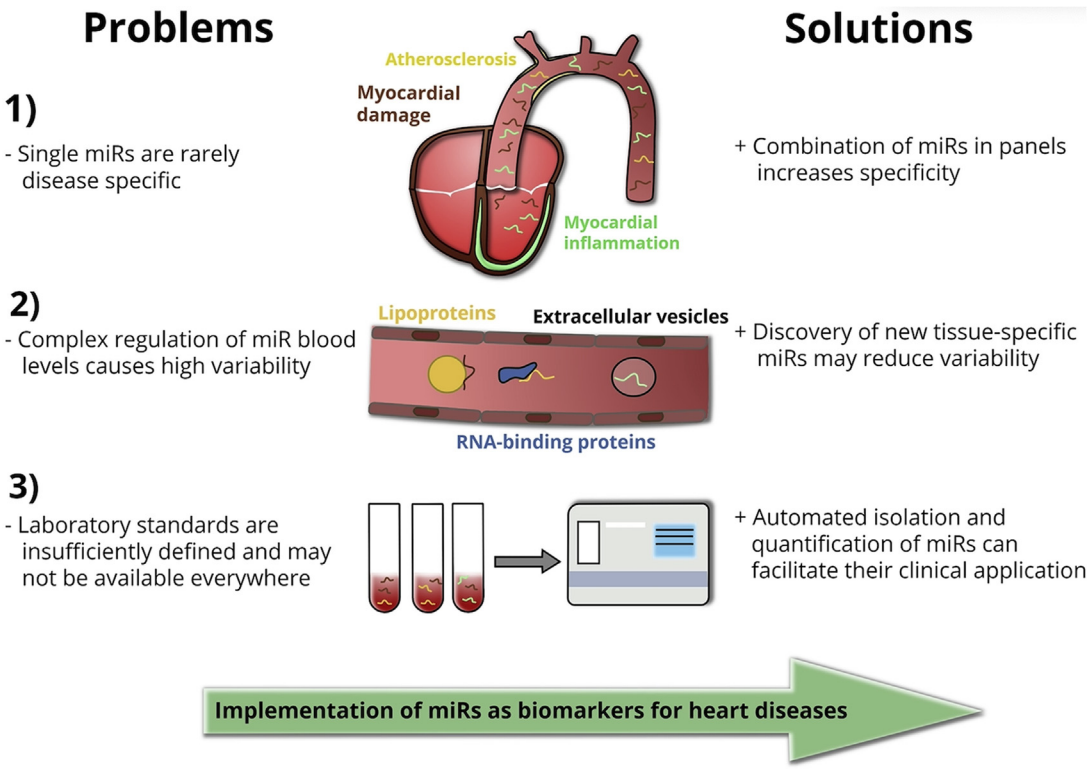
FIGURE 1 MiR-Based Diagnostics in Heart Diseases



The release of microRNAs (miRs) in different cardiovascular pathologies causes a complex signature of up- and down-regulated miRs in the peripheral blood. Here, we present a synopsis of the current challenges and possible future solutions to establish miRs as biomarkers for cardiovascular disease.

endomyocardial biopsy, hsa-miR-Chr8:96 has the potential to identify myocardial inflammation and fill the need for a reliable noninvasive biomarker that can easily be applied to routine patient care. In a broader context, the current study might have an impact on the entire field of miR-based diagnostics in cardiovascular disorders. The chosen scientific approach addresses a current limitation that prevents the implementation of miRs as biomarkers in clinical routine. Although the use of miRs for diagnostic purposes is of high interest and could find a wide range of applications, to date, none of them have made the transition from "bench to bedside" to be used in clinical practice as a standard diagnostic tool. This is mainly caused by 3 problems with miR-based diagnostics, which still need to be solved (**Figure 1**).

First, in order to function as next-generation biomarkers, which are an improvement on the existing diagnostic options, the candidate miRs need to be highly disease specific to be able to discriminate between different conditions (eg, causes of cardiac injury) (3). Second, evidence suggests that complex regulation of miRs in different (patho)physiological states causes a variability in miR levels in patient blood samples (1). Thus, blood levels of miRs depend on the balance between cellular export into the blood stream and clearance through the uptake of circulating miRs by recipient cells (1). These mechanisms are prone to various stimuli, which can interfere with circulating miR levels and result in a subsequent decline in specificity. Third, non-standardized preanalytical and analytical processes for the measurement of miRs may result in high intra- and interindividual variability of the miR levels (2,4-6). Thereby, innovative studies in basic science are a prerequisite for meeting the challenges of implementing miRs as biomarkers for heart diseases.

The proposed approach by Blanco-Domínguez et al of investigating disease-specific findings, such as

evaluating elevated or decreased levels of certain cell lines followed by screening for affected miRs, may be useful for the future identification of novel, disease-specific miRs. Moreover, further research is needed to deepen our knowledge about potentially confounding conditions, such as dilated cardiomyopathy and other inflammatory processes. One possible approach to integrate hsa-miR-Chr8:96 into the diagnostic algorithm of acute myocarditis and to increase its specificity could be to combine it with a different, myocardial-damage marker, such as troponin T. Another approach would be to use a combination of multiple differentially expressed miRs, in a so-called miR-panel. MiR-panels are less likely to be influenced by confounding conditions that might impact single miRs and thereby could improve their diagnostic value. They have been extensively studied in the field of cancer research, and recently published data suggest the potential use of a miR-panel as a sensitive screening tool for gastric cancer (4). In the cardiovascular field, a recently published meta-analysis of miR expression studies provides evidence for a novel miR-panel containing 16 miRs that are differentially expressed in patients with heart failure (5). In addition, an example of the combination of miR-panels and "traditional" biomarkers is provided by Wong et al (2) to detect heart failure. The combination of a miR-panel, containing 8 specifically selected miRs, with the well-established biomarker N-terminal pro-B-type natriuretic peptide increased the specificity of diagnosis compared with N-terminal pro-B-type natriuretic peptide alone for identifying patients with nonacute heart failure (2). These studies emphasize the importance of further research to increase specificity of miRs as cardiovascular biomarkers and decrease the impact of biological confounders.

In order to overcome the remaining hurdles to introduce miR-based diagnostic approaches into clinical cardiology, a standardization of the pre-analytical and analytical processes would be an important first step. These processes include sample collection (including information about pre-existing medication), RNA isolation, and also the measurement of miRs in patient samples (2-4,6). Furthermore, to allow these procedures to also be performed in smaller hospitals, the isolation and quantification processes need to be highly standardized and only use simple, automated methods that can be performed in any standard laboratory setting. Using these techniques, novel miRs could then be investigated in prospective clinical trials with large cohorts of patients (6). In conclusion, during the process of implementing miR diagnostics into clinical routine, there is still some work to do. However, recent studies have taught us that innovative study design can help overcome the limitations of miR diagnostics. This gives us hope that in the future we will be able to tap the full potential of miRs as biomarkers for cardiovascular disease.

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