HHS Public Access

Author manuscript

Annu Rev Pathol. Author manuscript; available in PMC 2020 January 24.

Published in final edited form as:

Annu Rev Pathol. 2019 January 24; 14: 211–238. doi:10.1146/annurev-pathmechdis-012418-012827.

Opportunities for microRNAs in the Crowded Field of Cardiovascular Biomarkers

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Abstract

Cardiovascular diseases exist across all developed countries societies. Biomarkers that can predict or diagnose diseases early in their pathogeneses can reduce their morbidity and mortality in afflicted individuals. microRNAs are small regulatory RNAs that modulate translation and have been identified as potential fluid-based biomarkers across numerous maladies. We describe the current state of cardiovascular disease biomarkers across a range of diseases, including myocardial infarction, acute coronary syndrome, myocarditis, hypertension, heart failure, heart transplantation, aortic stenosis, diabetic cardiomyopathy, atrial fibrillation, and sepsis. We present the current understanding of microRNAs as possible biomarkers in these categories and where their best opportunities exist to enter clinical practice.

Keywords

microRNAs; biomarkers; cardiovascular disease; hypertension; sepsis; aortic stenosis

INTRODUCTION

In the field of cardiovascular disease, biomarkers are essential tools in a clinician's armamentarium. The best of our biomarkers can aid in diagnosing an acute myocardial infarction (AMI), indicate long-standing heart failure, and predict the rejection of a transplanted heart. However, not all biomarkers are robust, and many fields within cardiovascular disease management await useful biomarkers to aid in diagnosis and assessing prognosis. Across all of these scenarios there is the opportunity for better

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

biomarkers. It is with this appreciation that we critically evaluate microRNAs (miRNAs) as a new category of biomarkers for cardiovascular diseases. In this review, we analyze what the opportunities are for using miRNA biomarkers across particular disease states, specifically comparing miRNA biomarkers to what is currently used for the disease when appropriate. We also provide context for the intricacies of miRNA biomarker studies so each reader has the tools to critically evaluate any of those described herein or forthcoming miRNA studies for their potential clinical use in cardiovascular disease.

A PRIMER ON miRNAS AND BIOMARKERS IN CARDIOVASCULAR DISEASE

A Brief Description of miRNA Biogenesis

miRNAs are short (18–22 nucleotide) regulatory RNAs that bind mRNAs and decrease protein translation. miRNAs are generally cotranscribed with neighboring genes or co transcribed within a cluster of miRNAs (a polycistronic cluster). These primary miRNAs (pri-miRNAs) are then processed by Drosha into pre-miRNAs and cleaved by Dicer into mature miRNAs. One half of a mature miRNA (either the 5p or 3p sequence) is loaded into Argonaute 2 (Ago2) as part of RISC (the RNA-induced silencing complex). Although complicated, controversial, and with many exceptions to the rule, a six base pair seed sequence at the 5′ end binds to a complementary region along the 3′ untranslated region of an mRNA, thus enabling specific regulation of scores of mRNAs by a single miRNA (1, 2). This entire mechanism has been extensively described and reviewed elsewhere (3–5).

The Strict Nomenclature of miRNAs

miRNAs have a strict naming convention (6, 7). A miRNA name begins with a three-letter code for the species (e.g., hsa for *Homo sapiens* and cel for *Caenorhabditis elegans*). This is followed by miR and then a number of some sequential order. If miRNAs have the same seed sequence, indicating a shared family function, there may be a letter, in alphabetical order, after the number. Examples include hsa-miR-181a, hsa-miR-181b, and hsa-miR-181c. Each processed miRNA has two strands: a dominant strand (more abundant and loaded in RISC) and a passenger strand (degraded, less abundant), one from each of the 5p and 3p ends. These were denoted as the s (sense) and as (antisense) or * strands (star, denoting passenger) in earlier literature. A complete miRNA name would be hsa-miR-181a-3p or hsa-miR-181a-5p. The one exception to this naming convention is the let-7 family of miRNAs, which kept their original names. Once a species is established, the species code is not commented on further in most manuscripts. Equally, if the 5p or 3p designation is not stated, it is generally assumed that the dominant strand is being referenced.

The True Number of Human miRNAs is Unknown

The number of human miRNAs is controversial. miRBase (http://www.mirbase.org/) has been considered the central repository of all known miRNAs. Version 21 of miRBase listed 2,588 human miRNAs, a relatively stable number compared with prior versions of the database (6). However, since that version appeared, with the explosion in small RNA sequencing (RNA-seq) and generic novel miRNA discovery tools, thousands of additional

human miRNAs have been proposed (8–11). Most of these have been collected into the new miRCarta database (https://mircarta.cs.uni-saarland.de/) that adds an additional 12,857 human miRNA precursors to our collection (12). Are all of these additional sequences truly in the miRNA oeuvre? Many groups suggest otherwise, indicating that miRBase is rife with non-miRNA species, and these newly minted novel miRNAs are mostly dead on arrival (13–17). In response to these concerns, MiRGeneDB (https://mircarta.cs.uni-saarland.de/) was developed as a hand-curated classification of miRNAs based on strict criteria, including genetic conservation and canonical development (14, 18). This database lists only 586 human miRNA genes. Clearly, the collection of small RNA–expressed species requires greater clarity and perhaps additional nomenclature to describe miRNA-like small RNAs (17). Regardless, for the purpose of utilizing miRNAs as biomarkers, any sequence that gives a consistent signal indicating the presence of disease can be useful.

Many miRNAs are Expressed in a Cell Type–Specific Fashion

A factor in understanding the function and biomarker utility of miRNAs is the appreciation of the cells in which they are expressed. If a miRNA is expressed in a cardiomyocyte, it may have more relevance as a cardiovascular biomarker than one that also is expressed in a noncardiovascular cell (e.g., a hepatocyte). Early expression localization studies were performed in tissue (19–21). These limited our understanding of the contribution of the various cell types that are expressed across a variety of tissues—such as endothelial cells, red blood cells, and fibroblasts—to the changing miRNA levels in diseased tissue. This led to problems that plague the miRNA literature (7, 22, 23). Better characterization of miRNAs at the cellular level has been slow to develop. We have been actively engaged in the realization of miRNA cellular localization (24, 25), culminating in a cellular microRNAome that is based on miRNA data from 46 primary cell types and 42 cancer or immortalized cell lines (8). This work was complemented by two other papers in 2017 describing miRNA expression in many additional cell types (9, 26). These combined data are now accessible as a University of California Santa Cruz genome browser barChart that shows miRNA expression levels across 78 primary cell types and 51 cancer or immortalized cells (Figure 1) (17). For the first time, any investigator can quickly determine the cellular location of their miRNA of interest to determine its relevance to disease (Table 1). This information is vitally important to miRNA biomarker studies as seen below.

MyomiRs

Between organ-specific data and cell-specific data, it has emerged that a group of miRNAs is found either exclusively or more abundantly in myocytes. These are termed myomiRs. As described in Table 2, some of these miRNAs are found within myosin heavy chain genes (27). Most of these myomiRs are also expressed in skeletal muscle, with miR-208 being the most specific of the myomiRs for cardiac muscle. This group of miRNAs is particularly important in cardiovascular disease. Because of their exclusivity to muscle cells, they should be found only in blood-based fluids (plasma and serum) if they are spilled from injured cells or perhaps be present if muscle cells use these miRNAs to signal to other cells (28). Of this group, miR-1 (miR-1–1 and miR-1–2 have identical sequences from different loci) has mainly been described in cardiac and skeletal muscle cells (8). However, it is also found in low levels in many organs (17, 29), suggesting that some noncardiac- and skeletal muscle

cell expresses miR-1 in vivo. MyomiRs are an important group of miRNAs in cardiovascular biomarker studies, as discussed below.

Biomarkers

Biomarkers may be biochemical, molecular, histologic, radiographic, or physiologic. They may be obtained from whole blood, serum, plasma, body fluids, or tissues. For the purposes of this review, we discuss current biochemical and molecular biomarkers and newer, potential miRNA biomarkers. Biomarkers have multiple uses. They can be indicators of a pathologic or physiologic process; they can be used to monitor the progress of or provide the prognosis for a disease; they can be used to monitor the efficacy or toxicity of a therapeutic agent; or they may represent a therapeutic target (30). They may also be used to stratify patients for the purposes of a clinical trial. Thus, their usefulness has been well established.

However, they should be used only once they have been well validated for their intended purpose. They may also undergo considerable improvement in terms of sensitivity or specificity, or both. An excellent example of this is the refinement of the use of blood measurements of cardiac-specific troponin as an indicator of myocardial damage (31).

CURRENT BIOMARKERS OF CARDIOVASCULAR DISEASE

Myocardial Infarction

Of all the cardiovascular diseases, the diagnosis of an AMI, usually as the result of an acute coronary artery event, is of the most societal importance. Fortunately, robust science has produced biomarkers that rapidly detect an AMI. The measurement of cardiac troponins [either Troponin I (cTnI) or Troponin T (cTnT)] has emerged as our gold standard diagnostic test. Cardiac troponins are cardiomyocyte-specific proteins that are spilled into the circulation when a cardiomyocyte dies (32). They can be measured rapidly in clinical chemistry laboratories and as point-of-care testing (33). American Heart Association guidelines recommend taking two measurements, one at first assessment and a second 3–6 hours later (34). A test is considered positive if at least one value is >99th percentile of the upper reference limit. With the advent of a new high-sensitivity test (hs-cTnT), this biomarker is firmly established as a key diagnostic tool in confirming a myocardial event. However, troponins can be elevated in non-AMI settings, including myocarditis and end-stage renal disease, although they are generally at lower levels than following an AMI and have different kinetics over time due to their sustained release (also known as troponin leak) or accumulation. Any cardiac injury can cause a release of troponins, with the extent of the injury correlating roughly with the level of the troponin. Thus, it is a sensitive but not specific test for an AMI, and opportunities may exist to find biomarkers with more discrimination.

Hypertension

Hypertension, or high blood pressure, is a polygenic and multifactorial disease, and its course can be altered by many environmental factors. Hypertension is injurious to the body’s organs and is associated with increased incidences of stroke, myocardial infarction, and aortic aneurysm, among other problems. The diagnosis is easily made with the use of a

sphygmomanometer to measure blood pressure, and the criteria for the diagnosis and treatment are well established (35, 36). Biomarkers beyond those obtained by the sphygmomanometer are clearly not needed for diagnosing hypertension; however, they may be of great value in helping to (*a*) stratify a patient for a therapeutic approach, (*b*) serve as prognostic indicators of hypertension-related sequelae or comorbidities, and (*c*) indicate a specific etiology of hypertension. It is in these roles that miRNA biomarkers may hold value.

Rejection of Orthotopic Heart Transplant

The gold standard for diagnosing heart transplant rejection is a pathologist's review of endomyocardial biopsy material (37, 38). However, simpler blood-based methods have emerged. A gene expression profiling method called AlloMap (CareDx, Brisbane, CA) is used clinically to rule out cardiac rejection in patients (39). A second method, still in development, is a cell-free DNA assay that can detect increased levels of DNA from the donor heart in a patient's blood, indicating heart injury (40). A third method utilizes brain natriuretic peptide (BNP) levels as indicators of rejection (41). The pros and cons of these methods as biomarkers are discussed in greater detail elsewhere, but no current blood-based test is useful at distinguishing between cellular and antibody-mediated rejection, among other limitations (42).

Myocarditis

Myocarditis can be difficult to diagnose, and having specific biomarkers could greatly improve our diagnostic abilities. Currently, endomyocardial biopsy remains the gold standard, as for heart transplantation (43). Since this is an invasive procedure and not widely offered, other methods are used as well. Even if myocarditis is present, the heterogeneous nature of the disease results in only ~50% of cases being diagnosed positively by biopsy (44). Other biomarker modalities that can be used include imaging studies and measurement of levels of troponin, BNP, and N-terminal pro-B-type natriuretic peptide (NT-proBNP). These tools have diagnostic overlap with myocardial infarction and other causes of heart failure, so there is not yet a robust biomarker specific to myocarditis.

Diabetic Cardiomyopathy

Diabetic cardiomyopathy is a well-recognized clinical entity that affects some patients with type 1 or type 2 diabetes mellitus. It is slowly progressive, and there are a number of risk factors, including a patient's age, the duration of diabetes, and poor glycemic control. It associates with elevated serum creatinine and microalbuminuria, two nonspecific biomarkers of disease (45, 46). However, the discovery of specific biomarkers that could predict its presence before it becomes clinically manifest could lead to early and potentially specific treatments beyond simply controlling blood sugar and making diet and lifestyle modifications. Currently, no such biomarker exists.

Aortic Stenosis

Aortic stenosis is the most common valvular disorder in developed countries. Once a diagnosis of stenosis is made by auscultation of a murmur and follow-up echocardiogram, a patient is usually managed medically until an aortic valve replacement is needed. One of the

major complications of aortic stenosis is left ventricular hypertrophy, with fibrosis leading to heart failure and death. The only blood-based biomarkers are BNP and NT-proBNP, which are nonspecific and usually indicative of decompensation, which occurs late in the management of the patient (47). Imaging modalities to identify myocardial fibrosis are being developed, but have not yet proven their value for aortic stenosis.

Heart Failure with Preserved Ejection Fraction

Heart failure with preserved ejection fraction (HFpEF) is, as the name implies, a cause of heart failure in which the ejection fraction is maintained, in contrast to the more common heart failure with reduced ejection fraction (HFrEF). HFpEF was previously called diastolic dysfunction or diastolic heart failure, and it is a catchall term for a variety of patients with diverse clinical phenotypes. It has remained a challenge to treat and discriminate across the different subsets of HFpEF. A number of typical cardiovascular and renal biomarkers are elevated in HFpEF (BNP, ST2, galectin 3, matrix metalloproteinases, osteopontin, cystatin C, troponins, and inflammatory cytokines) (48). None of these have much specificity for HFpEF, and diagnosing this entity remains a challenge. Although HFpEF and HFrEF can be distinguished by the determination of the ejection fraction, the differences between these entities remain an open scientific question.

Atrial Fibrillation

Atrial fibrillation can be readily diagnosed via electrocardiography (EKG) and can be designated as either paroxysmal or persistent. While patients can often be treated either electrically or pharmacologically, with reversion to a normal sinus rhythm, a percentage will revert to atrial fibrillation. In addition, atrial fibrillation is often the sequelae of underlying heart disease. Thus, while making the diagnosis of atrial fibrillation by EKG is established, a biomarker (or biomarkers) that could predict who is most prone to having atrial fibrillation for the first time or a recurrence could have a significant impact on the therapeutic strategies used for maintaining a normal sinus rhythm.

Sepsis

Although sepsis has long been characterized as a syndrome of disordered immune response to infection, a recent paradigm shift has altered the focus of sepsis research onto its cardiovascular dysfunction and resultant organ edema, ischemia, and failure. Further, despite numerous attempts to define its clinical features (49, 50), sepsis often presents nonspecifically and is frequently underrecognized early in its course. Thus, reliable diagnostic biomarkers could significantly improve sepsis outcomes, as poor recognition and delay in treatment is strongly associated with increased mortality (51). Procalcitonin, a precursor of the hormone calcitonin, has been demonstrated to have moderate discriminatory capability in the diagnosis of sepsis, and it has been incorporated sporadically into clinical practice (52). In order to gain acceptance by clinicians, miRNA-based diagnostic biomarkers would likely need to demonstrate superior test characteristics compared with procalcitonin.

A HISTORY OF miRNA BIOMARKER STUDIES

In 2008, Muneesh Tewari's group (53) indicated that miRNAs were stable in blood fluids and could be useful as biomarkers of disease. This seminal paper initiated a new enterprise to identify miRNAs that could serve as biomarkers for all neoplastic and nonneoplastic diseases. Thousands of publications on a litany of diseases using a variety of miRNA biomarker strategies have since been published. While this canon is too extensive to describe fully, certain generalities apply to these data sets.

miRNA biomarkers are found in a variety of biological fluids. Most miRNA biomarker studies are performed using serum or plasma. Fewer studies have used urine, saliva, whole blood, or peripheral blood mononuclear cells (PBMCs). Within serum or plasma, miRNAs can be found in protein complexes, bound to Ago2, or located in exosomes derived from most human cell types in various and changing ratios.

miRNAs can be assayed by different methods. Most miRNA biomarker studies have relied on miRNA profiling using hybridization arrays or parallel quantitative polymerase chain reaction (qPCR) systems containing hundreds of miRNAs or directed qPCR approaches on a more limited set of miRNAs (54). Other methods include droplet digital PCR and small RNA-seq. Most of these methods rely on a normalization control, which may be an intrinsic RNA, an extrinsic (spike-in) RNA, or a global normalization method.

Robust miRNA biomarker studies tend to have two stages. Generally, in the first stage, an agnostic, array-based approach is used on a moderate-size population (20–50 samples) to identify several potential individual targets or a small collection of miRNAs that in combination predict a certain disease or outcome. Then a second, larger population is assayed for only this subset of miRNAs, usually by qPCR. Another common approach is to use literature searches and prior similar biomarker studies to prioritize a more focused list of miRNAs, which are then assayed directly in one large study.

Practical Considerations for Using miRNAs as Biomarkers

The overwhelming excitement about miRNA biomarkers (and the huge number of patent filings) quickly gave way to some underappreciated complexities of assaying for miRNAs. Perhaps the initial publication warning of potential confounders was again written by Tewari's group (55). They demonstrated that many of the miRNAs identified as being associated with various neoplasms were, in fact, hematologic-specific miRNAs that correlated with blood cell levels. For example, miR-150 is found exclusively in lymphocytes and correlates with a patient's absolute lymphocyte count, which can vary between healthy and diseased patients. Thus, many of these miRNA biomarkers merely indicated what a complete blood count would have shown. This lack of knowledge of miRNA expression patterns has hampered a significant number of miRNA biomarker studies, in which miRNAs unrelated to the disease process are described.

We followed up Tewari and colleagues' work by investigating nonneoplastic miRNAs and focusing on whether a miRNA that was claimed as a biomarker for a particular disease was biologically plausible as a specific marker for that disease (25). Unfortunately, the vast

majority of miRNAs failed to show usefulness based on these criteria. Six miRNAs claimed as specific biomarkers were biomarkers for nine or more diseases, which makes them hard to claim as specific. We did note that myomiRs were frequently upregulated in a variety of cardiac diseases that injure muscle, such as myocardial infarction and myocarditis. So what causes the difficulties described in these publications?

Technical Causes of Variability in miRNA Biomarker Studies

The technical causes of variation in miRNA levels are well known (Table 3). The major cause of variation is likely sample preparation. As seen in one example from plasma, the extent of centrifugation can change the level of some miRNAs by >1,000-fold, but it may change others by <4-fold (56).

Another important source of variation is the normalization approach used. In tissue, the small nuclear U6 RNA (known as RNU6B or U6) can be used as a housekeeping gene to normalize miRNA levels. It has also been used for biomarker studies of plasma and serum. Unfortunately, RNU6B is not native to plasma or serum, being released only after the death of cells; thus, it should not be used as a normalizing control for this type of sample (25, 54), and studies based on U6 normalization should be questioned.

Different miRNA platforms using a variety of methods (hybridization, PCR) have their own biases that can affect miRNA levels. This issue was explored nicely by the miRQC (microRNA quality control) consortium (57). Finally, the statistical methods used for normalization—global methods or specific controls—can strongly influence biomarker discovery (58). One common flaw seen across many biomarker studies is the lack of a basal threshold for expression. Thus, groups find strong signals in the noise part of the data. Many of these purported miRNA biomarkers (often miRNAs with four-number names, such as miR-3168 or miR-1915) have little to no expression (although absolute expression is often not commented on) and are the result of spurious signals. These findings are rarely, if ever, replicated between studies (7).

Biological Causes of Variability in miRNA Biomarker Studies

In addition to technical causes of variation, biological factors also are worthy of consideration (Table 3). A major decision point in a biomarker study is the fluid source used for the miRNAs. Plasma and serum have both been used, although historically this decision was likely based on what samples were already present in a freezer, rather than planned in advance. miRNAs are known to vary between these fluids (54). For plasma, the choice of different preparations, such as platelet-poor plasma or platelet-rich plasma, can contribute to miRNA differences. miRNAs have also been reported to vary by sex (59, 60), patient’s age (61, 62), and time of day of collection (63). The last of these suggests there are fluctuations in the half-life of circulating miRNAs, which is a potential problem when trying to determine a temporal relationship with the disease of interest. Single nucleotide polymorphisms (SNPs) also exist in a small number of mature miRNAs. Even if the SNPs do not change the levels of the miRNAs, they can impact hybridization to miRNA-specific probes and qPCR primer binding or affect miRNA counting in alignment tools (64). A

number of publications have nicely addressed certain pitfalls and approaches to consider when developing and critically reviewing a miRNA biomarker study (7, 25, 65–67).

STUDIES OF miRNA BIOMARKERS IN CARDIOVASCULAR DISEASE

miRNA Biomarkers in Acute Myocardial Infarction and Acute Coronary Syndrome

Of all the cardiovascular miRNA biomarker opportunities, none has received as much attention as AMI and acute coronary syndrome (ACS). This is likely due to the high societal demand, clear phenotype, and potential financial reward. However, compared with many other cardiovascular diseases, the barrier for entry of a new, clinically useful biomarker is significantly higher due to the strength of troponin biomarkers in the current marketplace. Thus, to prove their usefulness, miRNA biomarkers for AMI and ACS must have better test statistics or other attributes that will make them more powerful than the troponins.

More than 100 manuscripts have evaluated miRNAs as biomarkers for this category, and it is beyond the scope of this review to cover them all. One of the first AMI biomarker studies was the work of Ai et al. (68) in 2010 that explored miR-1 and miR-133, finding that miR-1, but not miR-133, was elevated in participants with AMI. Many additional manuscripts explored these and other myomiRs, essentially reporting finding consistent elevations in cases of AMI and ACS (Table 2) (69). This is logical, as the death of cardiomyocytes will allow cellular components (i.e., miRNAs, genes, proteins) to spill into the blood. In the absence of myocardial or skeletal injury, the levels of these myomiRs in blood should be low to undetectable. Thus, myomiRs are essentially equivalent to troponins.

How do myomiRs compare with troponins? Here the data are more variable. Wang et al. (70) investigated the miRNAs miR-1, miR-133a, miR-499, and miR-208a in a population of 66 participants and showed that each miRNA was elevated in the setting of an AMI. Most importantly, they compared receiver operating characteristic (ROC) curves with cTnI and found that, at best, miR-208a was equivalent to the troponin. However, miR-208a is a expressed at relatively low levels and can be difficult to detect in blood-based fluids. Li et al. (71) studied plasma levels of these same four myomiRs in 99 participants. They also reported elevations in each myomiR, but all were inferior indicators compared with cTnT. Oerlemans et al. (72) performed a similar study in an emergency room setting by investigating 332 patients with suspected ACS. A combination of three miRNAs—miR-1, miR-499, and miR-21—had better test statistics (area under the curve; AUC) than hs-cTnT alone (AUC, 0.96 versus 0.86). In one of the strongest evaluations of myomiRs as biomarkers for AMI, the Wagner group (73) compared miR-208 and miR-499 with hs-cTnT in 510 participants. This paper was important in showing that miR-499 was superior to miR-208 and had some favorable test characteristics compared with hs-cTnT. Specifically, 93% of patients presenting within the first 3 h of having an AMI were positive for elevated miR-499, while only 88% were positive by hs-cTnT. It was suggested that a single miR-499 level might be superior to serial testing of hs-cTnT. Yet overall, miR-499 and hs-cTnT were essentially equivalent. Therefore, while several robust studies have been performed to specifically address whether myomiRs are superior to troponins, it seems that, at best, they behave equivalently and, at worst, are slightly inferior to troponins as biomarkers of AMI and ACS.

A number of non-myomiRs have also been evaluated in AMI and ACS. These include miR-941 (74), miR-323–3p (75), miR-652 (75), miR-221 (76), miR-328 (77), and miR-134 (77). Due to the large number of AMI studies that performed global surveys of miRNAs and failed to detect these miRNAs, it is challenging to think that these individual studies may be replicated and that measuring these miRNAs may be superior to measuring troponins. However, a secondary value of these miRNAs may be able to predict other facets of AMI and ACS and could be worthy of exploration.

One important study of the ability to predict a myocardial infarction is worth discussing. The Mayr group (78) robustly investigated 19 candidate miRNAs in 820 participants to assess their ability to predict incident myocardial infarction. They found elevated miR-126 was positively associated with risk, while miR-223 and miR-197 were inversely associated. miR-126 is modestly expressed in platelets, highly expressed in endothelial cells, and has well-known functions in both, thus a potential rationale for this finding exists (79). However, miR-126 has been claimed as a specific biomarker for a variety of diseases by the same group, including diabetes (25, 80). Rather than being a biomarker for a specific disease, it may be an indicator of abnormal platelet or endothelial cell function.

miRNA Biomarkers in the Rejection of Heart Transplants

As described above, three blood-based biomarkers of cardiac rejection exist (i.e., the gene expression panel, cell-free DNA, and NT-proBNP). Of these, the gene-expression panel is used clinically, although its test statistics indicate there is room for improvement, particularly in discriminating between cellular and antibody-mediated rejection. An evaluation of the differential expression of miRNAs in endomyocardial biopsy tissue from rejecting and nonrejecting hearts identified nine modulated miRNAs that were all inflammatory cell–enriched miRNAs, essentially demonstrating that more inflammatory cells were present in the rejecting tissue (as expected) (81). Three studies of miRNA biomarkers of acute cellular rejection from two groups yielded 13 circulating miRNAs, of which only miR-142–3p was shared by both groups (82–84). The larger study, by Duong Van Huyen et al. (82), evaluated 60 participants in a test cohort and 53 in a validation cohort. Despite the rigor of these three studies, one group identified miR-144 and the other group identified miR-451 as biomarkers of transplant rejection. These two miRNAs, in a bicistronic cluster, are expressed only in red blood cells and likely reflect hematocrit levels. A single study by Neumann et al. (85) investigated 40 patients with variable levels of transplant vasculopathy using miRNA profiling. They found miR-628 had the best test statistics (using the AUC) and could serve as a biomarker for the progression of coronary artery vasculopathy. While opportunities exist, more work is needed to settle on suitable miRNAs that can serve as biomarkers of rejection and transplant vasculopathy.

miRNA Biomarkers in Myocarditis

As seen above, myocarditis can be challenging to diagnose and confirm with our current set of biomarkers. Therefore, there is an opportunity to identify helpful miRNA biomarkers. Although there have been many studies showing miRNA dysregulation in myocarditis in human samples and animal models, the number of studies using blood-based biomarkers of myocarditis have been fewer. Corsten et al. (69), in a study of several heart diseases,

investigated 14 acute myocarditis plasma samples for several myomiRs, miR-223, miR-146a, miR-146b, and miR-155. They found modest but significant increases in miR-208b and miR-499, but no change in inflammation-related miRNAs. Although cTnT values were known, no direct head-to-head comparisons of superiority were reported. More recently, Wang et al. (86) studied 119 children with myocarditis and 120 age-matched controls, comparing miR-1, miR-146b, and other cardiovascular biomarkers. Based on ROC curves, these miRNAs were inferior to cTnI, interleukin (IL)-18, and tumor necrosis factor (TNF)-α.

miRNA Biomarkers in Hypertension

As stated above, miRNA biomarkers are not needed to diagnose hypertension, but they may be useful in stratifying patients for various therapeutic approaches, prognosticating comorbidities, or identifying an underlying etiology.

One recommendation for treating patients with hypertension is to encourage lifestyle modifications, specifically having them restrict salt intake. However, this is not always effective, and some patients have been classified as inverse salt sensitive. Thus, there is an opportunity to identify a biomarker that could predict this class of hypertensive patient. In a small study (10 participants), Gildea et al. (87) measured miRNAs in urinary exosomes and characterized them based on a patient's salt sensitivity status. miR-4516, intronic to the *PKD1* gene locus, was the only miRNA that was higher in salt-sensitive patients and lower in inverse salt-sensitive patients compared with controls. Qi et al. (88) used whole blood and qPCR to screen potential miRNAs as biomarkers for salt sensitivity in 91 participants. Their strongest signal was for miR-361–5p, which was associated with the risk of salt sensitivity. These studies suggest an opportunity for using urine or blood to categorize a patient's sensitivity to salt and provide the appropriate dietary advice.

Another opportunity for biomarkers is in cases of white-coat hypertension, in which a patient has elevated blood pressure readings in a medical center, but is otherwise normotensive. Several studies have screened for potential blood miRNA biomarkers to look for associations with white-coat hypertension. Huang et al. (89) found that three miRNAs—miR-30a, miR-29, and miR-133 (normalizedtocel-miR-54)—have potential as screening tools for white-coat hypertension. Cengiz et al. (90) also measured 10 plasma miRNAs (normalized to U6) in 90 participants (30 with white-coat hypertension, 30 hypertensive, and 30 normotensive). Of these miRNAs, miR-21, miR-122, miR-637, and let-7e expression levels were significantly upregulated in the hypertensive group compared with the normotensive group. miR-296–5p levels were significantly downregulated in the hypertensive patients and upregulated in the patients with white-coat hypertension compared with the normotensive patients. miR-122 is exclusively expressed in hepatocytes, suggesting that its elevation is related to low-level damage or microvesicle release from the liver.

Changes in vascular smooth muscle and arterial vessel wall thickness are common sequelae of hypertension, and noninvasive serial monitoring of these changes could alter how aggressively a hypertensive patient is treated. Cengiz et al. (91) measured plasma miR-21 in a group of 28 hypertensive patients and 28 matched controls. They found miR-21 to be

significantly increased both in the hypertensive patients and individuals with greater carotid intimal thickening.

In another interesting study, Karolina et al. (92) attempted to identify circulating miRNA profiles of metabolic syndrome by comparing different groups. They performed miRNA profiling of 46 healthy controls and 50 patients with metabolic syndrome, 30 with hypertension, 89 with hypercholesterolemia, and 50 with type 2 diabetes. miRNA profiles were determined from whole blood and exosomes, with comparable results for both. A cluster of three miRNAs—miR-130a, miR-195, and miR-92a—distinguished hypertensive patients from metabolic syndrome patients.

Kontaraki et al. (93) studied PBMCs in hypertensive men ($n = 60$) and healthy normotensive controls ($n = 29$). They measured the levels of five miRNAs (normalized to U6) that were purported to have effects on vascular smooth muscle phenotypic expression. Compared with the healthy controls, in hypertensive patients, the miRNAs miR-143, miR-145, and miR-133 were all significantly decreased, while miR-21 and miR-1 were significantly higher. Interestingly, the levels of miR-143, miR-145, and miR-21 were negatively correlated with 24-h ambulatory diastolic blood pressures, while miR-133 was positively correlated. Although these findings will not supersede the use of the sphygmomanometer in diagnosing hypertension, they may suggest pathways modulated in hypertension.

Some patterns of miRNA expression are beginning to emerge from these studies, raising the possibility that miRNA biomarkers will be useful for diagnostic, prognostic, or etiologic characterization of the hypertensive patient. For a more comprehensive review of miRNAs, their SNPs, and blood pressure regulation, the reader is referred to Marques & Charchar (94).

miRNA Biomarkers in Diabetic Cardiomyopathy

Diabetic cardiomyopathy is an important complication in a subset of diabetic individuals. There may be an opportunity to identify early miRNA biomarkers that can predict disease. De Gonzalo-Calvo et al. (95) studied myomiRs in serum from 72 participants with type 2 diabetes and myocardial steatosis, a feature of diabetic cardiomyopathy. miRNAs were normalized to a cel-miR-39 control. De Gonzalo-Calvo et al. (95) found a modest but significant association between miR-1 and miR-133a and myocardial steatosis, and they further showed that these miRNAs enhanced their ROC predictive model that was based on clinical factors. Then they investigated mice fed a high-fat diet, replicating the increased serum miR-1 and miR-133 levels. As described for other cardiovascular diseases, the serum increase of these two myomiRs likely reflects myocardial injury and the leakage of these myomiRs into the circulation. No other studies of blood-based biomarkers exist for diabetic cardiomyopathy.

A study of right atrial appendage and left ventricle tissues from 28 patients with type 2 diabetes and 38 nondiabetic patients explored myomiRs and miR-126 relative to U6 (96). In heart tissue from diabetic patients, researchers found lower levels of miR-1, miR-133, miR-126, and miR-499, and higher levels of miR-208 compared with tissue from the nondiabetic patients. That discrepancy between most myomiRs and miR-208 is difficult to

reconcile, but it could suggest miR-208 dysregulation in cardiac remodeling. The general overall reduction in the myomiRs in diabetic tissues could be the result of a changing cell ratio in which more fibroblasts and thus fewer myocytes are present.

Many additional miRNAs have been implicated functionally in diabetic cardiomyopathy based on animal and cellular studies. They are beyond the scope of this review, but the reader is referred to additional manuscripts for further insights (97–102).

miRNA Biomarkers in Aortic Stenosis

Multiple studies have investigated aspects of miRNAs serving as biomarkers of aortic stenosis, primarily related to myocardial complications. Garcia et al. (103) investigated plasma miR-133a in 74 participants with aortic stenosis. Interestingly, Garcia et al. (103) demonstrated that elevated preoperative miR-133a predicted improved normalization of left ventricular mass after surgery. Also of importance, they sampled blood from the coronary sinus (venous return from the heart) and right atrium (systemic venous return) and noted significantly higher values of miR-133a from the sinus (140.4 versus 72.3 relative expression units normalized to cel-miR-39), establishing the heart as the source of miR-133a in blood.

Chen et al. (104) investigated miR-1, miR-133a, and miR-378a in plasma from 152 participants with and without aortic stenosis. When normalized to U6, they noted that these miRNAs were lower in patients with aortic stenosis and that a low level of miR-378a alone could distinguish between patients with and without left ventricular hypertrophy. Beaumont et al. (105) investigated serum levels of seven miRNAs from 57 participants with or without aortic stenosis, showing there were reduced levels of miR-133a and miR-19b (normalized to cel-miR-39) in patients with aortic stenosis. The sum of these studies is a developing understanding of the relationship of miR-133a with aortic stenosis outcome.

Other studies have investigated other miRNA biomarkers for aortic stenosis. Fabiani et al. (106) investigated plasma miR-21 as a potential biomarker of myocardial fibrosis in severe aortic stenosis. They correlated elevated miR-21 with biopsy-based collagen levels in 23 patients. This confirmed the findings of Villar et al. (107), who showed that elevated miR-21 (normalized to cel-miR-39) correlated with the severity of aortic stenosis in both plasma and tissue biopsy samples from >100 participants. Coffey et al. (47) attempted a broad miRNA profiling study in 51 individuals with or without aortic stenosis. Although they found four miRNAs that varied by disease state (including miR-451a), these markers failed to be validated, and the authors concluded that no real biomarkers were discovered in the study. Finally, the Omland group (108) found that elevated miR-210 serum levels correlated with aortic stenosis in a study of 67 participants.

miRNA Biomarkers in Heart Failure with Preserved Ejection Fraction

In back-to-back manuscripts, Wong et al. (109) and Watson et al. (110) explored miRNAs in plasma from participants with HFpEF versus those with HFrEF. In Wong et al. (109), 39 HFrEF, 19 HFpEF, and 28 control participants were compared in a miRNA array platform. Numerous miRNAs varied across these three groups, with seven miRNAs being discriminatory for HFpEF. The second study investigated plasma from 270 participants with

HFpEF, HFrEF, or no heart failure (110). After initial miRNA profiling and subsequent validation in the wider cohort, five miRNAs (miR-375, miR-146a, miR-30c, miR-328, and miR-221) were found to vary in heart failure. While none of these miRNAs outperformed BNP in predicting heart failure, the AUC test statistic was optimized when they were added to the log BNP value.

miRNA Biomarkers in Atrial Fibrillation

A variety of studies of miRNA biomarkers for atrial fibrillation have described a number of potential biomarkers. One of the more interesting miRNAs in this group is miR-328. Lu et al. (111) used a canine model of atrial fibrillation to identify four miRNAs (miR-223, miR-328, miR-664, and miR-517) that were elevated in atrial myocardium, with miR-328 having the greatest increase. Elevation of miR-328 was also identified at the time of open-heart surgery in the right atrium of patients with atrial fibrillation ($n = 12$) compared with those without atrial fibrillation ($n = 10$). In additional studies in a transgenic mouse model, they further demonstrated that overexpression of miR-328 increased vulnerability to atrial fibrillation, potentially through the regulation of the target genes *CACNA1C* and *CACNB1*, both of which encode for L-type calcium channels (111).

More support for a role of miR-328 in atrial fibrillation comes from the Framingham Heart Study (112). miRNA profiling was performed on the whole blood of 2,467 participants. Four miRNAs—miR-328, miR-150, miR-331, and miR-28—were negatively nominally associated with prevalent atrial fibrillation. However, only lower miR-328 was significant after adjustments for age, sex, and technical factors. Thus, while miR-328 elevations appear to occur in myocardium involved in atrial fibrillation, lower miR-328 is seen in blood.

Liu et al. (113) used small RNA-seq to obtain a plasma miRNA expression profile in patients with paroxysmal atrial fibrillation ($n = 5$), persistent atrial fibrillation ($n = 5$), and healthy controls ($n = 5$). They found differences in miRNAs between these groups. Of note, miR-328 was not variable. They followed up four of these miRNAs in a separate population of patients with paroxysmal atrial fibrillation ($n = 30$), persistent atrial fibrillation ($n = 30$), and controls ($n = 30$). Only elevated miR-150 (in contrast to the Framingham Heart Study's reduction) was replicated in this larger cohort.

Natsume et al. (114) performed miRNA profiling of the serum of 10 patients with atrial fibrillation and 5 healthy controls to identify predictors of atrial fibrillation. Four miRNAs—miR-99a–5p, miR-192–5p, miR-214–3p, and miR-342–5p—were elevated in atrial fibrillation patients relative to controls, and the latter two had the highest accuracy based on ROC curves.

Dawson et al. (115) measured miRNAs in plasma and the right atrial appendages of patients with atrial fibrillation with ($n = 16$) or without congestive heart failure ($n = 17$) and controls ($n = 30$). Plasma levels of miR-29b and miR-21 were significantly decreased in patients with atrial fibrillation. Interestingly, in both human and canine right atrial appendages, levels of miR-29b were also significantly decreased by atrial fibrillation. The authors further went on to demonstrate a potential role for miR-29b in cardiac fibrosis.

McManus et al. (116) studied plasma miRNA biomarkers of prevalent atrial fibrillation in 112 patients with and 99 patients without atrial fibrillation. Atrial tissue was also obtained from patients undergoing cardiac surgery ($n = 31$). The plasma miRNAs miR-21 and miR-150 were both twofold lower in patients with atrial fibrillation compared with patients without. Both miRNAs increased threefold 1 month after ablation. Atrial levels of miR-21 but not miR-150 were lower in patients with atrial fibrillation compared with patients who did not have atrial fibrillation.

miRNA Biomarkers in Sepsis

A number of investigators have examined the role of circulating miRNAs for use as diagnostic biomarkers in sepsis (117–119). While most miRNAs have not been validated in multiple studies, a few miRNAs have been repeatedly identified across studies as being associated with sepsis, including miR-150, miR-223, and miR-146a (120–127). Several of these studies are confounded by methodologic flaws, however, and at present none of these miRNAs are used as biomarkers in clinical practice.

miR-150 has been investigated as both a diagnostic and a prognostic biomarker. Ma et al. (122) used small RNA-seq to compare miRNA expression in whole blood between cohorts of septic patients and nonseptic but critically ill patients. They observed that miR-150 expression was higher in sepsis, although the expression levels were not normalized (122). Several investigators have demonstrated that plasma miR-150 levels are correlated with disease severity and prognosis. Vasilescu and colleagues (120) examined the expression of miR-150 in the white blood cells of septic patients and found that miR-150 levels were inversely correlated with disease severity and levels of IL-10, IL-18, and TNFα. Similarly, other investigators have shown that serum and plasma levels of miR-150 are lower in patients with higher Sequential Organ Failure Assessment scores and in nonsurvivors of sepsis (121, 128). However, because miR-150 has been identified as a lymphocyte-specific miRNA, these findings may simply be a function of the circulating lymphocyte count and may not add value above a complete blood count with differential.

Similarly, investigators have examined the diagnostic and prognostic abilities of miR-223, a miRNA that is specific to neutrophils and macrophages. Initial studies were promising, as plasma miR-223 levels were found to be significantly lower in septic patients compared with nonseptic patients undergoing cardiac surgery (123), and miR-223 plasma expression was significantly lower among nonsurvivors of sepsis independent of illness severity and other relevant clinical variables (124). The prognostic potential of miR-223 in sepsis was further supported by a separate study demonstrating that plasma miR-223 levels are reduced in more severe sepsis compared with mild disease (125). A more recent analysis in a larger cohort demonstrated mixed results for the utility of miR-223 as a sepsis biomarker. Benz et al. (129) measured serum miR-223 levels in 221 critically ill patients (septic = 137, nonseptic = 84). There was no difference in miR-223 expression between the two groups, suggesting that it may have limited value as a diagnostic biomarker. However, reduced miR-223 levels were correlated with mortality in the intensive care unit, suggesting that miR-223 may have prognostic capability (129). It is unclear whether miR-223 expression

outperforms or has additive value to more traditional and widely used systems for scoring the severity of illness.

Although the association between miR-146a and sepsis has been examined several times, the results are inconsistent and contradictory. An early study examining miR-146a expression in sepsis was performed by Wang et al. (123), who found that (similar to miR-223) plasma miR-146a levels were reduced in patients with sepsis compared with levels in nonseptic acutely ill patients. Because miR-146a is known to regulate Toll-like receptor 4 signaling, this observation may have a plausible biological rationale (130). Subsequent studies, however, have inconsistently corroborated these findings, with one group finding no correlation between plasma miR-146a levels and sepsis among patients presenting to the emergency room (128), while another group observed increased expression of plasma miR-146a in pediatric sepsis (126). Thus, the clinical utility of miR-146a as a diagnostic biomarker of sepsis is uncertain.

RECOMMENDATIONS FOR BEST PRACTICES

As is evident from this collection of manuscripts, the technical approaches used to measure miRNA levels have varied widely (Table 4). It is clear that to generate robust data about miRNA biomarkers, the next generation of studies needs to adhere to stricter guidelines and follow recommendations so that results can be replicated (7, 65, 131–133). Some technically driven best practices, based on these and other reviews of miRNA biomarker studies, are to ensure the inclusion of large, well-characterized populations; steady normalization signals (e.g., spike-in RNAs); and consistent preparation of appropriate blood-based fluids; to avoid using as biomarkers miRNAs that have only low expression; and to use robust statistical analyses of the signal. Biologically based best practices include ensuring that individuals are well-matched between groups, using consistent fluid collection practicesand investigating biologically relevant targets.

CONCLUSIONS AND FUTURE CONSIDERATIONS

The outlook for using miRNA biomarkers for cardiovascular disease is complex. In the areas in which miRNA biomarker studies have made the most progress (e.g., AMI and ACS), other robust biomarkers exist. Where there is more opportunity and need for cardiovascular diagnostics, miRNA studies are inconsistent and less robust. Those who are working in this field would be well advised to learn from first-generation miRNA studies, focus on opportunities where there is a lower barrier of entry for miRNA-based diagnostics, and then perform robust and reproducible studies using biologically applicable samples. Only then will miRNAs succeed as a class of biomarkers for cardiovascular disease.

ACKNOWLEDGMENTS

M.K.H. was supported by the US National Institutes of Health (grant no. 1R01HL137811) and the American Heart Association (Grant-in-Aid no. 17GRNT33670405). P.V.H. was supported by the US National Institutes of Health (grant nos. UL1TR001450 and 1R01GM113995). A.J.G. was supported by the US National Institutes of Health (grant nos. 1K23HL135263-01A and 1R01GM113995).

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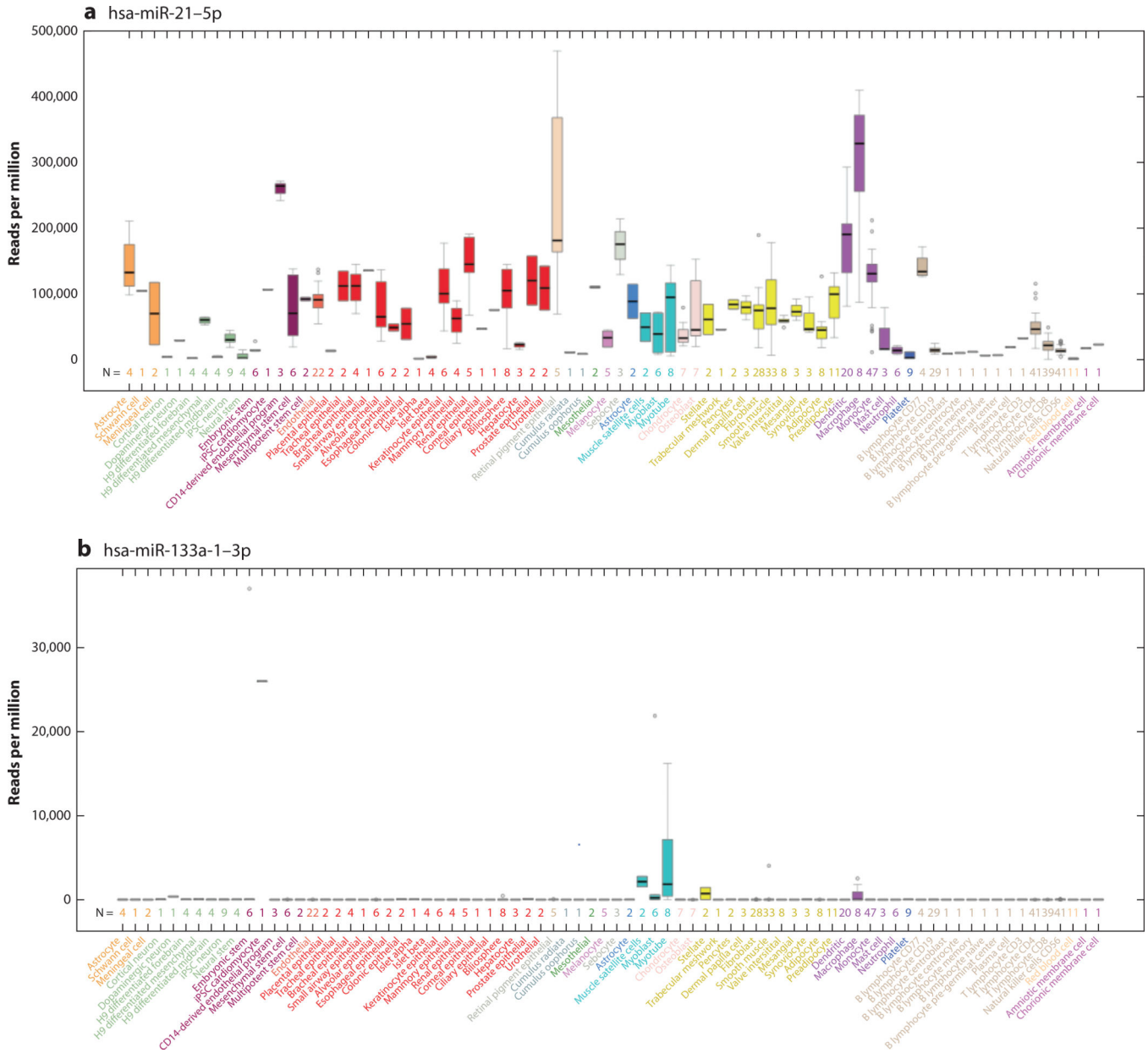

Figure 1.

University of California Santa Cruz Genome Browser barChart of the cellular expression patterns of microRNAs (miRNAs). (*a*) hsa-miR-21–5p expression is ubiquitous across most cell types, although the absolute expression level is variable. (*b*) hsa-miR-133a-1–3p shows cell type expression restricted to induced pluripotent stem cell cardiomyocytes, muscle satellite cells, myoblasts, myotubes, stellate cells, and macrophages at varying levels of expression.

Table 1

Cellular localization of microRNAs described in biomarker studies in the review

microRNA[a]	Cell-type enrichment
Let-7e	Ubiquitous
miR-19b	Ubiquitous
miR-21	Ubiquitous
miR-28	Ubiquitous
miR-29	Ubiquitous
miR-30a	Nonhematologic cells
miR-30c	Ubiquitous
miR-99a	Ubiquitous (nonhematopoietic)
miR-122	Hepatocytes
miR-126	Endothelial cells, platelets
miR-134	Platelets, iPSC neurons, islet β-cells
miR-143	Smooth muscle cells, fibroblasts, cardiac muscle cells, skeletal muscle cells
miR-144	Red blood cells
miR-145	Smooth muscle cells, fibroblasts, cardiac muscle cells, skeletal muscle cells
miR-146a	Melanocytes, macrophages, lymphocytes, platelets
miR-146b	Dendritic cells, lymphocytes, macrophages
miR-150	Lymphocytes
miR-155	Lymphocytes
miR-182	Epithelial cells
miR-192	Epithelial cells
miR-197	Neutrophils, macrophages, monocytes
miR-200a	Epithelial cells
miR-200b	Epithelial cells
miR-200c	Epithelial cells
miR-210	Ubiquitous
miR-214	Smooth muscle cells, fibroblasts, RPE cells, iPSC neurons
miR-215	Epithelial cells
miR-221	Ubiquitous
miR-223	Macrophages, neutrophils
miR-296	No strong cell signals
miR-323	iPSC neurons
miR-328	Platelets
miR-331	Ubiquitous
miR-342	Macrophages, lymphocytes
miR-361	Lymphocytes
miR-375	Islet β-cells (high expression), colonic epithelial cells (low expression)

microRNA[a]	Cell-type enrichment
miR-378a	Ubiquitous
miR-451	Red blood cells
miR-517	Embryonic stem cells, chorionic membrane cells
miR-637	No strong cell signals
miR-652	Platelets, macrophages, RPE cells
miR-664	Lymphocytes (low expression)
miR-941	Neutrophils, macrophages
miR-4516	No strong cell signals

Abbreviations: iPSC, induced pluripotent stem cell; RPE, retinal pigment epithelium.

Ubiquitous indicates widespread expression, but not necessarily in all cell types.

Table 2

Myocyte microRNAs (myomiRs) with increased or exclusive expression in skeletal and cardiac myocytes

microRNA	Gene locus	Chromosome location	Cell type expression
miR-1–1	NA	20	Cardiac, skeletal (and others at low levels?)
miR-1–2	Intronic to *MIB1*	18	Cardiac, skeletal (and others at low levels?)
miR-133a	Intronic to *MIB1*	18	Cardiac, skeletal
miR-133b	Intronic to *LINCMD1*	6	Skeletal
miR-206	Downstream of *LINCMD1*	6	Skeletal
miR-208a	Intronic to *MYH6*	14	Cardiac
miR-208b	Intronic to *MYH7*	14	Skeletal
miR-499a	Intronic to *MYH7B*	20	Cardiac, skeletal
miR-499b	Intronic to *MYH7B*	20	Cardiac, skeletal

NA, not applicable.

Table 3

Technical and biological factors affecting fluid-based studies of microRNA biomarkers

Technical factors	Biological factors
Extent of centrifugation	Fluid source (serum, plasma, PBMCs, exosome)
Spike-in control	Sex
Platform	Time of day
RNA isolation method	Hemolysis
Storage method	Genetic variation
Analysis method	Patient phenotype
Fluid collection method	Age
Alignment method (for small RNA sequencing)	
RNA storage (stability)	
Lower level of expression limitation	

Abbreviation: PBMCs, peripheral blood mononuclear cells.

Table 4

Fluid-based studies of microRNA biomarkers of cardiovascular diseases

microRNAs investigated[a,b]	Source	Method	Study size	Type of study	Reference
Acute myocardial infarction or acute coronary syndrome					
miR−1; −133	Plasma	qPCR	93 AMI; 66 controls	D	68
miR−1; −133a; −208b; −499	Plasma	qPCR	67 AMI; 32 controls	D	71
miR−1; −208a; −499; −21; −146a	Serum	qPCR	332 patients with chest pain	D	72
miR−208b; −499	Plasma	qPCR	510 AMI; 87 controls	D	73
Multiple microRNAs;[b] miR−941	PBMC	ELOSA QC assay; qPCR	72 patients with chest pain	D	74
Multiple microRNAs; miR−323; −27b; −652; −103	Plasma	Exiqon qPCR panel; qPCR	240 ACS; 120 controls	D	75
Multiple microRNAs	Plasma	Qiagen miRNome PCR array	27 patients with chest pain; 16 controls	D	76
miR−328; −134	Plasma	qPCR	359 AMI; 30 controls	D	77
Multiple microRNAs; miR−24; −126; −140; −150; −197; −223; −486	Plasma	TaqMan Array A and B Cards; qPCR	820 Bruneck study participants	P	78
Heart transplantation					
miR−326; −142; −101; −144; −27a; −424; −339	Serum	qPCR	10 patients at 3 time points	D	84
miR−142; −101; −424; −27a; −144; −339; −326	Serum	qPCR	63 patients	D	83
miR−10a; −21; −31; −92a; −142; −155; −451; −126; −221; −296; −208; −181a; −181b; −182	Plasma	qPCR	30 patients with rejection; 30 patients without rejection; 53 validation participants	D	82
Multiple microRNAs; miR−628; −155; −34a; −98; −204	Plasma	TaqMan Array A and B Cards; qPCR	40 patients; samples used twice	D	85
Myocarditis					
miR−1; −133a, −146a; −146b; −155; −208b; −23; −499	Plasma	qPCR	14 patients with acute myocarditis; 20 with remote myocarditis; 20 controls	D	69
miR−1; −146b	Serum	qPCR	119 pediatric patients with myocarditis; 120 controls	D	86
Hypertension					
Multiple microRNAs; miR −1268b; −5002; 4516; −3183; −3940; −4649; −320a	Urinary exosomes	Microarray; qPCR	10 hypertensives	D	87
Multiple microRNAs; miR−15b; −19a; −382; −26b; −361; −423; −210; −361	Whole blood	Small RNA sequencing; qPCR	56 salt-sensitive hypertensives; 56 salt-resistant hypertensives	D	88
miR−30a; −29; −133	Plasma	qPCR	35 white-coat hypertensives; 35 hypertensives; 35 normotensives	D	89

microRNAs investigated[a,b]	Source	Method	Study size	Type of study	Reference
miR−21; −122; −125a; −126; −130a; −155; −195; −296; −637; let−7e	Plasma	qPCR	30 white-coat hypertensives; 30 hypertensives; 30 normotensives	D	90
miR−21	Plasma	qPCR	28 hypertensives; 28 normotensives	D	91
Multiple microRNAs; miR−103; −17; −183; −197; −23a; −509; −584; −652; −130a; −195; −92a; −150; −192; −27a; −320a	Whole blood and exosomes	miRCURY LNA microRNA Array; qPCR	50 patients with metabolic syndrome; 30 hypertensives; 50 with type 2 diabetes; 89 with hypercholesterolemia; 46 controls	D	92
miR−143; −145; −133, −21; −1	PBMCs	qPCR	60 hypertensives; 29 normotensives	D	93
Diabetic cardiomyopathy					
miR−1; −133a; −133b; −208a; −208b; −499	Serum and exosomes	qPCR	78 with type 2 diabetes from the PIRAMID study	D	95
Aortic stenosis					
miR−133a	Plasma	qPCR	74 patients	P	103
miR−1; −133a; −378a	Plasma	qPCR	112 patients; 40 controls	D	104
miR−19b; −133a; −21; −29; −1; −208a; −499	Serum	qPCR	28 patients; 29 controls	Y	105
miR−21	Whole blood	qPCR	23 patients undergoing surgery	P	106
miR−21	Plasma	qPCR	75 patients; 32 surgical controls; 25 controls	D	107
Multiple microRNAs; miR−22; −23; −382; −451a; −21	Plasma	Affymetrix GeneChip miRNA 2.0 Array; qPCR	24 patients; 27 controls	D	47
miR−210; −22; −425	Serum	qPCR	57 patients; 13 controls	D	108
Heart failure with preserved ejection fraction					
Multiple microRNAs; miR −1225; −1233; −125a; −1299; −130a; −1322; −145; −17; −1825; −183; −186; −190a; −193b–3p; −193–5p; −204; −211; −301a; −320d; −326; −361; −423; −431; −485; −494; −509; −545; −550a; −625; −629; −638; −671; −92b	Whole blood and plasma	miRCURY LNA microRNA Array; qPCR	118 patients with heart failure; 58 controls from the SHOP and SLAS studies	D	109
Multiple microRNAs; miR−30c; −146a; −221; −328; −375	Serum	TaqMan Array A and B Cards; qPCR	90 patients with HFpEF; 90 with HFrEF; 90 controls from the STOP-HF Study	D	110
Atrial fibrillation					
Multiple microRNAs	Whole blood	Array	153 with prevalent AF; 107 with incident AF; 2,185 without AF (controls)	P	112

microRNAs investigated[a,b]	Source	Method	Study size	Type of study	Reference
Multiple microRNAs; miR−19a; −146a; −150; −375	Plasma	Small RNA sequencing; qPCR	35 with persistent AF; 35 with paroxysmal AF; 35 controls	D	113
Multiple microRNAs; miR−27b; −30a; −328; −99a, −192; −214, −342; −125b; −130b; −362; −424	Serum	TaqMan Array A and B Cards; qPCR	30 with paroxysmal AF; 30 with chronic AF; 55 controls	D	114
miR−29b; −21; −133; −15	Plasma	qPCR	33 with persistent AF; 32 with congestive heart failure; 30 controls	D	115
86 microRNAs	Plasma	qPCR	112 with AF; 99 controls from the miRhythm Study	D	116
Sepsis					
miR−150; −182; −342–5p, −486	PBMCs	Agilent hybridized microarray	24 with sepsis; 32 healthy controls	D	120
miR−146a; −223; −126; −132; −155; let−7i	Serum	qPCR	50 with sepsis; 30 controls with SIRS; 20 healthy controls	D	123
miR−574–5p; −297	Serum	Affymetrix microarray	78 sepsis survivors; 64 sepsis nonsurvivors	P	134
Multiple microRNAs	Serum	Solexa sequencing with qPCR validation	117 sepsis survivors; 97 sepsis nonsurvivors	P	124
miR−15a, −16	Serum	qPCR	166 with sepsis; 32 with SIRS; 24 healthy controls	D	135
miR−223; −15b; −483; −499; −122; −193b	Plasma	qPCR	166 with sepsis; 24 healthy controls	D, P	125
miR−181b	Plasma	qPCR	36 sepsis; 17 controls in intensive care unit	D	136
miR−146a	Plasma	qPCR	14 with sepsis; 14 with SIRS	D	127
Multiple microRNAs	Plasma	qPCR	22 with sepsis; 22 with SIRS; 17 healthy controls	D	122
miR−122; −193b; −483; −574	Serum	Affymetrix microarray	232 with sepsis; 24 healthy controls	P	137
miR−21; −125b; −132; −146a; −155; −223	Plasma	qPCR	40 pediatric patients with sepsis; 20 pediatric patients with SIRS; 15 healthy pediatric controls	D	126
miR−223	Serum	qPCR	137 with sepsis; 84 without sepsis; 75 healthy controls	D, P	129
miR−150; −146a; −223	Plasma	qPCR	69 with sepsis; 24 healthy controls	D, P	128
let−7a; miR−150; −1249; −199b	PBMCs	Geniom Biochip microarray	22 with sepsis; 20 healthy controls	D	138
miR−15a; −27a; −34a	Plasma	qPCR	62 with sepsis; 32 healthy controls	P	139

microRNAs investigated[a,b]	Source	Method	Study size	Type of study	Reference
miR−30d; −30a; −192; −26a; −23a; −191–5p	Plasma	qPCR	29 with sepsis; 44 with SIRS; 16 controls	D	140

Abbreviations: ACS, acute coronary syndrome; AF, atrial fibrillation; AMI, acute myocardial infarction; D, diagnostic biomarker study; ELOSA, enzyme-linked oligosorbent assay; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; P, prognostic biomarker study; PBMCs, peripheral blood mononuclear cells; QC, quality control; qPCR, quantitative polymerase chain reaction; SIRS, systemic inflammatory response syndrome; Y, pathophysiologic study.

[a]The microRNAs listed reflect those assayed, which were not necessarily found to have significant associations with disease.

[b]Multiple microRNAs implies the use of a multiplexed array system or small RNA sequencing.