Review Article

Circulating MicroRNAs as Potential Novel Biomarkers in Cardiovascular Diseases: Emerging Role, Biogenesis, Current Knowledge, Therapeutics and the Road Ahead

Kumar Rahul, Satish Kumar[1], Bhupendra Kumar, Vandana Chaudhary[2]

Departments of Cardiovascular and Thoracic Surgery, [1]Medicine and [2]Physiology, King George's Medical University, Lucknow, Uttar Pradesh, India

ORCID:

Kumar Rahul: 0000-0002-8929-2489
Satish Kumar: 0000-0001-5206-9825
Bhupendra Kumar: 0000-0002-9365-6479
Vandana Chaudhary: 0000-0002-8425-1506

Abstract

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality worldwide. Although considerable progress has been made in the diagnosis, treatment, and prognosis of CVD, there still remains a critical need for novel diagnostic biomarkers and new therapeutic interventions to reduce the incidence of diseases. Recently, there has been increasing evidence that circulating MicroRNAs (miRNAs), which are endogenous, stable, single-stranded, short, noncoding RNAs (ncRNAs), can be used as diagnostic biomarkers. miRNAs are small ncRNA having approximately 21–25 nucleotides in length, and they mainly act as transcriptional regulators of gene expression in diverse biological processes such as cellular proliferation, differentiation, and tumorigenesis and death. miRNAs regulate in several cardiovascular pathologies, not only limited to hypertrophy, heart failure, arrhythmias, hypertension, myocardial infarction, dyslipidemias, and congenital heart diseases but are also present and circulate in various body fluids such as blood, pericardial fluid, and saliva and act as potential novel biomarkers in various cardiovascular pathologies. Furthermore, miRNAs represent potential novel therapeutic targets for several of above-mentioned CVD. This review presents detailed information about miRNAs regarding its structure and biogenesis, functions, expression profiles in serum/plasma of living organisms, diagnostic and prognostic potential as a novel biomarker and therapeutic applications in various CVD.

Keywords: Cardiovascular disease, microRNA, noncoding RNAs

Introduction

Cardiovascular diseases (CVD) are one of the most common causes of mortality in both developing and developed nations worldwide.[1] Even though there have been improvements in primary prevention, the prevalence of CVD continues to increase in recent years. It is also important to know about the pathophysiology of CVD at molecular level that may help in finding a novel biomarker which may aid in early diagnosis and treatment. While nearly 80% of genes in the human body undergo transcription, only 1% to 2% of them get translated into proteins, which leaves many noncoding RNA (ncRNA) transcripts.[2,3] ncRNAs are composed of small nuclear and nucleolar RNAs, Y-RNAs, microRNAs (miRNAs), and long ncRNAs. ncRNAs are very important in regulating gene expression and also in epigenetic applications. Furthermore, they may be one of the most important etiologic factors for the development of cardiovascular disease. To date, miRNAs are the most studied and characterized ncRNAs in the literature.[4]

Received: 17-05-2022 **Revised:** 11-07-2022 **Accepted:** 19-07-2022
Published Online: 02-09-2022

Access this article online

Quick Response Code:

Website: http://www.ijcva.com

DOI: 10.4103/ijca.ijca_8_22

Address for correspondence: Dr. Kumar Rahul, Department of Cardiovascular and Thoracic Surgery, King George's Medical University, Lucknow, Uttar Pradesh, India.
E-mail: drrahul64@yahoo.co.in

How to cite this article: Rahul K, Kumar S, Kumar B, Chaudhary V. Circulating microRNAs as potential novel biomarkers in cardiovascular diseases: Emerging role, biogenesis, current knowledge, therapeutics and the road ahead. Int J Cardiovasc Acad 2022;8:31-40.

miRNAs are endogenous, conserved, single-stranded ncRNA s of 21-25 nucleotides in length.[5] Lin-4 was the first miRNA discovered in Caenorhabditis elegans in 1993. The second miRNA or the first miRNA to be discovered in humans was let-7 discovered in 2000 with its target protein lin-41.[6] Unlike the first miRNA, let-7 is highly conserved in many species including humans where it is regulated at transcriptional and posttranscriptional levels. Let-7 recognizes 3'UTR in the lin-41 gene and binds to it and hence downregulates lin-41 translation. Lin-41 is associated with different protein ubiquitin ligase in an autoubiquitination assay.[7] In addition, mouse lin-41 is found to be more pronounced during the early development of mouse limb bud, nervous tissue, and craniofacial tissue.[8] The let-7 family of miRNAs have also been found to modulate the immune response against opportunistic human pathogen like *Pseudomonas aeruginosa*.[9] Since the discovery of lin-4 and let-7 attention of researchers have been drawn toward this new promising RNA biology and its revolution. Moreover, the primary miRNA database was released in 2002 with only 218 entries, and it continued to increase over the years. The latest miRNA base Sequence Database includes 28,645 entries standing for hairpin precursor miRNAs which includes 35,828 mature miRNAs in 223 species. To date, over 2500 miRNAs have been discovered in the human body.[10,12] Furthermore, many novel computational programs have been found to predict target mRNAs of each specific miRNA, such as Target scan (http://www. targetscan. org), miRanda (http://www. microRNA. org), and Tarbase (http://www. microrna. gr/tarbase). A diverse role of dysregulated miRNAs in various pathologies including CVD in the human body were reported in several literature.[11,12] This review presents detailed information about miRNAs regarding its structure and biogenesis, function, expression profiles in serum/plasma of living organisms, diagnostic and prognostic potential as a novel biomarker, and therapeutic applications in various diseases.

Biosynthesis of MicroRNAs s

miRNA biogenesis starts with the processing of RNA polymerase II/III transcripts post or co-transcriptionally.[13] About half of all presently identified miRNAs are intragenic and are processed mainly from introns and relatively few exons of protein-coding genes, while the remaining are intergenically transcribed independent of the host gene and are regulated by their own promoters.[14,15] miRNAs at several instances are also transcribed as a single long-length transcript chain known as clusters, that may have similar seed regions and in such cases, they are considered to be a family.[16] The biogenesis of miRNA is classified into canonical and noncanonical pathways [Figure 1].

The Canonical Pathway of MicroRNAs Biosynthesis

The canonical biosynthesis pathway is the dominant pathway by which miRNAs are processed. In this pathway, primary miRNAs are transcribed from their genes and then processed into primi RNAs by the microprocessor complex, consisting of an RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and a ribonuclease III enzyme Drosha.[17] DGCR8 recognizes an N6-methyladenylated GGAC and other motifs within the primi RNA, while Drosha cleaves the primi miRNA duplex at the base of the characteristic hairpin structure of primi miRNA. This results in the formation of a 2 nt 3′ overhang on precursor-miRNA (pre-miRNA).[18,19] Once pre-miRNAs are generated, they are exported to the cytoplasm by an exportin 5 (XPO5)/RAs-related nuclear protein (Ran) guanosine triphosphate (GTP) complex and then processed by the RNase III endonuclease Dicer.[20] This processing involves the removal of the terminal loop, resulting in a mature miRNA duplex.[21] The directionality of the miRNA strand determines the name of the mature miRNA form. The 5p strand arises from the 5′ end of the pre-miRNA hairpin while the 3p strand originates from the 3′ end. Both strands derived from the mature miRNA duplex can be loaded into the Argonaut (AGO) family of proteins (AGO1-4 in humans) in an ATP-dependent manner.[22] For any given miRNA, the proportion of AGO-loaded 5p or 3p strand varies greatly depending on the cell type or cellular environment, ranging from near equal proportions to predominantly one or the other.[23] The selection of the 5p or 3p strand is based in part on the thermodynamic stability at the 5′ ends of the miRNA duplex or a 5′ U at nucleotide position 1. In general, the strand with lower 5′ stability or 5′ uracil is preferentially loaded into AGO and is deemed to be the guide strand. The unloaded strand is called the passenger strand which will be unwound from the guide strand through various mechanisms based on the degree of complementarity. The passenger strands of miRNA that contain no mismatches are cleaved by AGO2 and degraded by cellular machinery which can produce a strong strand bias. Further, miRNA duplexes with central mismatches or non-AGO2-loaded miRNA are passively unwound and are degraded.[24]

Noncanonical MicroRNAs Biosynthesis Pathways

To date, multiple noncanonical miRNA biogenesis pathways have been elucidated [Figure 1]. These pathways make use of different combinations of the proteins involved in the canonical pathway mainly Drosha, Dicer, XPO5, and AGO2. In general, the noncanonical miRNA biogenesis can be grouped into Drosha/DGCR8-independent and Dicer-independent pathways. Pre-miRNAs produced by the Drosha/DGCR8-independent pathway resemble Dicer substrates. An example of such pre-miRNAs is mirtrons, which are produced from the introns of mRNA during splicing.[25,26] Another example is the 7-methylguanosine (m7G)-capped pre-miRNA. These nascent RNAs are directly exported to the cytoplasm through exportin 1 without the need for Drosha cleavage. There is a strong 3p strand bias most likely due to the m7G cap preventing 5p strand loading into AGO.[27] On the other hand, Dicer-independent miRNAs are processed by Drosha from endogenous small hairpin RNA (shRNA) transcripts. These pre-miRNAs require

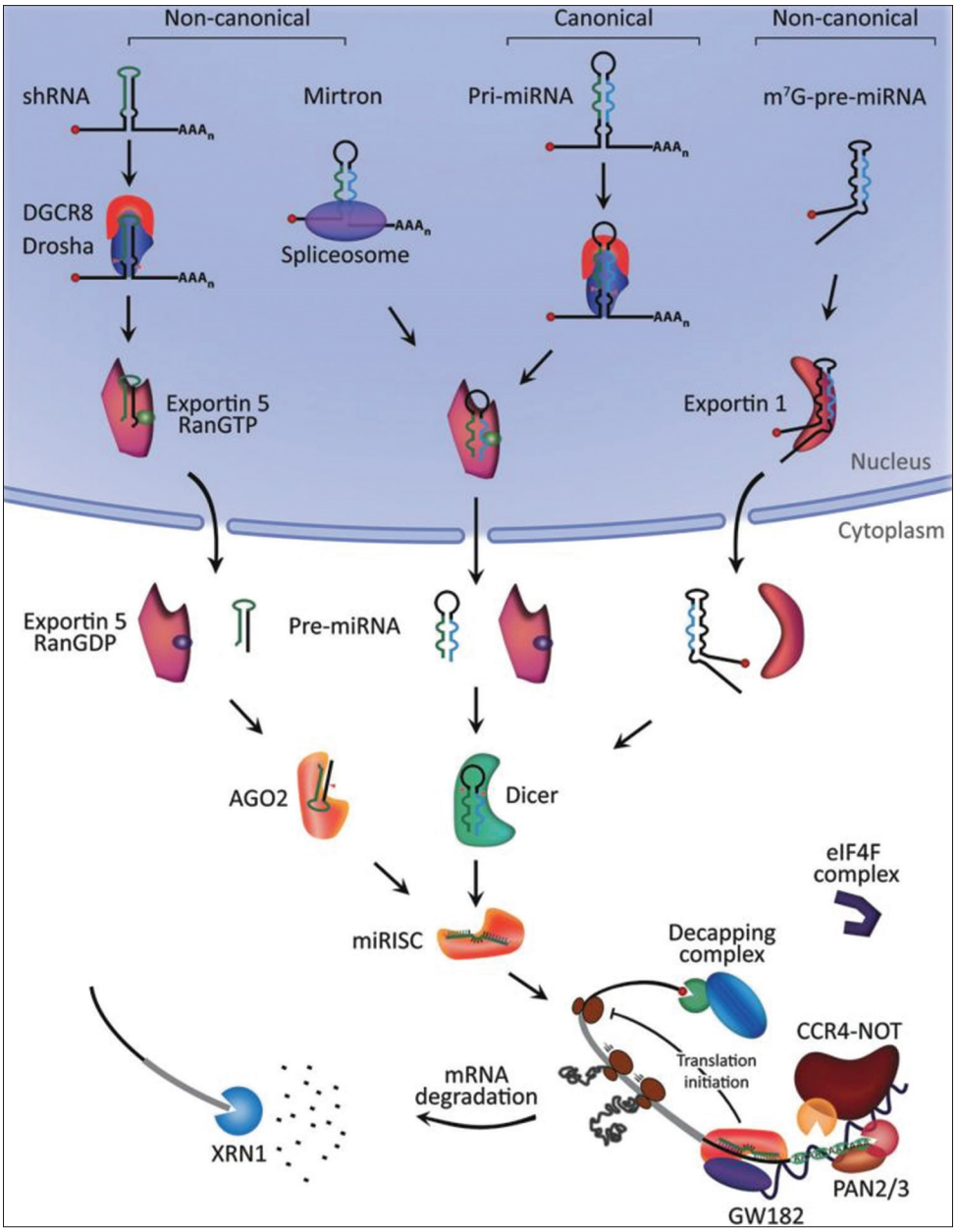

Figure 1: MicroRNA biogenesis and mechanism of action

AGO2 to complete their maturation within the cytoplasm because they are of insufficient length to be Dicer-substrates.[28] This, in turn, promotes the loading of the entire pre-miRNA into AGO2 and AGO2-dependent slicing of the 3p strand. The 3′-5′ trimming of the 5p strand completes their maturation.[29]

Figure 1 shows MicroRNA biogenesis and mechanism of action. Canonical miRNA biogenesis begins with the generation of the primi-miRNA transcript. The microprocessor complex, comprised of Drosha and DGCR8, cleaves the primi-miRNA to produce the pre-miRNA. The pre-miRNA is exported to the cytoplasm in an Exportin5/RanGTP-dependent manner and processed to produce the mature miRNA duplex. Finally, either the 5p or 3p strands of the mature miRNA duplex is loaded into the AGO family of proteins to form a miRNA-induced silencing complex (miRISC). In the noncanonical pathways, small hairpin RNA (shRNA) is initially cleaved by the microprocessor complex and exported to the cytoplasm via Exportin5/RanGTP. They are further processed via AGO2-dependent, but Dicer-independent, cleavage. Mirtrons and 7-methylguanine capped (m7G) pre-miRNA are dependent on Dicer to complete their cytoplasmic maturation, but they differ in their nucleocytoplasmic shuttling. Mirtrons are exported via Exportin5/RanGTP while m7G-pre-miRNA are exported via Exportin1. All pathways ultimately lead to a functional miRISC complex. In most cases, miRISC binds to target mRNAs to induce translational inhibition, most likely by interfering with the eIF4F complex. Next GW182 family proteins bound to AGO e recruit the poly (A)-deadenylases

PAN2/3 and CCR4-NOT. PAN2/3 initiates deadenylation while the CCR4-NOT complex completes the process, leading to the removal of the m7G cap on target mRNA by the decapping complex. Decapped mRNA may then undergo 5′−3′ degradation via the exoribonuclease XRN1 Hayder *et al*.[30]

MicroRNA in Cardiac Development and Physiology

From the embryonic period to the fully developed heart, miRNAs are constantly found in cardiac tissues. miR-21, miR-29a, miR-129, miR-210, miR-211, miR-320, miR-423, and let-7c are highly expressed in normal fetal heart tissue which plays a major role in embryonic and postnatal cardiac development.[31] Decreased levels of above-mentioned miRNAs are due to depleted levels of Dicer, an enzyme whose role is essential for miRNA processing and its absence may lead to pericardial edema, poorly developed ventricular myocardium, and rapid necrosis which were studied in mouse models. Similarly, tamoxifen inducible recombinase deletion of dicer causes heart failure and death in the adult heart. In other words, inhibition of miRNA expression is due to dicer deletion. Deletion of dicer during the embryonic phase may result in hypoplastic ventricular muscle and pericardial edema.[32,33] Postnatal deletion of dicer in heart muscle cells may result in cardiomyocyte contractility dysfunction and disarrays which ultimately results in dilated cardiomyopathy and progresses to heart failure.[34] miRNA 1 and miRNA 133 plays a crucial role in cardiac development which has been proved experimentally in mice model. miRNA-1 (miR 1–1, miR 1–2) and miR-133 (miR 133a–1, miR 133a–2, and miR 133b) and miR-206 are muscle-specific miRNA which are regulated by different enzymes in different cells. The main functions of miRNA-1 on heart development are favoring apoptotic mechanisms and inhibition of cardiac development.[35,36] miRNA-1 targets Hand 2, a transcription factor which favors the proliferation of cardiomyocytes in zebrafish embryo hearts.[37] This suggests that during cardiac development rise in miRNA-1 level causes a decrease in Hand 2 transcription factor level and for a healthy heart this needs to be balanced in miRNA homeostasis. If there is an excessive increase in miRNA-1 in early embryogenesis, it may lead to dysregulation of cardiac myocyte proliferation, myoblast differentiation, and ventricular septal defect by interfering with cardiac morphogenesis.[38] Histone deacetylates 4 (HDAC4) is another transcriptional repressor protein of muscle gene expression and miR-1 targets this protein and promotes myogenesis. The role of miR-133 in cardiac development works as a regulator of cardiogenesis

MicroRNA in Cardiac Remodeling

The term "remodeling" is adaption in response to any chronic stress in the heart, for example chronic hypertension can lead to hypertrophy in cardiac myocytes which is required to maintain adequate cardiac output. However, if cardiac remodeling process remains for long duration it may lead to heart failure, myocardial infarction, and other cardiac diseases. miR-1 and miR-133 are found to be downregulated in a heart where cardiac remodeling processing is active, especially in pressure overload-induced hypertrophy. Calcium-dependent gene expressions are crucial for myocyte growth and differentiation. Calmodulin, Mef2a, and Gata4 are the main calcium-dependent transcription factors and miR-1 inhibits these transcription factors as shown by a study in mice model.[39] Other classes of miRNA such as miR-208, miR-195, miR-214, and miR-21 are overexpressed in hypertrophied heart.[39] During pressure-induced stress, cardiac-specific intron of alpha myosin heavy chain gene 6 encodes a cardio-specific miR-208 and then miR-208 further upregulates beta myosin heavy chain, thereby promoting cardiac hypertrophy.[40] A recent study by Bang *et al*.[41] showed that in response to stress, cardio-fibroblast releases miRNA enriched exosomes, miR-21 also known as "star" miRNA which is potent paracrine acting RNA molecule and their overexpression may result in cardiomyocyte hypertrophy. If miR-195 is overexpressed, it may lead to cardiac hypertrophy. Upregulation of microRNA-195 in failing hearts can result in dilated cardiomyopathy which was experimentally shown in transgenic mice model.[41,42]

MicroRNA in Cardiovascular Disease

Arrhythmia

miRNA levels are found to be altered from its normal hemostasis in both atrial and ventricular arrhythmia. Both upregulation and downregulation of miR-1 may cause arrhythmias, especially in ventricle and atria, respectively. In ventricular arrhythmia, miR-1 represses KCNJ2 and GJA1. KCNJ2 encodes Kir2.1 which is a potassium channel subunit and GJA1 encodes connexin 43 which is a cardiac gap junction. This leads to slowed conduction and depolarization of cytoplasmic membrane which has arrhythmogenic potential and elimination of miR-1 has shown to improvement in arrhythmic potential in infarcted rat hearts. This has opened a window for future anti-arrhythmic therapy-targeting miR-1.[43] Another mechanism by which overexpression of miR-1 causes arrhythmia is by CaM-KII-dependent phosphorylation of the L-type Ca2 + channels and ryanodine receptors 2, leading to a burst of calcium from the sarcoplasmic reticulum responsible for the arrhythmia.[44] miR-328 is associated with atrial fibrillation. Lu *et al*.[45] in their study, found that miR-328 was elevated by 3.5-fold in atrial fibrillation as compared to those without atrial fibrillation.

Hypertension

Several studies have highlighted the involvement of miRNAs in blood pressure regulation, particularly by affecting the renin-angiotensin-aldosterone system. Marques *et al*.[46] compared both miRNA and mRNA expression in renal tissue from untreated hypertensive and normotensive individuals using microarray technology. The authors screened 850 miRNAs and identified nine miRNAs which exhibited expression differences with two of the miRNAs (miR-181a and miR-663) linked to

repression of renin expression in the human kidney.[46] Boettger *et al*.[47] demonstrated that miR-143/145 knocked out mice exhibited significantly reduced blood pressure compared with wild-type littermates and identified angiotensin-converting enzyme mRNA as a target for miR-145. Furthermore, it was found that miR-145 was overexpressed in atherosclerotic plaques of hypertensive versus normotensive patients undergoing carotid endarterectomy implicating miR-145 as a potentially key regulator of blood pressure mediated vascular damage.[48]

Pulmonary hypertension

Pulmonary arterial hypertension is characterized by an increase in the resistive and reactive components of pulmonary vascular impedance which ultimately leads to right ventricular failure.[49,50] Over 30 circulating miRNAs have been associated with the development and progression of pulmonary arterial hypertension.[51] The levels of these miRNAs correlate with pulmonary arterial dispensability and pulmonary vascular resistance index and decrease response to oxygen and or inhaled nitric oxide.

Heart failure

No doubt that heart failure remains a significant burden in present-day world and for the treating physician with the leading cause of hospital admissions elderly population. According to Health Management Organization, Kaiser permanent coronary artery disease (CAD), hypertension, diabetes mellitus, atrial fibrillation, and valvular heart disease are the five major causes of heart failure globally.[52] Advancement in miRNA research has raised the possibility of its appearance in heart failure cases using circulating miRNAs as a novel biomarker tool [Figure 2]. Goren *et al*.[53] found that levels of specific miRNAs such as miR-423-5p, miR-320a, miR-22, and miR-92b are found to be elevated in a patient with systolic heart failure. Further, a study by Devaux *et al*.[54] revealed that panels of four miRNAs, namely miR-16, miR-27a, miR-101, and miR-150 are associated with improved prediction of left ventricular contractility 6 months after acute myocardial infarction (AMI). Among various miRNAs, expressions of miR-122, 423-5p,-210,-499,-622 are found to be increased in patients with heart failure. The significance of the increased level of miR-423-5p has been further supported by Tijsen *et al*.[55] where six other miRNAs were found to be elevated, but miR-423-5p was strongly correlated with the clinical diagnosis of heart failure. miR-1 plasma levels were found to be elevated in acute myocardial-induced heart failure.[56] A recent study of myocardial biopsy in 34 Chinese patients were performed who developed recent heart failure and were compared with a group of patients without heart failure undergoing routine cardiac surgery. It was found that miR-1,-21,-23,-29,-130,-195, and -199 levels were significantly raised in the heart failure group.[57]

Acute myocardial infraction

Several studies have investigated the role of miRNAs in identifying patients with AMI. Although a number of promising candidates have emerged including miR-1, miR-133a, miR-133b, miR-208a, miR-499, and miR-499-5p further validation is required for most of them [Figure 3]. In a study conducted by Devaux *et al*.[58] the authors measured the levels of six miRNAs in 1155 patients attending the cardiac emergency department with acute-onset chest pain. 224 patients were diagnosed with AMI and their miRNA assay was performed which showed significantly raised levels of miR-208b, miR-499, and miR-320a in AMI compared with other diagnostic and investigative tools. However, none were able to outperform cardiac troponin T (cTnT) or high-sensitivity cTnT (hsTnT) or add to their diagnostic ability.[58] This highlights a major obstacle in the identification of miRNAs as diagnostic biomarkers in AMI. Further, the diagnostic criteria for AMI place significant emphasis on cardiac troponins as it becomes extremely difficult if not impossible, for any new biomarkers to outperform them. Furthermore, such use of miRNAs as biomarkers in acute coronary syndromes is also

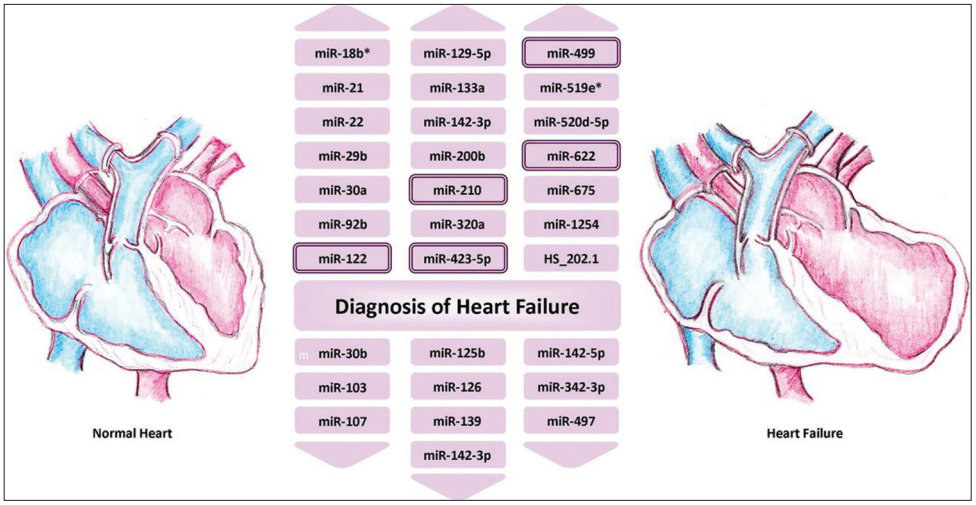

Figure 2: Circulating microRNAs associated in the diagnosis of heart failure. MicroRNAs with a double border have been linked to heart failure in more than one study

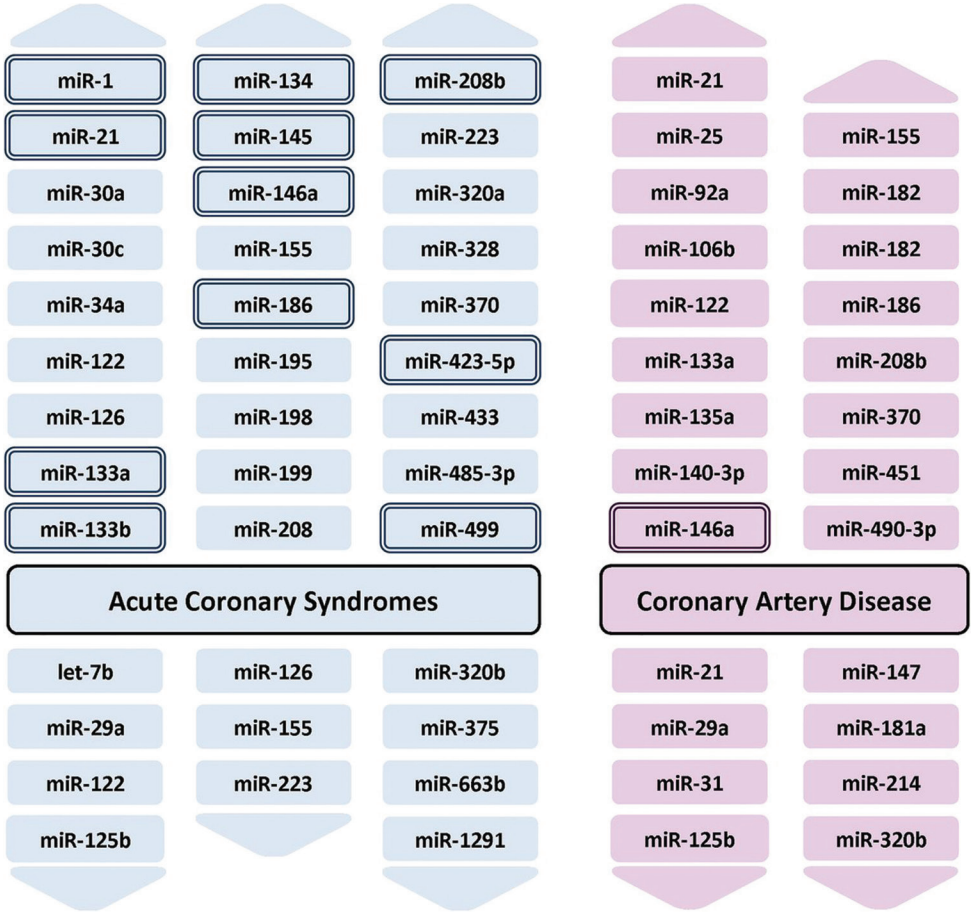

Figure 3: Circulating microRNAs associated with acute coronary syndromes and coronary artery disease. MicroRNAs with a double border have been linked to the associated trait by more than one study

limited by the time taken to investigate the level of candidate miRNAs in a single sample. Owing to this at present this cannot be achieved within a timeframe of clinical utility. In one study, which recruited 175 patients with AMI admitted within 12 h of symptom onset and 90 healthy controls as the control group, the results demonstrated that circulating miR-19a levels were significantly raised in patient groups compared to the control group. The authors concluded that circulating miR-19a possibly has a prognostic value which can be used as a promising molecular target for early diagnosis and prognosis of AMI provided the results are available in desired less time frame.[59]

However, the role of miRNAs as a diagnostic biomarker in less time-sensitive diagnoses and also their role as prognostic biomarkers may herald its diagnostic potential.

Coronary artery disease

A number of miRNAs have been linked to a diagnosis of CAD [Figure 3]. Zampetaki *et al*.[60] identified three miRNAs (miR-126, miR-197, and miR-223) that were predictive of incidental CAD in a prospective study of cardiovascular disease (with 10-year follow-up). Furthermore, the addition of these three miRNAs to the Framingham Risk Score improved classification to a greater degree than hsTnT. While further validation is required, these results suggest a potential role for miRNAs in predicting the risk of future AMI due to CAD.

Atherosclerosis

Atherosclerosis is a complex and chronic inflammatory interaction of the blood vessel wall with altered lipoprotein monocyte-derived macrophages, T-cells, and the normal arterial elements. Patients with CAD have reduced expression of different miRNAs such as miR-126, miR-145, and miR-155 in their blood.[61] The detailed role of miRNAs 126,145 and 155 *in vivo* studies have been studied by Wei *et al*.[62] They found that downregulation of miR145 in smooth muscle cells disrupts smooth muscle cell normal hemostasis and favors atheroma formation. In one study, it was shown that miR-33 which is located in the SREBF2 gene is responsible for reducing the expression of cholesterol transporter ABC transporter A1 which leads to a decrease the level of high-density lipoprotein (HDL) which might act as a trigger factor for atherosclerosis.[63]

miRNA in aortic valve disease

In aortic stenosis, miRNAs were also found to be altered in stenotic aortic valve leaflets compared to insufficient ones. The reduced levels of miR-26a and miR-30b were responsible for a higher risk of developing valvular leaflet fusion. The change

of morphology is due to accelerated calcium accumulation compared to regurgitant aortic valves without morphological fusion of valve leaflets. These miRNAs were involved in the biological processes that modulate calcification-related genes *in vitro*.[64] Several studies have recorded an important association of miRNAs with posttranscriptional regulation of gene expression in aortic valve stenosis. miR-141 was found to be a regulator of the levels of bone morphogenetic protein 2, whereby unrestrained activity led to calcification of the aortic valve mediated by a stimulation of osteogenesis. miR-141 was markedly attenuated in patients with aortic stenosis associated with the bicuspid aortic valve [Figure 4]. Yanagawa *et al*.[65] proposed this new key role of miR-141 in the modulation of aortic valve calcification disorders, highlighting the strategic therapeutic target that emerged in the assessment of progressive calcification in stenotic aortic valve disease. Song *et al*.[66] analyzed the progression of calcified aortic valve disease and suggested the crucial role of myofibroblastic and osteoblast-like phenotypes. The authors reported substantial differences in the levels of two miRNA classes: MiR-486 and miR-204. The levels of miR-486 were increased in calcific aortic valves where the myofibroblastic transition was induced by upregulation of the expression of Runx2 and Osx and miR-204 levels were deficient, which leads to high cellular and valvular pro-osteogenic activity.

MicroRNAs in mitral valve disease

Over the recent years, miRNAs have represented an emerging class of widely circulating biomarkers in various pathological states including degenerative mitral valve disease.[67] Concerning the prolapse of the mitral valve, only a small number of circulating miRNAs have been studied focusing on the degenerative disorders of the mitral valve. These findings were limited to the experimental animal models. Hulanicka *et al*.[68], in their study, evaluated plasma miRNA levels as potential biomarkers for chronic myxomatous mitral disease in dogs. Investigators evaluated the expression of 9 miRNAs that had already been discovered and were involved in CVD.[68] Using a real-time polymerase chain reaction (PCR) method, they found that the plasma levels of two out of nine miRNAs were significantly downregulated, recording an evident involvement of these molecules in developing endocardiosis and mitral valve lesion in dogs. Various miRNAs that have been studied including miR-125, miR-126, miR-21, miR-29b, and miR-30b showed a significant expression in the pathogenesis of mitral valve disease.

Congenital heart disease

miRNA plays an important role from embryogenesis to a fully developed adult heart. Several congenital cardiac defects have been found to be associated with altered expression of miRNA. Alteration of miR-1 and miR-181c levels is found to be associated with Ventricular septal defects (VSD). In humans presenting with VSD, their cardiac cells show decreased expression of miR-1 and increased expression of miR-181c.[69,70] Congenital heart disease in Down syndrome patients has been found to have overexpressed five major miRNAs, namely miR-99a, let-7c, miR-125b-2, miR-155, and miR-802 in chromosome 21.[71] Several cardio facial neural crest defects may result due to abnormalities in neural crest cell migration and dicer deletion, which is mainly responsible for the miRNA maturation. Noonan's Syndrome, DiGeorge Syndrome, LEOPARD Syndrome, and cardio-facio-cutaneous syndrome are some of the examples. Tetralogy of Fallot (TOF) is the most common congenital cyanotic heart disease. The association of TOF with miRNAs has been recently reported by O'Brien *et al*.[72] in 33 infants with nonsyndromic TOF without a 22q11.2 deletion. They found that 33 miRNAs were downregulated in patients who have nonsyndromic TOFs. Another study in 2015 by this group found that overexpression of miR-421 in the ventricular cell is associated with TOFs.[73] Liang *et al*.[74] demonstrated that miRNA-940 is most significantly downregulated in patients with TOF.

Circulating MicroRNAs as a diagnostic indicator of cardiovascular disease

The detection of miRNAs in body fluids is done by various methods, namely real-time PCR or microarray assay. Circulating miRNAs are stable nucleic acids whose sequences can be amplified and are easily detectable with appropriate methods in a wide range of bodily fluids from blood, urine, saliva, cerebrospinal fluid, and breast milk. Despite the advancement in medical science, early and effective diagnosis or treatment for myocardial infarction still remains a clinical challenge.[75] The role of cardiac-specific miRNAs for the detection of AMI have been described by various researchers. Cardiac-specific miRNAs 1, 133, 208b, 499 may have the

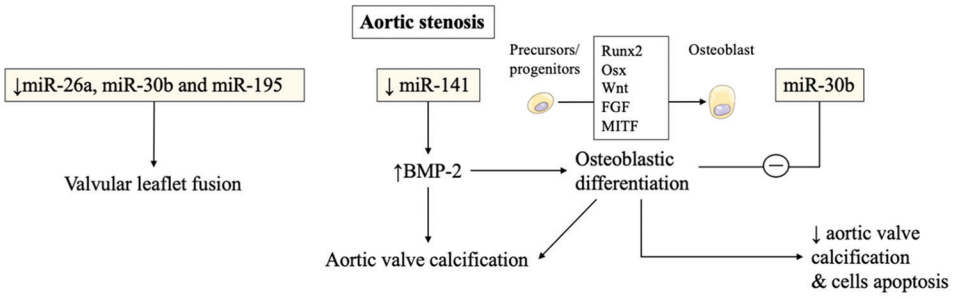

Figure 4: The role of miRNA in aortic stenosis severity and valve calcification. Reduced levels of miRNA-26a, miRNA-30b, and miRNA-195 contribute to further damage to the valve. The negative regulation exerted on osteoblastic differentiation by miRNA-30b favours a better prognosis

future for upcoming diagnostic markers for AMI.[76,77] miR-1, -21,-22,-23,-29,-122,-130,-195,-199,-320a, 423-5p,-499,-622 levels were found to be overexpressed in heart failure patients. Particularly increased levels of miR-423-5p were well correlated with heart failure in different studies. Similarly, levels of miR-122 and miR-370 were positively correlated with total cholesterol, triglycerides, and low-density lipoprotein cholesterol and CAD progression.[78]

Circulating MicroRNAs as the therapeutic role and future prospects

Since single miRNA can target multiple mRNA-making proteins on gene regulation, presently it is feasible to use miRNA as a therapeutic target. Elimination of miR-1 has shown to improvement in arrhythmic potential in infarcted rat hearts and this has opened a new window for future anti-arrhythmic therapy-targeting miR-1. *In vivo* delivery of lentivirus miR-24 can interact with tissue growth factor β 1 to decrease the cardiac fibrosis resulting from various heart conditions such as MI, cardiomyopathies, and heart failure.[79] miRNAs-based therapeutics have been more studied in atherosclerosis. The atheroprotective effect of miR-126 has been explained by Wei *et al*.[63] Similarly, anti-miR-33 therapy has been found to be atheroprotective by increasing circulating HDL in mice models.[80] Moreover, combining miRNAs with traditional biomarkers can be a logical approach to improve risk stratification and long-term prognosis in CVD. miRNA-based therapeutics may be more beneficial to treat CVD using novel platforms and computational tools in combination with traditional methods of analysis.

Conclusions

The discovery of miRNAs has opened a new door for insight into the biology of the cardiovascular system and our understanding of disease pathogenesis. The recent increase in interest of miRNAs in cardiovascular biology and diseases among researchers signifies its bright future in the coming days. Although much has happened within this short period, still it needs further research to completely understand cardiovascular disease pathogenesis. Contrary to its name "microRNA," it has a wider macro function in the heart, including cardiac embryogenesis, differentiation, and from hypertrophy to disease pathogenesis. miRNA might outweigh cardiac sensitivity troponins in the coming days for the early and accurate diagnosis of AMI with predictive future complications risk and their treatment. Therefore, it seems there is a possibility to treat cardiomyopathies in the near future. This will definitely be an important landmark and the availability of more durable and permanent treatment options for treating CVD.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zhou SS, Jin JP, Wang JQ, Zhang ZG, Freedman JH, Zheng Y, *et al.* miRNAS in cardiovascular diseases: Potential biomarkers, therapeutic targets and challenges. Acta Pharmacol Sin 2018;39:1073-84.
2. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, *et al.* The transcriptional landscape of the mammalian genome. Science 2005;309:1559-63.
3. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, *et al.* The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res 2012;22:1775-89.
4. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem 2012;81:145-66.
5. Lee RC, Feinbaum RL, Ambros V. The C. Elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843-54.
6. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001;294:853-8.
7. Rybak A, Fuchs H, Hadian K, Smirnova L, Wulczyn EA, Michel G, *et al.* The let-7 target gene mouse lin-41 is a stem cell specific E3 ubiquitin ligase for the miRNA pathway protein Ago2. Nat Cell Biol 2009;11:1411-20.
8. Schulman BR, Esquela-Kerscher A, Slack FJ. Reciprocal expression of lin-41 and the microRNAs let-7 and mir-125 during mouse embryogenesis. Dev Dyn 2005;234:1046-54.
9. Ren Z, Ambros VR. Caenorhabditis elegans microRNAs of the let-7 family act in innate immune response circuits and confer robust developmental timing against pathogen stress. Proc Natl Acad Sci U S A 2015;112:E2366-75.
10. Kozomara A, Griffiths-Jones S. miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 2014;42:68-73.
11. Condorelli G, Latronico MV, Cavarretta E. microRNAs in cardiovascular diseases: Current knowledge and the road ahead. J Am Coll Cardiol 2014;63:2177-87.
12. Karaca E, Aykut A, Ertürk B, Durmaz B, Güler A, Büke B, *et al.* MicroRNA expression profile in the prenatal amniotic fluid samples of pregnant women with down syndrome. Balkan Med J 2018;35:163-6.
13. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15:509-24.
14. de Rie D, Abugessaisa I, Alam T, Arner E, Arner P, Ashoor H, *et al.* An integrated expression atlas of miRNAs and their promoters in human and mouse. Nat Biotechnol 2017;35:872-8.
15. Kim YK, Kim VN. Processing of Intronic microRNAs. EMBO J 2007;26:775-83.
16. Tanzer A, Stadler PF. Molecular evolution of a microRNA cluster. J Mol Biol 2004;339:327-35.
17. Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the microprocessor complex. Nature 2004;432:231-5.
18. Alarcón CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF. N6-methyladenosine marks primary microRNAs for processing. Nature 2015;519:482-5.
19. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The drosha-DGCR8 complex in primary microRNA processing. Genes Dev 2004;18:3016-27.
20. Okada C, Yamashita E, Lee SJ, Shibata S, Katahira J, Nakagawa A, *et al.* A high-resolution structure of the pre-microRNA nuclear export machinery. Science 2009;326:1275-9.
21. Zhang H, Kolb FA, Jaskiewicz L, Westhof E, Filipowicz W. Single processing center models for human dicer and bacterial RNase III. Cell 2004;118:57-68.
22. Yoda M, Kawamata T, Paroo Z, Ye X, Iwasaki S, Liu Q, *et al.* ATP-dependent human RISC assembly pathways. Nat Struct Mol Biol 2010;17:17-23.
23. Meijer HA, Smith EM, Bushell M. Regulation of miRNA strand selection: Follow the leader? Biochem Soc Trans 2014;42:1135-40.
24. Khvorova A, Reynolds A, Jayasena SD. Functional siRNAs and miRNAs exhibit strand bias. Cell 2003;115:209-16.
25. Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass

Drosha processing. Nature 2007;448:83-6.
26. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other microprocessor-independent, Dicer-dependent small RNAs. Genes Dev 2008;22:2773-85.
27. Xie M, Li M, Vilborg A, Lee N, Shu MD, Yartseva V, *et al.* Mammalian 5'-capped microRNA precursors that generate a single microRNA. Cell 2013;155:1568-80.
28. Yang JS, Maurin T, Robine N, Rasmussen KD, Jeffrey KL, Chandwani R, *et al.* Conserved vertebrate mir-451 provides a platform for Dicer-independent, Ago2-mediated microRNA biogenesis. Proc Natl Acad Sci U S A 2010;107:15163-8.
29. Cheloufi S, Dos Santos CO, Chong MM, Hannon GJ. A dicer-independent miRNA biogenesis pathway that requires ago catalysis. Nature 2010;465:584-9.
30. Hayder H, O'Brien J, Nadeem U, Peng C. MicroRNAs: Crucial regulators of placental development. Reproduction 2018;155:R259-71.
31. Romaine SP, Tomaszewski M, Condorelli G, Samani NJ. MicroRNAs in cardiovascular disease: An introduction for clinicians. Heart 2015;101:921-8.
32. da Costa Martins PA, Bourajjaj M, Gladka M, Kortland M, van Oort RJ, Pinto YM, *et al.* Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. Circulation 2008;118:1567-76.
33. Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, *et al.* Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 2007;129:303-17.
34. Chen JF, Murchison EP, Tang R, Callis TE, Tatsuguchi M, Deng Z, *et al.* Targeted deletion of Dicer in the heart leads to dilated cardiomyopathy and heart failure. Proc Natl Acad Sci U S A 2008;105:2111-6.
35. Xu C, Lu Y, Pan Z, Chu W, Luo X, Lin H, *et al.* The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. J Cell Sci 2007;120:3045-52.
36. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature 2005;436:214-20.
37. Schindler YL, Garske KM, Wang J, Firulli BA, Firulli AB, Poss KD, *et al.* Hand2 elevates cardiomyocyte production during zebrafish heart development and regeneration. Development 2014;141:3112-22.
38. Mishima Y, Stahlhut C, Giraldez AJ. miR-1-2 gets to the heart of the matter. Cell 2007;129:247-9.
39. Ikeda S, He A, Kong SW, Lu J, Bejar R, Bodyak N, *et al*. MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes. Molecular and Cellular Biology 2009;29:2193-204.
40. van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. Science 2007;316:575-9.
41. Bang C, Batkai S, Dangwal S, Gupta SK, Foinquinos A, Holzmann A, *et al.* Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 2014;124:2136-46.
42. van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, *et al.* A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc Natl Acad Sci U S A 2006;103:18255-60.
43. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, *et al.* The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med 2007;13:486-91.
44. Terentyev D, Belevych AE, Terentyeva R, Martin MM, Malana GE, Kuhn DE, *et al.* miR-1 overexpression enhances Ca(2+) release and promotes cardiac arrhythmogenesis by targeting PP2A regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2. Circ Res 2009;104:514-21.
45. Lu Y, Zhang Y, Wang N, Pan Z, Gao X, Zhang F, *et al.* MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. Circulation 2010;122:2378-87.
46. Marques FZ, Campain AE, Tomaszewski M, Zukowska-Szczechowska E, Yang YH, Charchar FJ, *et al.* Gene expression profiling reveals renin mRNA overexpression in human hypertensive kidneys and a role for microRNAs. Hypertension 2011;58:1093-8.
47. Boettger T, Beetz N, Kostin S, Schneider J, Krüger M, Hein L, *et al.* Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Invest 2009;119:2634-47.
48. Wienholds E, Plasterk RH. MicroRNA function in animal development. FEBS Lett 2005;579:5911-22.
49. O'Rourke MF. Vascular impedance in studies of arterial and cardiac function. Physiol Rev 1982;62:570-623.
50. Wang Z, Chesler NC. Pulmonary vascular wall stiffness: An important contributor to the increased right ventricular afterload with pulmonary hypertension. Pulm Circ 2011;1:212-23.
51. Sucharov CC, Sucharov J, Karimpour-Fard A, Nunley K, Stauffer BL, Miyamoto SD. Micro-RNA expression in hypoplastic left heart syndrome. J Card Fail 2015;21:83-8.
52. Goyal A, Norton CR, Thomas TN, Davis RL, Butler J, Ashok V, *et al.* Predictors of incident heart failure in a large insured population: A one million person-year follow-up study. Circ Heart Fail 2010;3:698-705.
53. Goren Y, Kushnir M, Zafrir B, Tabak S, Lewis BS, Amir O. Serum levels of microRNAs in patients with heart failure. Eur J Heart Fail 2012;14:147-54.
54. Devaux Y, Vausort M, McCann GP, Kelly D, Collignon O, Ng LL, *et al.* A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. PLoS One 2013;8:e70644.
55. Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, *et al.* MiR423-5p as a circulating biomarker for heart failure. Circ Res 2010;106:1035-9.
56. Zhang R, Niu H, Ban T, Xu L, Li Y, Wang N, *et al.* Elevated plasma microRNA-1 predicts heart failure after acute myocardial infarction. Int J Cardiol 2013;166:259-60.
57. Lai KB, Sanderson JE, Izzat MB, Yu CM. Micro-RNA and mRNA myocardial tissue expression in biopsy specimen from patients with heart failure. Int J Cardiol 2015;199:79-83.
58. Devaux Y, Mueller M, Haaf P, Goretti E, Twerenbold R, Zangrando J, *et al.* Diagnostic and prognostic value of circulating microRNAs in patients with acute chest pain. J Intern Med 2015;277:260-71.
59. Mansouri F, Seyed Mohammadzad MH. Molecular miR-19a in acute myocardial infarction: Novel potential indicators of prognosis and early diagnosis. Asian Pac J Cancer Prev 2020;21:975-82.
60. Zampetaki A, Willeit P, Tilling L, Drozdov I, Prokopi M, Renard JM, *et al.* Prospective study on circulating MicroRNAs and risk of myocardial infarction. J Am Coll Cardiol 2012;60:290-9.
61. Glass CK, Witztum JL. Atherosclerosis. The road ahead. Cell 2001;104:503-16.
62. Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, *et al.* Circulating microRNAs in patients with coronary artery disease. Circ Res 2010;107:677-84.
63. Wei Y, Nazari-Jahantigh M, Neth P, Weber C, Schober A. MicroRNA-126, -145, and -155: A therapeutic triad in atherosclerosis? Arterioscler Thromb Vasc Biol 2013;33:449-54.
64. Nigam V, Sievers HH, Jensen BC, Sier HA, Simpson PC, Srivastava D, *et al.* Altered microRNAs in bicuspid aortic valve: A comparison between stenotic and insufficient valves. J Heart Valve Dis 2010;19:459-65.
65. Yanagawa B, Lovren F, Pan Y, Garg V, Quan A, Tang G, *et al.* miRNA-141 is a novel regulator of BMP-2-mediated calcification in aortic stenosis. J Thorac Cardiovasc Surg 2012;144:256-62.
66. Song R, Fullerton DA, Ao L, Zhao KS, Reece TB, Cleveland JC Jr., *et al.* Altered MicroRNA expression is responsible for the pro-osteogenic phenotype of interstitial cells in calcified human aortic valves. J Am Heart Assoc 2017;6:e005364.
67. Songia P, Branchetti E, Parolari A, Myasoedova V, Ferrari G, Alamanni F, *et al.* Mitral valve endothelial cells secrete osteoprotegerin during endothelial mesenchymal transition. J Mol Cell Cardiol 2016;98:48-57.
68. Hulanicka M, Garncarz M, Parzeniecka-Jaworska M, Jank M. Plasma miRNAs as potential biomarkers of chronic degenerative valvular disease in dachshunds. BMC Vet Res 2014;10:205.
69. Smith T, Rajakaruna C, Caputo M, Emanueli C. MicroRNAs in congenital heart disease. Ann Transl Med 2015;3:333.
70. Li J, Cao Y, Ma XJ, Wang HJ, Zhang J, Luo X, *et al.* Roles of miR-1-1 and

miR-181c in ventricular septal defects. Int J Cardiol 2013;168:1441-6.
71. Latronico MV, Catalucci D, Condorelli G. MicroRNA and cardiac pathologies. Physiol Genomics 2008;34:239-42.
72. O'Brien JE Jr., Kibiryeva N, Zhou XG, Marshall JA, Lofland GK, Artman M, *et al.* Noncoding RNA expression in myocardium from infants with tetralogy of Fallot. Circ Cardiovasc Genet 2012;5:279-86.
73. Bittel DC, Kibiryeva N, Marshall JA, O'Brien JE. MicroRNA-421 dysregulation is associated with tetralogy of Fallot. Cells 2014;3:713-23.
74. Liang D, Xu X, Deng F, Feng J, Zhang H, Liu Y, *et al.* miRNA-940 reduction contributes to human tetralogy of Fallot development. J Cell Mol Med 2014;18:1830-9.
75. Boon RA, Dimmeler S. MicroRNAs in myocardial infarction. Nat Rev Cardiol 2015;12:135-42.
76. Corsten MF, Dennert R, Jochems S, Kuznetsova T, Devaux Y, Hofstra L, *et al.* Circulating MicroRNA-208b and MicroRNA-499 reflect myocardial damage in cardiovascular disease. Circ Cardiovasc Genet 2010;3:499-506.
77. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, *et al.* Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circ Cardiovasc Genet 2011;4:446-54.
78. Gao W, He HW, Wang ZM, Zhao H, Lian XQ, Wang YS, *et al.* Plasma levels of lipometabolism-related miR-122 and miR-370 are increased in patients with hyperlipidemia and associated with coronary artery disease. Lipids Health Dis 2012;11:55.
79. Wang J, Huang W, Xu R, Nie Y, Cao X, Meng J, *et al.* MicroRNA-24 regulates cardiac fibrosis after myocardial infarction. J Cell Mol Med 2012;16:2150-60.
80. Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, *et al.* Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. J Clin Invest 2011;121:2921-31.