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Circulating miRNAs as biomarkers in cardiovascular diseases

C.M. KANETO[1], J.S. NASCIMENTO[2], M.S.J.G. PRADO[2], L.S.O. MENDONÇA[1]

[1]Department of Biological Science, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil
[2]Department of Health Science, Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil

Abstract. – Cardiovascular diseases (CVDs) have shown a high prevalence every year, presenting arterial hypertension as prime factor for their development, also driven by population growth, the aging of population and epidemiologic changes in disease. One of the main challenges in the study of CVD is the identification of reliable biomarkers that can be used in clinical practice and, in this context, microRNAs (miRNAs) have attracted much attention recently. MiRNAs are small non-coding RNAs, identified as post-transcriptional regulators of the expression of several genes both in physiologic and pathologic conditions. They have been studied as possible biomarkers, since they are highly expressed in the vascular system and are crucial modulators for the differentiation, contraction, migration and apoptosis of vascular cells, so modifications in their expression can cause several vascular alterations. Thus, this review aimed to compile the main studies regarding the role of miRNAs in the development of cardiac diseases, their potential applicability in the diagnosis, prognosis and treatment of these disorders. It was possible to verify that alterations in miRNAs expression are present in almost all cardiovascular diseases, such as the development of cardiac hypertrophy, coronary heart disease, heart failure and other conditions. Furthermore, growing evidence indicates that circulating miRNAs may become a potential tool for rapid and easy tests, since they are detected in peripheral blood, also allowing new therapeutic possibilities.

Key Words:
MicroRNAs, Gene expression, Cardiovascular diseases, Biomarkers, Cardiovascular diagnostic testing.

Introduction

According to the World Health Organization (WHO), 36 million (63%) deaths that occurred globally in 2008 were due to chronic noncommunicable diseases (CNCDs), including cardiovascular diseases (CVDs)[1]. It is estimated that 17.7 million people died from CVDs in 2015, accounting for 31% of all global deaths, and of these, about 7.4 million occurred due to coronary heart disease and 6.7 million due to stroke[2]. CVDs mainly affect low-income individuals, which are more exposed to behavioral risk factors and have less access to health care services. These diseases can lead to disability or incapacity, large costs in social and economic terms, losses in the productive sector and harmful effects on life quality of those affected, negatively reflecting the effects of globalization, urbanization and habits such unhealthy diet, insufficient physical activity and use of alcohol and tobacco[1]. The incidence of CVDs has also increased significantly with the aging of the population and this relation can be appreciated by consideration of the morbidity and mortality rates of age-related CVDs including coronary heart disease, heart failure, stroke and aortic stenosis, a frequent valve disease in the population over 75 years old[3]. The number of deaths by ischemic heart disease, the most common cause of cardiovascular death, increased by an estimated 41.7% from 1990 to 2013 and population aging was found to contribute to an estimated 52.5% increase in these deaths, whereas population growth, which is considered another driver on trends in mortality, contributed to an estimated 23.6% increase[4]. Considering that aging is a well-established cardiovascular (CV) risk factor, the rising number of older adults in many countries and the large effect of age-specific cardiovascular death rate on CV mortality rates[4], CVDs remain as global threat, imposing burden in terms of functional decline, disability and healthcare costs[2], reinforcing the importance of investments and policies aimed at targeting preventable risk factors that can reduce the impact of these diseases. Improving preventive and therapeutic measures is also important to accomplish good results

Corresponding Author: Carla Martins Kaneto, MD; e-mail: cmkaneto@uesc.br

and recent research focused particularly on underlying molecular mechanisms and on the identification of valid and novel diagnostic and prognostic biomarkers that could strength primary prevention[3]. The ideal biomarker must fulfill a number of criteria, such as: (1) It must be accessible through non-invasive methods; (2) it must have a high degree of sensitivity and specificity for the disease in question; (3) It should allow for early detection; (4) Must present sensitivity to relevant changes in the pathological process; (5) It must have a long half-life in the sample, and (6) Must be able to provide rapid and accurate detection[5]. In this context, microRNAs (miRNAs) can accomplish several of these criteria. They are stable in circulation, are often regulated in tissue and pathology in a specific manner and its expression can be detected with a high degree of sensitivity and specificity using the specific amplification of their sequences[6]. Although the presence of intact extracellular RNA in plasma has been described in 1972[7], in 2008 it was reported that miRNAs are also present in the circulation in all compartments of blood, including plasma, platelets, red blood cells and nucleated blood cells[6,8]. These circulating miRNAs are supposed to be stable in plasma even under adverse conditions like high temperatures, low or high pH and long-term room temperature storage. These qualities suggest that miRNAs biomarkers discovery and validation will be more efficient than protein-based biomarkers, in which critical problems in the generation of specific antibodies are often found due to the complexity of the protein composition, post-translational modifications, and the poor abundance of many proteins in serum and plasma[5,6]. Apparently, these circulating miRNAs are protected from the activity of endogenous RNAses and evidences suggest that this protection is achieved through their packaging in microparticles such as exosomes, microvesicles or apoptotic bodies[9], by binding to RNA ligands, such as Argonaute 2 and nucleophosmin 1[10,11] or even by binding to high density lipoproteins[12]. The presence of miRNAs in microparticles also led to the intriguing idea that circulating miRNAs could play a role in cell communication, suggesting that miRNAs are selectively secreted by a cell and act on distant target cells, possibly to regulate gene expression. This remains as an area of intense research and early studies reveal that miRNAs may actually function as mediators of cell-to-cell communication[13]. First described in 1993[14], miRNAs are small endogenous RNAs of approximately 22 nucleotides that play important roles in animals by regulating post-transcriptional gene expression. They are first transcribed as primary miRNAs by RNA Polymerase II and then are cut by an RNase III enzyme, Drosha, into precursors of approximately 70 nucleotides (premicroRNAs), which are transported to the cytoplasm. Another enzyme, called Dicer, converts premicroRNAs into mature microRNAs that are recruited into RNA-induced silencing complexes (RISC). These RISCs interfere with the translation or stability of target messenger RNAs by binding to them with total complementarity, leading to cleavage of messenger RNA, or with partial complementarity, leading to translation repression[15]. Researchers have also proposed an alternative mechanism of action, where binding of miRNAs leads to faster deadenylation of mRNAs, decreasing mRNAs stability and accelerating their degradation[16]. Evidence also suggests that miRNAs also play a central role as a critical cellular factor with great capability to fine-tune biological processes[17]. MiRNAs genes can vary in their location in the genome. Initial studies had suggested two distinct classes of miRNAs: those that originated from overlapping introns of protein coding transcripts and others that are encoded in exons[18], but clusters of miRNAs genes that coexpress polycistronicaly, transcribed as a single unit, were then discovered[19,20]. The current release (MiRBase 21) contains 28645 entries representing hairpin precursor miRNAs[21], but 10000 new miRNAs are expected to be described in miRBase release 22. However, the biological significance of the majority of annotated miRNAs remains unknown and requires functional validation. MiRNAs play a major role in the coordinated development of various organ systems and physiological conditions and temporal and spatial expression of distinct sets of tissue-specific miRNAs is crucial in modeling tissue development and differentiation in processes ranging from embryonic development to neoplastic progression. In skin development, for example, miR-203 is expressed during differentiation of mouse skin, which controls the basal to suprabasal transition by regulating p63 expression[22,23]. MiR-127 was found to be essential for branching of lung in rat fetal lung cultures[24] and miR-124 was found to be essential for proper development of the nervous system[25,26]. Other important functional roles for miRNAs were documented in insulin secretion[27], adipocyte differentiation[28], lipid metabolism regulation[29] and lung epithelial pro-

genitor cells differentiation[30]. All of these and other works, including high-throughput miRNA profiling studies in specific organ systems, have established the relevance of miRNA in animal development and physiological conditions. Evidence also shows that miRNAs have been implicated in many human diseases. Unique miRNA signatures were found to be associated with various inherited, metabolic, infectious, non-infectious and neoplastic diseases, highlighting their potential to be reliable biomarkers. Many miRNAs have been linked to the initiation and progression of various neoplastic processes and it is estimated that approximately 50% of miRNAs are located at genomic sites that are disrupted or amplified in different types of cancer[31]. These miRNAs are supposed to be evolved in processes like apoptosis[32-34] and cell cycle regulation[35]. They can also influence the pathogenesis and manifestation of infectious diseases, modulating the pathogenicity of pathogens, the efficiency of host response and the resolution of inflammatory responses[17]. Alterations in miRNA expression have also implicated in other noninfectious diseases, including autoimmune diseases[36-38], hepatic metabolic diseases like type 2 diabetes, nonalcoholic fatty liver disease, steatohepatitis[39] and cardiovascular diseases[40-42]. More importantly, evidence shows that they can be readily detected in serum and plasma and that their expression patterns have a positive correlation with these diseases. Comprehensive analysis of miRNAs in serum and plasma to characterize blood miRNA profiles from healthy individuals and patients with different cardiovascular diseases found that these patients may have specific serum miRNA profiles and the opportunity to detect their expression by noninvasive means have led to investigations toward developing miRNAs as biomarkers, providing new perspectives on the pathophysiology of heart diseases like ischaemic heart disease, hypertension, hypertrophy, heart failure and atherosclerosis. Through this review we provide a brief overview of the recent reports investigating the pathophysiological relevance of circulating miRNAs for the cardiovascular system, concentrating mainly on recent findings on miRNAs in, hypertension, hypertrophy, atherosclerosis, coronary heart disease and heart failure.

Hypertension and Hypertrophy

Essential hypertension (EH) may increase the risk of several cardiovascular diseases, including coronary heart disease, stroke, kidney failure and heart failure and in recent years, due to actual living conditions, progressive lengthening in life expectancy and stress, the prevalence of EH in young patients is increasing and there is an urgent requirement for methods to prevent and early identify hypertension and its complications[43]. Several studies have reported the involvement of miRNAs in blood pressure regulation, particularly by affecting the rennin-angiotensin- aldosterone system. MiR-155, for example, has been found to regulate the expression of *AGTR1*, the angiotensin II type 1 receptor that correlates positively with blood pressure. An *AGTR1* allele with a 3' UTR region SNP (single nucleotide polymorphism) (+1166 A/C) is not recognized by miR-155 and is associated with a increased risk of EH[44]. Differential miRNA expression was also observed in renal tissue from hypertensive patients compared to normotensive individuals and miR-181a and miR-663 were linked to repression of rennin expression in human kidney[45]. Caré et al[46] also demonstrated that pressure overloaded mice has low cardiac miR-133 levels and inhibition with an anti-miR-133 oligonucleotide generated cardiac hypertrophy *in vivo*. MiR-1, which is encoded as a part of the same bicistronic unit of miR-133, is inversely related to cardiac hypertrophy[46], modulating the insulin-like growth factor-1 pathway directly, inhibiting insulin-like growth factor-1 and its receptor[47] or by downregulating secreted targets related to this pathway[48], being able to attenuate cardiomyocyte hypertrophy in the intact adult heart by regulation of cardiomyocyte growth responses through modulation of calcium signaling components such as calmodulin[49]. miRNA expression in early hypertrophic development investigation showed that only four of the 13 miRNAs that have previously been reported to be associated with late-stage pressure overload induced hypertrophy were induced during early hypertrophic growth. MiR-23a, miR-27b, miR-125b and miR-195 were associated with angiogenesis and cell growth and their expression in early hypertrophic was accompanied by upregulation of a marker of cardiac growth, indicating that different miRNAs are involved in early hypertrophic growth than in late stage pressure-overload induced heart failure[50]. Global miRNA expression was also evaluated in exercise-induced left ventricular hypertrophy (LVH). Using an experimental model of exercise-induced LVH, Martinelli et al[51] identified that miR-26b, miR-150, miR-27a

and miR-143 can modulated physiological cardiac hypertrophy and also indicate that previously established regulatory gene pathways involved in pathological LVH are not changed in physiological LVH. MiRNA expression levels were also quantified in peripheral blood mononuclear cells of untreated EH patients and revealed that miR-9 and miR-126 are related to EH, as they showed a distinct expression profile in hypertensive patients relative to healthy individuals and are associated with clinical prognostic indices of target-organ damage in hypertensive patients[52]. MiR-510 was also found to be upregulated in blood samples from EH patients, which was corroborated by methylation analysis, suggesting that this miRNA could be used as a novel biomarker for diagnosis and as a therapeutic target for hypertension[53]. There have been some studies that examined biological fluid miRNAs as biomarkers for hypertension, but most remain inconclusive due to the small sample sizes and differences in methodological standardization[54]. Most studies were undertaken to identify and validate the potential of circulating miRNAs as biomarkers for target-organ damage (TOD) of hypertension. One of the first studies that investigated the importance of miRNAs in cardiac hypertrophy showed that mR-208 is crucially involved in hypertrophic signaling[55]. Huang et al[56] assessed the expression levels of miR-29a, miR-29b and miR-29c in patients with EH and healthy individuals and found a positive relation between these miRNAs expression and higher left ventricular mass index (LVMI), suggesting that the miR-29 family may represent a potential marker of hypertension and TOD in EH patients. Similar results were obtained for miR-155[57], miR-7-5p and miR-26b-5p[58]. Approximately, twelve circulating miRNAs were found to be upregulated in hypertrophic cardiomyopathy patients, but only miR-29a was significantly associated with both hypertrophy and fibrosis evaluated with magnetic resonance[59]. MiR-29a levels were also found to be increased in patients with hypertrophic obstructive cardiomyopathy and correlating markers of cardiac hypertrophy, but not in hypertrophic non-obstructive cardiomyopathy patients, showing a specific signature to distinguish between hypertrophic non-obstructive and obstructive cardiomyopathies[60]. Recent reports also present data about paracrine miRNA crosstalk between cardiac fibroblasts and cardiomyocytes, leading to cardiomyocyte hypertrophy, demonstrating that cardiac fibroblasts secrete miR-21 as a paracrine signaling mediator of cardiomyocyte hypertrophy with potential as a therapeutic target[61]. Collectively, these results further support the idea that circulating levels of some miRNAs could serve as biomarkers for LVH.

Atherosclerosis and Coronary Heart Disease

The initiation and progression of atherosclerosis are complicated and multifaceted pathologies, which remain incompletely understood and have become a major public health problem all over the world. They involve chronic inflammation that is developed by interactions of different compounds and cells, including macrophages, vascular smooth muscle cells (VSMCs) and endothelial cells (ES)[62]. Rupture of unstable plaques lead to adhesion of thrombocytes, formation of thrombi, artery occlusion and ischemic disruptions, including myocardial infarction and stroke. It was demonstrated that various biological processes in all stages of atherosclerosis progression are associated with microRNAs[63,64]. Changes in miRNA expression during disruption of human atherosclerotic plaque stability have been mainly investigated in whole plaques without separation into cell types and a number of studies identified hyperexpression of miR-100, miR-127, miR-133a, miR-133b, miR-145 and miR-494 in unstable atherosclerotic plaques while higher levels of miR-21, miR-143 and miR-221 expression were associated with stable atherosclerotic plaques[63-69]. IL-6 (interleukin-6) expression was also found to be upregulated in coronary plaque, blood monocytes and serum of patients with coronary atherosclerosis (AS), whereas miR-365 expression was down-regulated, suggesting that this miRNA may regulate the pathogenesis and immune response in AS[70]. In addition to the investigation of miRNAs expression in the atherosclerotic plaque, circulating miRNAs expression is also of considerable interest and some of them could be considered as potential biomarkers of clinical atherosclerosis and coronary artery disease. Elevated miR-29a levels were found to be associated with atherosclerosis[71]. The level of circulating miR-21 was found to be increased in patients with subclinical atherosclerosis and myocardial infarction, as well as the level of miR-221, which was decreased in stroke[72,73], but none of them are specific to atherosclerosis, once increased levels were also observed in the blood of patients with different oncological diseases[74,75]. More recently, circulating miR-155-5p, miR-483-5p and

miR-451a were also identified as biomarkers for the early identification of atherosclerotic plaque rupture[76]. MiR-143 and miR-145 expression were found to be altered in blood plasma of patients with hypertension, coronary artery disease and myocardial infarction[77] and the levels of circulating miR-133, miR-1 and miR-208a are also supposed to be good diagnostic markers of coronary artery disease and myocardial infarction[78]. MiR-941 expression was found to be relatively higher in patients with acute coronary syndrome (ACS) and ST-segment elevation myocardial infarction (STEMI) than in patients with stable angina (SA) and non-ST elevation ACS and may be a potential biomarker of ACS or STEMI[79]. In a previous study Li et al[80] identified that circulating miRNAs MiR-122, MiR-140-3p, MiR-720, MiR-2861 and MiR-3149 levels are elevated during early stage of acute coronary syndrome (ACS) and then conducted a study to determine the origin of these elevated plasma miRNAs in ACS, concluding that the elevated plasma levels were mainly originated from monocytes and circulating endothelial cells. The impact of transient coronary ischemia on circulating miRNAs was also an object of interest and the circulating miRNAs kinetics in response to cardiac stress in patients with or without significant coronary stenosis was also evaluated. Jansen et al[81] found that patients with stenosis showed an increase of circulating miR-21, miR-126-3p and miR-222 in response to cardiac stress while patients without significant stenoses presented gradually increased miR-92a levels. It is possible that miRNAs could have also a protective effect. MiR-22 was found to lower the levels of pro-inflammatory cytokines by inhibiting the NLRP3 Inflammasome pathway, suppressing coronary arterial endothelial cells (CAECs) apoptosis and protecting CAECs in rats with coronary heart disease[82].

To investigate if miRNAs could have a role in prognostic determination, the relationship between miR-146a levels and the coronary collateral circulation (CCC), an alternative blood supply for ischemic myocardium which improves survival rates among patients with CAD, was observed and increased levels were observed in CAD patients with good CCC, while decreased levels where found in patients with poor CCC[83]. MIR-574 was found to promote cell proliferation and apoptosis inhibition and is suggested as a potential molecular target for CAD treatment, once its downregulation significantly inhibited vascular smooth muscle cells growth[84].

Heart Failure

Cardiac remodeling allows the heart to adapt to external stressors, but chronic activation of remodeling processes becomes pathological and is a significant component of cardiovascular diseases like heart failure (HF). Functional miRNA studies reported that a variety of miRNAs play a role in the mechanisms leading to heart failure, such as remodeling, hypertrophy, apoptosis and hypoxia[85,86] and others demonstrated that the etiology of heart failure (ischemic, aortic stenosis or idiopathic cardiomyopathy) was associated with differentially expressed miRNA patterns[87], suggesting that miRNAs play an active role in the onset and progression of heart failure.

Several studies have investigated the potential of circulating miRNAs for the diagnosis of chronic and acute heart failure. In chronic heart failure, multiple miRNAs with differential expression were identified and have been describe as candidates for future diagnostic markers. To determine the miRNA signature of failing myocardium, miRNAs expression was evaluated in a large cohort of patients with stable and advanced HF compared to normal adult and fetal samples and alterations of miRNA cistrons miR-1-1, miR-195, miR-199a, miR-199b and miR-221 were observed[88]. MiR-423-5p, miR-320a, miR-22 and miR-92b also presented elevated serum levels in 30 stable chronic systolic heart failure patients and correlated with important clinical prognostic parameters[89]. Genome-wide miRNA expression profiles of patients with non-ischemic heart failure with reduced ejection fraction (HF-REF) were also conduced and could identify and validate several miRNAs that show altered expression levels in these patients, discriminating them from controls both as single markers or when combined in multivariate signature and also correlating with disease severity as indicated by left ventricular ejection fraction[90]. Differentially expressed circulating miRNAs were also described in acute heart failure (AHF), including low levels of miR-103, miR-142-3p, miR-30 and miR-342-3p[91] and high levels of miR-499[92]. A panel of seven miRNAs (miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, and miR-652-3p) was found to present decreased expression in acute heart failure patients compared to healthy controls and patients with an acute exacerbation of chronic obstructive pulmonary disease and these decreasing miRNA levels were shown to be predictive for mortality in patients with AHF[93]. It is possible that miRNAs

expression might discriminate between HF with reduced ejection fraction (HFrEF) and HF with a preserved ejection fraction (HFpEF), providing a better insight in their differential pathophysiology as well. MiRNA profiling performed on plasma samples of 28 controls, 39 HFrEF and 19 HFpEF identified four miRNAs (MiR-125a-5p, miR-190a, miR550a-5p and miR638) that could distinguish HFrEF from HFpEF[94].

A large number of biomarkers are predictors of outcome in HF, but only a small number of studies have focused on the prognostic value of circulating miRNAs in patients with acute and chronic HF. miRNAs levels were measured in endothelial progenitor cells from HF patients and low levels of miR-126 were found and associated with cardiovascular death in ischemic HF patients, while high levels of miR-508a-5p were associated with cardiovascular death in non-ischemic HF[95]. Decreases in miR18a-50 and miR-652-3p expression during hospitalization were also found to be predictive for 180-day mortality[93]. MiRNAs expression was also investigated as biomarkers to monitor progression of HF in children with univentricular physiology and miR-129-5p was shown to be a sensitive and specific biomarker independent of ventricular morphology or stage of palliation[96]. Together, several researches have shown the diagnostic and prognostic capacities of circulating miRNAs in HF and indicate a potential role as a more individualized approach to treating patients with HF.

However, the studies presently available do not yet provide sufficient evidence for a clinical use of MiRNAs as biomarkers in cardiovascular diseases. Most of them do not start with a large miRNA panel screen in order to select the most differentially expressed miRNAs and it is also important to compare the predictive diagnostic and prognostic value of the miRNAs to already established CVD biomarkers to determine the individual and additional predictive value of miRNAs. Besides this, most reports have a small patients number, which decreases statistical power, so larges studies should be conducted to verify miRNAs potential as diagnostic and prognostic markers.

Conclusions

The aim of this review was not to analyze the role of individual miRNAs in a specific CVD but rather to present the broad spectrum of capabilities that these small non-coding RNAs possess using some examples. The elucidation of the involvement of miRNAs in the pathogenic mechanisms of CVDs is highly valuable for a better understanding of these processes at the molecular level and may eventually lead to the development of novel treatment approaches.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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