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# Micro RNAs: The Game Changers of Diagnosis and Therapy of Human Cardiovascular Diseases

Habibe Karadaş[1], Hamid Ceylan[1]

[1]*Atatürk University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Türkiye*

*habibe.karadas17@ogr.atauni.edu.tr*

***Abstract*** **–**. MicroRNAs (miRNA) are 21-23 nucleotide-long non-protein-coding RNAs discovered in *C. elegans* in 1993 that resist endogenous RNase activity. More than one hundred miRNAs were discovered in samples from healthy humans and identified as circulating miRNAs. These small RNAs function as regulatory elements in many cellular events. miRNAs act by reducing the expression of the target gene. Although the use of miRNAs in cardiovascular health has not yet been supported by clinical trials, recent studies have suggested that they can be used as clinical biomarkers in the diagnosis and treatment of cardiovascular diseases. However, the promising results of current studies support potential future applications of miRNA therapeutics. In this paper, the action mechanisms of miRNAs and their potential use as a biomarker in the diagnosis and treatment of cardiovascular diseases are mentioned.

*Keywords – Cardiovascular Disease, Non-coding RNAs, MicroRNAs, Diagnosis, Biomarker*

## I. INTRODUCTION

The central dogma of gene regulation in biology has traditionally focused primarily on protein-coding genes through the DNA-mRNA-protein central dogma [1]. However, whole-genome sequencing studies have shown that approximately 1.5% of the total RNA molecule consists of the genome responsible for protein coding, and the majority are non-protein coding regulatory elements called non-protein coding RNA (npcRNA) [2]. These ncRNAs include small nucleolar RNAs (snoRNAs) and long noncoding RNAs (lncRNAs), such as large intergenic noncoding RNAs (lincRNAs) [3]. While the functions of many npcRNAs are still largely unknown, recent research has uncovered their mechanisms, including their important biological functions, and their role in various pathological conditions, including neurodegenerative diseases, cardiovascular disease, and cancer.

### *A. What is Micro RNA?*

For the first time in 1993, two different simultaneous experiments conducted independently, the scientists showed that the non-protein coding RNA they discovered in *Caenorhabditis elegans* and named lin-4 is a post-transcriptional regulator of the expression of the Lin-4 gene [4]. These small non-protein coding RNAs, called miRNAs, function by reducing the expression of the target gene [5]. A large number of different miRNAs encoded in the human genome have been discovered. These miRNAs have a role in regulating the apoptosis pathways of the targeted genes, different functions such as cell development and differentiation. A single miRNA can bind to hundreds of different target mRNAs, so there is no specificity between miRNAs and target mRNAs [6]. miRNAs regulate gene expression of the relevant mRNA by binding to the 3'UTR regions of their target mRNAs [7].

### *B. Micro RNA biogenesis*

miRNA biogenesis begins with RNA polymerase II enzyme-mediated transcription in the cell nucleus, followed by a long miRNA (pri-miRNA) with mature miRNA sequence in a hairpin structure. [8]. The hairpin structure formed is

recognized by the DGCR8 protein, and then the DROSHA enzyme combines with DGCR8 to form the (PASHA) complex, and this complex structure converts pri-miRNA to precursor miRNA. The pre-miRNA is transported from the nucleus to the cytoplasm via the nuclear pores by EXPORTIN-5. The free pre-miRNA in the cytoplasm is recognized by DICER and cleaved to form a double-stranded miRNA 21–23 nucleotides long. In the next step, the miRNA formed is single-stranded by the Ago-2 protein, and mature miRNA is formed. The miRNA interacts with some proteins, along with Ago-2, to form the RNA-induced silencing complex (RISC). RISC acts in two different ways: mRNA destabilization or translational suppression [9].

### *C. Stability of circulational miRNAs and their uses as biomarkers*

More than one hundred miRNAs were discovered in samples from healthy humans and identified as circulating miRNAs. These circulating miRNAs show resistance to endogenous RNase activity [10]. miRNAs are found in the circulation in microparticles such as exosomes, microparticles, and apoptotic bodies, which explains how miRNAs are protected from endogenous RNase activity [11].

miRNAs are regulators and mediators of metabolism. miRNAs have been detected in many different body fluids, such as serum, saliva, blood, and tears, and have an important function as extracellular messengers in intercellular communication. [12] Recently, researchers have suggested that it can be used as a diagnosis and treatment strategy for diseases by comparing circulating miRNA levels in samples taken from patients and healthy individuals [13].

In 2008, miRNAs were identified in human blood plasma. In the study by Mitchell et al., it was observed that circulating miRNAs show resistance to endogenous RNase-mediated degradation [14]. The resistance that miRNAs develop against endogenous RNase activity is explained by their localization in microparticles and miRNA-protein complexes in the extracellular matrix. Later, miRNAs were detected in many body fluids, such as breast milk, cerebrospinal fluid, saliva, and urine. These results allowed a comparison of circulating miRNA levels in healthy and sick subjects and their use as diagnostic or prognostic biomarkers [15].

### *D. The role of miRNAs in heart failure*

Heart failure is a clinical diagnosis in which the heart cannot provide the adequate flow of blood needed to carry out the body's metabolic activities [16]. It may occur as a result of factors such as heart failure, long-term arrhythmias, acute myocardial infarction (MI), and exposure to various cardiotoxic drugs [17]. Levels of some circulating miRNAs are biomarkers that can be used in heart failure. Recent studies and increasing evidence have revealed that miRNAs are effective in the emergence and development of heart failure [18].

Recent studies investigating the role of miRNAs in diseases such as acute coronary syndrome and coronary artery disease, miR-423-5p, miR-622, miR-499, miR-210, and miR-122 have been shown to be miRNAs associated with heart failure [19].

These changes in the amounts of approximately 30 miRNAs, whose levels both increase and decrease in circulation, have been associated with heart failure and various pathologies arising from it. Decreased levels of these circulating miRNAs, including miR-18a, miR-652, miR-199a, miR-106a, and miR-26b, are observed in subjects with heart failure. In patients with acute heart failure, the levels of circulating miRNAs miR-301a, miR-18a, miR-223, miR-423, and miR-652 were found to decrease within the following 2 days, and these findings were found to be associated with increased risk [20].

### *E. The role of miRNAs in acute myocardial infarction*

Acute myocardial infarction (AMI) disease is caused by coronary artery occlusion that causes cardiovascular damage. [21]. Myocardial remodeling after AMI; enlargement of the heart chamber and thinning of the ventricular wall; results from cell and tissue apoptosis and fibrosis [22].

Some circulating miRNAs can be used as biomarkers for AMI. In an animal model of AMI, serum levels of miR-1, which was noticed to regulate myocardial development and differentiation, peaked 6 hours after the heart attack and returned to normal values 72 hours later [23] [24]. When patients with acute myocardial

infarction and healthy controls were compared, the levels of miR-21 were significantly higher in patients with AMI. miR-21 has been shown to be a novel biomarker that predicts left ventricular remodeling after AMI [25].

It has been determined that differences in the levels of some circulating miRNAs are associated with AMI. Most miRNAs affected by AMI regulate protein expression unrelated to the cardiovascular system. This raises issues regarding the specificity of miRNAs that can be used as biomarkers in AMI. For example, some conditions that cause muscle damage affect muscle-specific miR-1 levels. Thus, changes in miR-1 may be a sign of muscle or tissue damage but are not specific to AMI. Among the AMI-associated miRNAs, substantial evidence supports the idea that miR-208a may be a diagnostic biomarker specific to AMI [26].

miR-208a is specific to the heart, and its expression level is significantly affected in most patients with AMI. MiR-208 is rapidly detected in AMI patients. It was determined that miR-208 levels started to decrease in the first 3 hours after AMI, and there was no significant difference in comparisons with controls after 24 hours. These studies have shown that miR-208 may be a suitable and important biomarker for the early diagnosis of AMI, but it cannot be a reliable biomarker in the long term. In this case, other miRNAs, such as miR-133 or miR-499, can be used as long-term biomarkers [27].

### *F. Role of miRNAs in arrhythmia*

Arrhythmia describes a group of symptoms in which the heartbeat changes abnormally. Rhythmic arrhythmias can occur in different ways, such as tachycardia or bradycardia. It has been suggested that changes in the levels of several circulating miRNAs may be associated with arrhythmias.

Analyzing the sera from patients with arrhythmia and comparing them to controls without heart disease, it was shown that miR-150 levels in platelets were reduced by 3.2 times and serum levels by 1.5 times [25]. It is possible that lower levels of platelet miR-150 are involved in the conditions involved in the development of arrhythmias, such as inflammation and fibrosis [29].

Early studies have shown that miR-1 levels are significantly reduced by approximately 86% in the atrial tissue of patients with atrial fibrosis compared to those without atrial fibrosis. Lu et al. showed that in mouse models, overexpression of miR-328 increases vulnerability to arrhythmia, and inhibiting expression of miR-328 reduces vulnerability to arrhythmia. In atrial samples obtained, miR-328 levels increased 3.5-fold in patients with atrial fibrosis compared to patients without atrial fibrosis [30].

Since long-term arrhythmias lead to impaired atrial and cardiac functions, the duration of the arrhythmia is an important factor in predicting the clinical outcomes that may occur after the arrhythmia [31]. miR-483-5p levels were elevated in atrial fibrosis patients 1 and 2 years after arrhythmia diagnosis, regardless of arrhythmia duration. On the other hand, miR-34a-5p levels increased after 1 and 2 years, while miR-125b-5p levels decreased at baseline [32].

### *G. The role of miRNAs in ischemic stroke*

Ischemic stroke (IS) is a disease that causes the blood flow to the brain to drop below a critical level due to the occlusion or narrowing of the blood vessels to the brain, resulting in paralysis-related disability in the individual.

Several miRNAs, particularly miR-16 and miR-126, have been shown to regulate various pathophysiological processes that lead to and follow IS [33]. Neurorestorative effects were observed in diabetic mice with stroke in a treatment modality derived using mouse brain endothelial cells and using exosomes rich in miR-126 [34].

Early detection of IS is critical to saving lives and minimizing damage to the brain. Several miRNAs have been shown to be associated with early stages of ischemic stroke and can be used as early diagnostic biomarkers. It has been stated that miR-335 may be the most suitable and promising biomarker candidate for early detection of IS [35].

### *H. Therapeutic effects of miRNAs*

Treatments using miRNAs are based on the idea that they specifically affect miRNA levels in certain diseases [36]. Depending on the mechanism of the pathophysiology, this includes both suppression of miRNA and elevation of miRNA levels or their replacement with artificially produced copies. The second method mentioned can be done using miRNA mimic technology,

which aims to silence the target gene by generating miRNA-like RNA fragments. These artificial RNA fragments mimic endogenous miRNAs and activate RISC, which specifically binds to target mRNAs and suppresses genes. miR mimics act in a target gene-specific manner, unlike endogenous miRNAs. Although mir-mimics have been widely studied in cancer research, only a few studies on cardiovascular disease have been published so far [37].

Modulation of miRNAs appears to prevent cardiomyocyte apoptosis, reduce infarct size, and reduce cardiac dysfunction. Today, new methods are being developed by synthesizing miRNA mimics and antagonists for the treatment of diseases [38]. Many studies have shown that artificial miRNAs and miRNA antagonists behave like real miRNAs and bind to, inhibit, or degrade mRNA. Inhibition of miR-92a by intravenous administration of an antagomir leads to both the formation of new blood vessels and the functional recovery of damaged tissues in models of ischemia and myocardial infarction [39].

Systemic and local applications of these two methods in animal models were used to examine the consequences of regulating miRNA levels using mimics and antagomirs and to determine their therapeutic potential. In an exemplary study, it was observed that the development of atherosclerotic lesions in mice was reduced following systemic treatment with miR-126-5p mimic and administration of miR-126-3p via apoptotic bodies. [40]. Likewise, beneficial effects were observed when endothelial miRNAs with harmful effects were also inhibited. In a different experiment, the administration of miR-92a-3p antagomir to mice reduced atherosclerosis and improved the lesion phenotype as a result of inhibition or damage to the interactions between miR-103-3p and its proatherogenic targets. [41].

Systemic administration of antisense oligonucleotides against miR-208a-3p in hypertensive rats reduced cardiac stress and subsequent pathological hypertrophy. However, after inducing hypertension by administering angiotensin II to mice, treatment with miR-29b-3p attenuated the progressive deterioration of cardiac function in mice and reversed the signs of hypertensive cardiopathy. These studies show the therapeutic effects and complications of miRNAs in hypertension [42].

The need for new treatments has increased due to the inability of cardiomyocytes to regenerate lost contractile tissue and reproduce damaged tissue. In one study, the therapeutic effects of this miRNA were investigated by administering miR-199a-5p via an adeno-associated virus vector to pigs immediately after myocardial infarction was induced. 30 days after miR-199a-5p administration, MI resulted in an increase in muscle mass and contractility and a decrease in scar formation. Despite these results, uncontrolled expression of this miRNA caused the sudden deaths of 70% of the pigs. This shows that the control of the treatment dose should be done more sensitively. [43].

### *I. Advantages and disadvantages in clinical applications*

Studies show that miRNA-based drugs have between 30 and 1000 targets, making it difficult to eliminate all undesirable effects. There are many hurdles to overcome, including the mechanism of action and stability of miRNA-based drugs [36].

Some miRNAs, which were used in the treatment of different diseases in the past, can be reused in cardiovascular diseases. For example, RG-101 and Miravirsen were the first antimiR drugs to enter clinical trials. Both target miR-122 to treat chronic hepatitis C virus infection [44]. Both drugs initially showed promising results in reducing the hepatitis C viral load, but were later withdrawn from the market due to their undesirable immunological effects and jaundice, respectively. Liver-specific circulating miRNA-122 levels have been reported to regulate cholesterol and fatty acid metabolism and are an independent predictor of clinical outcomes in chronic systolic heart failure [45]. Therefore, miR-122 targeted drugs can be reused to treat dyslipidemia and cardiovascular diseases after being adapted to reduce their undesirable effects [46].

The miR-132-3p inhibitor (CDR132L) was developed to treat heart failure and has been extensively studied in the laboratory and clinic. CDR132L, currently scheduled for Phase II testing, could be the first miRNA-targeted drug in cardiovascular therapy [47].

The main principle of miRNA therapeutic strategies is to try to stop diseases by reducing protein synthesis in certain diseases, such as cardiovascular diseases, cancer, and viral

infections. Circulating miRNAs are stably present because of their transport by exosomes and association with various proteins. In addition, circulating levels of miRNAs change early and rapidly in response to the pathophysiological changes that occur as a result of the development of cardiovascular diseases. This allows them to be used as biomarkers that facilitate diagnosis and treatment. miRNAs are not yet considered reliable as diagnostic or prognostic biomarkers of cardiovascular diseases. However, many studies show that miRNAs can fill some gaps and even replace existing diagnostic standards [31].

## II. CONCLUSION

Despite preclinical and early clinical trials and promising basic research, no approved miRNAs are yet available for the treatment of any cardiovascular disease. miRNAs play an active role in multiple physiological processes and signaling pathways. Although they are elusive therapeutics, their mechanisms of action make them interesting candidates. Factors such as side effects and dose should be comprehensively identified and addressed through basic and clinical studies. Despite the current downsides, there is a consensus that miRNAs can be used as biomarkers and that miRNA-based therapies can be developed.


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