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MicroRNA: A new therapeutic strategy for cardiovascular diseases

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Abstract

Myocardial infarction, atherosclerosis and hypertension are the most common heart-related diseases that affect both the heart and the blood vessels. Multiple independent risk factors have been shown to be responsible for cardiovascular diseases. The combination of a healthy diet, exercise and smoking cessation keeps these risk factors in check and helps maintain homeostasis. The dynamic monolayer endothelial cell integrity and cell-cell communication are the fundamental mechanisms in maintaining homeostasis. Recently, it has been revealed that small non-coding RNAs (ncRNAs) play a critical role in regulation of genes involved in either posttranscriptional or pretranslational modifications. They also control diverse biological functions like development, differentiation, growth, and metabolism. Among ncRNAs, the short interfering RNAs (siRNAs) and microRNAs (miRNAs) have been extensively studied, but their specific functions remain largely unknown. In recent years, miRNAs are efficiently studied as one of the important candidates for involvement in most biological processes and have been implicated in many human diseases. Thus, the identification and the respective targets of miRNAs may provide novel molecular insight and new therapeutic strategies to treat diseases. This review summarizes the recent developments and insight on the role of miRNAs in cardiovascular disease prognosis, diagnostic and clinical applications.

Keywords

MicroRNAs; Endothelial cells; Cardiac fibrosis; Atherosclerosis; Myocardial infarction; Stroke; Hypertension

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1. Introduction

Heart diseases are the leading cause of death in the United States and around the world [30]. Hypertension, tobacco exposure, high cholesterol, obesity, diabetes, unhealthy diets, and alcohol seem to have an additive effect for the causation of cardiac diseases. Myocardial infarction (heart attacks), cerebrovascular disease (stroke), atherosclerosis and hypertensions (raised blood pressure) are the most common cardiovascular diseases, which involve heart and blood vessels. Number of recent studies has shown that miRNAs are essential for the normal development and physiology of various organs, including the heart. Studies have also started to characterize the link between micro RNAs (miRNAs) and different aspects of cardiac pathogenesis such as chamber morphogenesis, conduction, and contraction. Moreover, congenital anomalies of the heart can be associated with the dysregulation of specific miRNAs [50].

Recently, investigators have demonstrated that RNA functions not only as an intermediate molecule between DNA and protein, but is also involved in the complicated process of gene regulation and expression. Some of the RNAs are the functional molecules that are not capable of translating into proteins. Hence, these RNAs are called noncoding RNAs (ncRNAs). Among the several classes of ncRNAs, miRNA is the most extensively studied and has gained prominence in current research. These ncRNAs are found within intergenic or intragenic regions of host genes, which make up approximately 10% of the human genome [14]. Recently, a rapid progress has been made in profiling miRNA and reported it in various diseases and cell types. MiRNAs are involved in a number of biological processes, including cell proliferation, apoptosis, stress response, hematopoiesis, and oncogenesis. Studies have highlighted that miRNA could be a potential molecular therapeutic strategy for various diseases, including heart diseases [88]. MiRNAs are tissue and lineage specific, and many more to be discovered. The recently developed high-throughput approaches revealed the miRNA size, and their target, and the connectivity of the miRNA-dependent regulatory network. One step further, the expression levels of miRNAs and their decay rates have been identified in individual cell types. These works together help us to understand miRNA-dependent gene regulation to study the response of the entire network. Studies have shown that miRNAs stably regulate many developmental and cellular processes, including numerous eukaryotic plants, and mammals with vary in expression at extracellular and intracellular fractions [48]. During the past decade, numerous research articles have shown a wide knowledge about the basic mechanisms of miRNAs, biogenesis and its functions in the circulatory system. This review will discuss the biosynthesis of the miRNA and its functional role in cardiovascular diseases, as well as the challenges in miRNA-based therapy.

2. Noncoding RNAs

The ncRNAs are the functional entity of a cell in regulating the gene expression. These regulatory RNAs have function but do not encode proteins. In 1950s, discoveries of ribosomal RNA (rRNA) and transfer RNA (tRNA) are known as the principle RNA molecules that participate in gene expression. In the early 1980s, the existence of small

nuclear RNAs (snRNAs) was discovered. Recently, the other ncRNAs, such as small cajal body specific RNAs (scaRNAs), small nucleolar RNAs (snoRNAs), long noncoding RNAs (lncRNAs), piwi-interacting RNAs (piRNAs) and circular RNAs (circRNAs) were discovered for the labile genes. The discovery of miRNAs was a huge revolution because it depicted their importance of posttranscriptional events in gene expression, particularly in eukaryotic organisms [11].

2.1. SiRNAs

The siRNAs are small double stranded RNAs and well studied among the ncRNAs of approximately 20-25 base pair (bp) in length. The major role of siRNA is to involve in RNA interference (RNAi) pathway in regulating the gene expression. This RNAi-mediated gene regulation can be executed by either siRNA or miRNA, but there are subtle differences between the two. However, siRNA based strategies have some disadvantages, mostly RNase susceptibility. The use of RNAi-based therapeutics from a clinical standpoint, is a new platform that is steadily developing [74].

2.2. PiRNAs

Comprising a length of approximately 24-30 bp. PiRNAs are the Dicer-independent ncRNAs that associated with PIWI subfamily proteins. PiRNAs are highly abundant in germ cells. Some are involved in the formation of heterochromatin or RNA destabilization, which mediates gene silencing [9].

2.3. SnoRNAs

SnoRNAs are intermediate-sized ncRNAs (60–300 bp) responsible for post-transcriptional modifications and assist in folding and stability of rRNA [46].

2.4. Sca-RNAs

The scaRNAs (60-425 bp) are a subset of snoRNA family, which is large family of conserved ncRNAs that primarily guide biochemical modifications of particular nucleotides (e.g., methylation and pseudouridylation) of rRNAs and snoRNAs. Without the specific modifications controlled by the scaRNAs, the spliceosome fails to function properly [59]. Dysregulation of alternative splicing has been associated with the regulation of heart development and cardiovascular diseases [49]. It has been already reported that 125 snoRNAs including 12 scaRNAs were down regulated in the right ventricle from 16 infants with tetralogy of Fallot [65]. These suggest that scaRNAs playing an important role in heart development and cardiovascular diseases.

2.5. LncRNAs

LncRNAs are a heterogeneous group of non-coding transcripts more than 200 bp long that plays an important role in many biological processes. These constitute the largest portion of the mammalian non-coding transcriptome [62]. Mostly, lncRNAs are known to mediate epigenetic modifications of DNA by recruiting chromatin remodeling complexes to specific loci [62]. These transcripts are identified by searching for chromatin signatures that associated with active transcription in the regions across which transcriptional elongation

takes place. Recent studies have shown that some lncRNAs are regulated during acute myocardial infarction (eg, Novlnc6) and heart failure (eg, Mhrt), whereas others control hypertrophy, mitochondrial function and apoptosis of cardiomyocytes and therefore, considered as a powerful foe in cardiac functions [87].

2.6. CircRNAs

The circRNAs are one among the ncRNAs and have most recently been identified and characterized, and predominantly present in the cytoplasm [31]. The circRNAs, unlike the other known RNAs, the 5’ and 3’ ends present in the RNA molecules are covalently joined together and forms a circular loop. Even though, circRNAs arise from protein-coding genes in the cell but have not been shown to code for proteins. These circRNAs are more stable than other ncRNAs because of its circular nature and do not have 5’ or 3’ ends for endonuclease-mediated degradation. A study has shown that the circRNAs can act as antagonists of miRNAs [61].

2.7. MiRNAs

MiRNAs are a new class of highly conserved RNAs of approximately 22 bp in length, genetically encoded, endogenous, noncoding single-stranded RNAs. It has been shown that the first evidence for small regulatory RNAs called the miRNAs at the genetic loci of lin-4 and let-7, which regulate the timing of *Caenorhabditis elegans* during development [51]. MiRNAs mainly down regulate the gene expression and in rare instances, miRNAs have been reported to up regulate target gene expression [16, 90]. The siRNAs and miRNAs are a class of small, non-coding RNAs that are processed inside the cell by an enzyme called Dicer and incorporated into an RNA inducing silencing complex (RISC). However, siRNA is considered a double-stranded whereas, miRNA is a single-stranded RNA and possesses a small hairpin-like structure [98]. Based on their chemical composition and/or functions, these two classes of RNAs cannot be distinguished easily because of their equal size with 5’-phosphate and 3’hydroxy termini and functionally interchangeable [45]. While both are involved in translational repression or mRNA cleavage, they differ on the degree of complementary between the small RNA and its target. SiRNAs cause degradation of a target mRNA molecule in a sequence specific manner. On the other hand, miRNAs typically suppress the translation of many different mRNA sequences because of its partial complementary pairing. Interestingly, both are important for therapeutic use because of the roles they play in controlling gene expression.

3. MiRNAs biosynthesis and function

The biosynthesis of miRNAs is a complex, multi-step process, which begins in the nucleus and the maturation process ends in the cytoplasm of the cell (**Fig 1**). The synthesis process starts from the miRNA genes that are transcribed either by RNA polymerase II or III arising out of introns of protein-coding genes or from independent genes of long primary miRNA (pri-miRNA) transcripts [48]. These gene transcripts have their own promoting sites to express independently and are organized in clusters for the transcriptional regulation. The resulting pri-miRNAs are cleaved by the microprocessor enzyme complex DROSHA and DGCR8 (DiGeorge Critical Region 8), which are present in the nucleus. The DGCR8

determines the precise cleavage site at the pri-miRNA [34]. The cleaved precursor miRNA (pre-miRNA) molecules contain a local hairpin structure and are approximately 100 bp in length [44]. The maturation process of miRNA is first carried out by DROSHA, a RNAse III family protein in the nucleus. Then, the pre-miRNAs are exported from the nucleus to the cytoplasm through nuclear pores via a GTP-GDP gradient by the enzyme exportin-5 [44]. In the cytoplasm, DICER, derived from an RNA III family interact with another protein partner transactivation response RNA binding protein (TRBP) together cleaves the pre-miRNAs into short double-stranded RNA. This ultimately forms the mature miRNAs. The length of an average mature human miRNA contains a hairpin loop of 33 base pairs [98]. Multiple overlapping proteins and RNA interactions involve and play a great role in the miRNAs biogenesis regulation [48]. There are three major pathways involved in miRNA biosynthesis.

The mature miRNAs gene expression was regulated according to the direction of RISC along with argonaute protein (Ago) assembly [33] to the target mRNA. This leads to the imperfect or perfect complementary of miRNA: mRNA interaction at the 3’ UTR region, resulting in either translational inhibition or transcript degradation, respectively [16, 90]. Studies have shown that p53/p73/p63 functions as transcription factors, but it also regulates miRNAs processing machinery DROSHA-DGCR-8, DICER-TRBP2 and argonaute proteins. In particular, these transcription factors that regulate the processing of miRNAs are let-7, miRNA-200c, miRNA-143, miRNA-107, miRNA-16, miRNA-145, miRNA-134, miRNA-449a, miRNA-503, and miRNA-21 [7]. Since the discovery of miRNAs, number of biological, clinical research is performed on numerous aspects, including cardiovascular diseases. Over expression and suppression of miRNAs revealed the importance of miRNAs in pathophysiology.

4. MiRNAs mimics and inhibitors

The individual miRNA mimics and inhibitors are the useful tools in modulating the specific cell phenotype. MiRNA mimics would be a small and chemically modified double-stranded RNAs that imitate the mature miRNAs. These miRNA mimics enhance the function of endogenous miRNA, leading to a decrease in protein expression. Studies have shown that miRNA mimics could be a useful tool in modulating the failing human heart [77]. MiRNA inhibitors are single stranded oligonucleotides that bind irreversibly to the endogenous miRNAs and inactivate its function. In contrast to miRNA mimics, miRNA inhibitors are also known as an antagomir that suppresses the function of endogenous miRNA and lead to an increase in protein expression. Antagomirs are also served as potential tools for preventing cancer [85] and therefore, could be used in the clinical practice to repress tumor growth. Moreover, the use of anti-sense miRNA treatment is an important strategy to overcome other pathological states like spinal cord injury [71] and ischemic stroke [92]. Thus, RNA mimics and inhibitors are useful in identifying and confirming the pairs of miRNA/target genes that are predicted by computational tools.

5. MiRNAs in cardiovascular diseases

Recently, several studies have reported that the dysregulation of miRNAs expression in tissues is linked to cardiovascular diseases. In addition, studies demonstrated that miRNAs

also existed in blood, in the form of circulating miRNAs and are resistant to endogenous ribonuclease activity and can be present in a remarkably stable form during pathological conditions. More importantly, circulating miRNAs are currently explored as biomarkers in a wide range of cardiovascular conditions, including atherosclerotic disease. In the following sections, we are discussing an overview on the role of miRNAs and its association with various cardiovascular diseases.

5.1. MiRNAs: Atherosclerosis

Atherosclerosis (ATH) or arteriosclerosis is an inflammatory disease in which hypercholesterolemia plays a major role in the origin and development of the pathology. During ATH, the artery wall thickens as a result of accumulation of calcium and fatty materials such as cholesterol and triglycerides [56]. Early stages of ATH are characterized by hyperplasia of vascular smooth muscle cells (VSMCs), which forms a multilayer compartment internally to tunica media called as neointima and the infiltration of low-density lipoproteins (LDLs) in the arterial wall. Accumulation of LDL, monocytes and macrophages forms a vascular plaque. This atherosclerotic plaque becomes fragile and may rupture or erode from either immature or advanced lesions associated with thin, collagen-poor fibrous caps. Formation of a lesion in the neointima compromises the elasticity of the arterial walls and leads to angina. This results in flow-limiting stenosis and therefore, causes cardiovascular diseases such as peripheral artery diseases (PAD), coronary artery diseases (CAD), myocardial infarction, and aneurysms [99].

Animal and human evidence suggests that initiation of ATH is from the dysfunction of ECs of the arterial wall, which is subjected to different noxious stimuli and injuries [79]. The major stimuli/injuries that cause EC-dysfunction includes diabetes mellitus, dyslipidemia, hypertension, smoking, and aging. This injuries-mediated oxidative stress and proinflammatory mediators become predominant in causing ATH. An understanding of the role of miRNAs in ATH may provide novel targets and therapeutic opportunities for controlling disease and other chronic inflammatory disease states, and is depicted in **Fig.2**. Knowledge of miRNA expression pattern and function in distinctive cell types in human is limited because of difficulty in obtaining samples. The currently available information about the patterns of miRNAs observed in different cell types is discussed below.

5.1.1. Endothelial cells—Emerging evidence has revealed that miRNAs constitute a new class of intra- and intercellular signaling molecules to modulate inflammation in endothelial cells (ECs). The miRNA expression profile revealed that the association of a group of miRNAs shows an important role in ATH and the role of individual miRNAs in ECs is poorly understood. Many miRNAs such as MiRNA-126, miRNA-31 and miRNA-17-3p regulate vascular inflammation by controlling the expression of the adhesion molecules VCAM-1, ICAM-1 and E-SEL [76]. It has been established that miR-146a has a potential role in plaque destabilization and the onset of acute coronary syndrome and a novel regulator of inflammation during ATH. This effect is partly by activating the NF-κB signal-transduction pathway [32]. Studies have also shown that miRNA-10 is playing an important role in the regulation of ATH, specifically governing the NF-κB signaling pathway [25]. Recently, it has been found that endothelial miRNA-126-5p maintains a proliferative reserve

in ECs through suppression of the Notch1 inhibitor delta-like 1 homolog (Dlk1) and thereby prevent atherosclerotic lesion formation [72]. Moreover, mRNA-126 promotes angiogenesis by the inhibition of suppressors of the phosphatidylinositol kinase (PI3K) pathway resulting in VEGF and FGF induced endothelial cell proliferation and tube formation [28].

Furthermore, miRNA- 146 represses the pro- inflammatory NF- κB pathway as well as the MAP kinase pathway and downstream early growth response transcription factors. This study also demonstrates that HuR, an RNA binding protein that promotes EC activation by suppressing expression of endothelial nitric oxide synthase (eNOS or NOS2), is a novel miRNA- 146 target [13]. Recent studies have illustrated the role of miRNA-223 in the regulation of high-density lipoprotein-cholesterol (HDL-C) uptake and in the inhibition of cholesterol biosynthesis. Moreover, miRNA-223 plays an important role in cholesterol metabolism [91]. Recently, the novel role of miRNA-26a and its therapeutic potential for atherosclerosis associated with apoptotic cell death has been reported [102]. Thus, miRNAs from ECs are contributing significantly during ATH pathogenesis.

5.1.2. Smooth muscle cells (SMCs)—Many reports have shown that miRNAs undergo specific fate, phenotypic changes and differentiation in VSMCs [57]. It has been shown that miRNA-21 can be up regulated during an injury and be participated also in cell proliferation and survival by targeting PTEN and Bcl2 in smooth muscle cells from blood vessels. Depletion of miRNA-21 resulted in decreased cell proliferation [41]. Studies have further supported the role of miRNA-221 and miRNA-222 in the SMCs proliferation and differentiation [57]. Recently, researchers reported that miRNA-145 is essential for differentiation [15] but later the in vivo miRNA-143/145 KO mice studies revealed that these miRNAs are not the only essential component for the VSMCs development [6]. A study also shown that the family of miRNA-130/301 targets TGF-BMP pathway members SMAD4 and SMAD5 as well as peroxisome proliferator-activated receptor (PPARγ), to control pulmonary VSMCs proliferation during PH [5]. These studies suggest that VSMCs are important for the miRNA-mediated regulation of ATH pathogenesis.

5.1.3. Monocytes and macrophages—Monocytes are the precursors of tissue macrophages and myeloid-derived dendritic cells that influence plaque development followed by recruitment into the intima and differentiation to foam cells [2]. Foam cell macrophages play a crucial role in plaque destabilization and rupture, which makes them the centerpiece in the study of atherogenesis for a long time. Until now, the infiltrated macrophages have been one of the focal points of ATH research and are believed to be critical in regulating changes in the local environment [12]. MiRNAs can also affect monocyte infiltration via several mechanisms. For instance, miR-124a modulates CCL2 expression to facilitate the rolling and attachment of monocytes to the vessel wall [64]. Several miRNAs such as miRNA-17, miRNA-20a and miRNA-106a also regulate macrophage infiltration through the direct suppression of signal-regulatory protein-α [104]. Moreover, high levels of miRNA-145 expressed by VSMCs can decrease macrophage infiltration and, thus, could be a potential target in developing new therapeutic strategy to alleviate ATH [58]. MiRNA-223 directly targets numerous chemo-attractants, such as chemokine C-X-C motif ligand 2 (CXCL2) and CCL3 and regulates the infiltration of

myeloid cells. [22]. There is an increased expression of miRNA-155 and a decreased expression of the colony-stimulating factor 1 receptor (CSF1R) and B-cell lymphoma 6 (BCL6) was observed by the inflammatory macrophages present in the atherosclerotic lesions [97]. MiRNA-342-5p derived from macrophages is responsible for the up regulation of atherogenesis, leading to the increase of nitro-oxidative stress during atherosclerotic lesion formation. This may be due to the up regulation of NOS2 in proinflammatory macrophages, which removes the repression of miRNA-155. MiRNA-143, miRNA-145 and miRNA-365 were down regulated, and miRNA-21, miRNA-146, miRNA-214, and miRNA-352 were up regulated in neointimal formation models [41]. Moreover, miRNA-342-5p promotes inflammatory macrophage activation through Akt pathway during ATH. Thus, targeting miRNA-342-5p in activated macrophages during atherogenesis may be a promising therapeutic strategy because it prevents the initiation of a cascade of molecular events that sensitize macrophages to inflammatory stimulation [96].

5.2. MiRNAs: Hypertension

Hypertension is the persistent elevation of systemic blood pressure, which is one of the most common medical conditions involving the cardiovascular diseases such as MI and stroke as well as the leading cause of kidney failure [53]. Despite continuous advancement in treatment options, it is an increasingly important health problem, affecting as many as one billion people worldwide with high morbidity and mortality. The role of miRNAs in the regulation of hypertension is summarized in **Fig. 3**. Recently, miRNAs have been considered as potential biomarkers for the regulation of PH. It has been shown that there is a down regulation of miRNA-204 in PH associated pulmonary arterial smooth muscles' cells both in animal and human samples [37]. MiRNA-21 is predicted to be a PH-modifying miRNA in regulating targets integral to the bone morphogenetic protein (BMP) and Rho/Rho kinase signaling. Therefore, miRNA-21 considered as an important regulator to control PH pathogenic signaling [67]. Another study has unveiled that miRNA-124 controls the proliferative, migratory and inflammatory phenotype of pulmonary vascular fibroblast. Additionally, it regulates fibroblast proliferation via direct binding to the 3' UTR of polypyrimidine tract-binding protein 1 (PTBP1) and subsequent regulation of Notch1/PTEN/FOXO3/p21Cip1 and p27Kip1 signaling [93]. MiRNA124 and miRNA-135a are also associated with mineralocorticoid receptor (alternative name: nuclear receptor subfamily 3 group C member 2-*NR3C2*) regulation. It is a ligand-dependent transcription factor that regulates the expression of ionic and water transporters in response to steroid hormones, primarily aldosterone. MiRNA-124 and miRNA-135a also attenuate signaling in the renin–angiotensin–aldosterone (RAA) system and thus participate in the regulation of blood pressure [75]. Sun and coworkers also studied the complexity of RAA system, exercise and miRNAs in the development of PH. They observed that miR-155 was an essential regulator of endothelial nitric oxide synthase (eNOS) expression and endothelium-dependent vasorelaxation while mediating inflammation in PH. Nitric oxide generated by the eNOS plays a significant role in maintaining cardiovascular homeostasis. Therefore, inhibition of miRNA-155 may be a new therapeutic approach in improving endothelial dysfunction during the development of cardiovascular diseases [78]. A recent molecular network analysis study forecasted that the miRNA-130/301 family is a master regulator in controlling the

cellular proliferation in PH by unexpectedly interacts with each other by targeting PPARγ [5]. These studies suggest that miRNAs are the important regulator for PH.

5.3. MiRNAs: Myocardial Infarction (MI)

Ischemic heart disease resulting in loss or dysfunction of cardiomyocytes is the leading cause of death worldwide. The survivors of acute MI eventually develop chronic heart failure, with over 5 million cases in the United States alone [30]. The cause may be due to a variety of factors, including MI, hypertension or genetic mutation, which overall result in lack or poor flow of oxygen to heart, heart tissues. This results to coronary artery occlusion, cardiac arrest and subsequently death happen. This phenomenon is called acute MI, and more commonly known as heart attack. Role of tissue-specific miRNAs has been documented in both the physiological and pathological conditions of myocardial infarction. Circulating miRNAs in patients with MI have been examined very recently. It was reported that miRNAs served as a critical modulating regulator, which participates in almost all aspects of cardiovascular diseases and vascular biology [1].

The expression levels of miRNAs vary between tissues. MiRNA-1, miRNA-133a and -133b are expressed strongly in heart and skeletal muscle. However, after acute MI, there was no significant difference in the expression of miRNA-1, miRNA-133a and miRNA-133b, which normally exhibits at high levels in the heart [1, 17]. Studies also show that after ischemic preconditioning in the heart, miRNA-1, miRNA-21and miRNA-24 were significantly increased. This increased miRNAs further triggers cardio-protection by up regulating eNOS, heat shock protein-70 (HSP-70), and heat-shock factor 1 (HSF-1) [100]. MiRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting HSP 20. Therefore, miRNA-320 could be a new therapeutic target for ischemic heart diseases [69]. It has also been studied that miRNA-21 regulates matrix metalloproteinase 2 (MMP-2) expression at the fibrosis area of the infarcted zone via a PTEN pathway [70]. Another study has shown that miRNA-133a of adult cardiac progenitor cells (CPCs) increases cardiac function after MI by the reduction of fibrosis, hypertrophy coupled to an increase in vascularization and cardiomyocyte proliferation [40]. A study has shown that MiRNA-24 is playing a critical role in cardiac fibroblast function and cardiac fibrosis after MI through a furin-TGF-β signaling pathway [94].

An emerging role of miRNA-26 family and their implications for different cell types during cardiovascular disease condition was reviewed recently [38]. The miRNA-29 family is another important miRNA that acts as a negative regulator of gene expression by targeting the mRNAs that encode proteins involved in fibrosis, including multiple collagens, fibrillins, and elastin. Down-regulation of miRNA-29 with anti-miRNAs in vitro and in vivo induces the expression of collagens, whereas over-expression of miRNA-29 in fibroblasts reduces collagen expression and thereby protects the heart from MI [89] as explained in Fig.4. Meanwhile, some other studies have shown that miRNA-29 family induces apoptosis by activating p53 and targeting p85alpha and cell division control protein 42 (Cdc42) [68]. In addition, a recent report has declared that miRNA-28 promotes myocardial ischemia through the inhibition of Aldehyde dehydrogenase 2 (ALDH2) expression in *Mus muculus* cardiomyocytes and thus can be served as a potential target for therapy [55]. Recently, a

study reported that patients with ischemic heart disease have been substantial up regulation of miRNA-377 in biopsy samples from myocardial tissues. Further more, transplantation of miRNA-377 knockdown human CD34 cells into the ischemic mouse myocardium promoted cardiac repair and attenuated ventricular function. This study also identified that STK35 kinase is the direct target of miRNA 377 during MI [42]. These studies documented that miRNAs are important in regulating the pathological conditions of MI.

5.4. MiRNAs: Cardiac Hypertrophy

Cardiac hypertrophy or hypertrophic cardiomyopathy is an inheritable disease and also called the disease of the sarcomere, where the heart muscle becomes thicker in some parts of the heart. This thickening of the muscle forces the heart to work harder to pump blood to circulate different parts of the body [29]. Hypertrophic cardiomyopathy exists as physiological and pathological forms. In the physiological form, there is an enlargement of the heart in healthy individuals due to heavy exercise and is not associated with any kind of cardiac disorder or damage. In the pathological form, the size of the heart initially increases to compensate for the damage to cardiac tissue, but later leads to a decline in left ventricular function [52]. Pathological hypertrophy has been extensively studied and reviewed in relation to miRNAs that are present in the heart [18, 43]. To understand the specific role of miRNAs in cardiac hypertrophy, van Rooij et al. have reported the first microarray analysis data using two different animal models of pathological cardiac hypertrophy. They found that miRNA expression patterns in the two models were similar, indicating the presence of similar miRNA-controlled hypertrophic mechanisms [88]. Dysregulation of IGF-1 signaling has also been involved in pathological hypertrophy, which is regulated by miRNAs. MiRNA-378 and is mostly expressed in cardiac myocytes, during cardiac hypertrophy [19, 63]. The comprehensive role miRNAs in hypertrophic cardiomyopathy have summarized in **Fig. 5**.

MiRNA-1 and miRNA-133 have shown to be down regulated in exercised trained rats and cardiac-specific Akt transgenic mice, which are models of physiological cardiac hypertrophy. MiRNA-133 is one of the key regulators of cardiac hypertrophy and can be targeted in therapy. Moreover, MiRNA133 works negatively on RhoA/CDC42 signaling and therefore, regulated hypertrophy [10]. Recent study has shown that miRNA-1 also negatively regulates hypertrophy by acting on Mef2a and Gata4, two transcription factors in calcium signaling [39]. Furthermore, a recent study determined that expression of miRNA-652 increases in the hypertrophic heart. The suppression of miRNA-652 improved the cardiac function, reduced fibrosis and fewer apoptosis in the hypertrophic heart. Thus, silencing of miRNA-652 protects the hypertophic heart and has the therapeutic potential against cardiac dysfunction [4]. Another study showed miRNA-223 was down regulated in endothelin-1 induced hypertrophic cardiomyocytes and in the hypertrophic heart. Therefore, over expression of this miRNA will rescue the hypertrophy by up regulating TNNI3K, a novel cardiotroponin I (CTI)-interacting and cardiac hypertrophy related kinase [95]. Studies have shown that miRNA-212 and miRNA-132 families have major roles in both cardiac hypertrophy and cardiomyocyte autophagy by regulating Fox signaling pathway [86]. Furthermore, a study reveals that miRNA-208a is required for proper cardiac conduction and expression of the cardiac transcription factors homeo domain-only protein, GATA4 and the

gap junction protein connexin 40 (Cx40). Therefore, miRNA-208a modulates cardiac hypertrophy and electrical conduction [63]. These studies suggest that miRNAs have a specific role in cardiac hypertrophic disease remodeling.

5.5. MiRNAs: Cardiac fibrosis

Cardiac fibrosis is characterized by excessive accumulation of extracellular matrix (ECM), collagens and α-smooth muscle actin that ultimately destroys tissue architecture and eventually abolishes normal function [81]. During cardiac disease, there are fibroblast activation and proliferation that lead to inappropriate secretion of ECM proteins. Both an increased synthesis and decreased degradation of ECM components can lead to fibrosis. Fibrosis causes imbalance in the turnover of ECM proteins and impairment in cardiac contractility. This alters the electromechanical properties of the myocardium, often leading to arrhythmias, which is a major cause of mortality in heart disease. MMPs are a family of proteolytic enzymes that degrade ECM components and therefore, up regulate matrix remodeling, whereas a tissue inhibitor of metalloproteinase is down regulated in cardiac fibrosis [60]. Collagens are the major ECM protein, but other important ECM proteins such as fibronectins, elastin and fibrillins also play a significant role during the development of fibrosis. The release of profibrotic mediators after tissue injuries, such as TGF-β and platelet-derived growth factor (PDGF) are caused fibroblasts remodeling by differentiating into myofibroblasts [35]. It has been shown that MiRNA-7a/b protects cardiomyocytes form cell death during ischemia/reperfusion injury by effectively inhibiting MMPs expression and cardiac fibroblast migration suggesting its therapeutic value in treating cardiac diseases [54]. Although, the mechanism of the development of fibrosis has been recently uncovered, and the therapeutic options are yet to emerge as an effective antifibrotic agent.

Studies have shown that TGFβ mediated down regulation of the miRNA-29 family members (miRNA-29a, 29b and 29c) and their target genes, including ECM proteins during myocardial infarction play a significant role in cardiac fibrosis. Although the functional consequences of the change in the miRNAs during cardiac fibrosis are unknown, it has been shown that there is a down-regulation of miRNA-149 and an increased expression of miRNA-21, miRNA-214 and miRNA-223 in cardiac fibrosis [35]. The role of miRNAs in cardiac fibrosis is explained in **Fig. 6**. A recent report demonstrates that miRNA-24 regulates TGF-β1 pathway and thereby has a functional involvement MI fibrosis remodeling. It has also been observed that miRNA-24 could decrease the synthesis of ECM proteins and hence, the differentiation of fibroblasts to myofibroblast. MiRNA-24 also reduces TGF-β secretion in cardiac fibrosis, which decreases by smad2/3 phosphorylation [94].

In addition to all these miRNAs, during cardiac fibrosis, miRNA-21 becomes predominant in cardiac fibroblasts in response to cardiac hypertrophy and failure. MiRNA-21 also regulates the extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase (MAPK) signaling pathway through inhibition of sprouty-1. Therefore, it promotes fibroblast survival and growth factor secretion. Silencing of miRNA-21 with an antagomir results in the reduction of cardiac ERK-MAPK activity, inhibition of interstitial fibrosis, and improvement of cardiac dysfunction [82]. MiRNA-21 also regulates cardiac fibrosis of the infarct zone via Bcl2 signaling [21] and MMP2-PTEN signaling pathways [70]. There is a

down regulation of miRNA-133a in angiotensin II-mediated hypertension and cardiac fibrosis, leading to a derepression of its target collagen, collagen type 1A1. Additionally, another study found that the expression of connective tissue growth factor, one of the major fibrosis-promoting factors, is regulated by miRNA-30 and miRNA-133 in human and animal cardiac tissue [23]. It was also displayed recently that the down-regulation of miRNA-122 in heart might be involved in myocardial fibrosis in aortic valve stenosis, probably through TGF-β_1 up-regulation [3]. MiRNA-15 and miRNA-378 families are another novel regulator of cardiac hypertrophy and fibrosis acting by the inhibition of the TGFβ [63, 84], and thus show the link between miRNA and TGFβ-pathway. A study has shown that miRNA-155 also plays an important role in regulating cardiac fibrosis in the diabetic heart [47]. Recently, it has been shown that MiRNA-503 promotes angiotensin-II induced cardiac fibrosis, and it would be a promising therapeutic target for reducing cardiac fibrosis [103].

5.6. MiRNAs; Cerebral ischemia (CI) and Stroke

Stroke targets approximately 2-4% of the total healthcare worldwide and is the second most common cause of death. In stroke etiology, miRNAs have distinct expression patterns that modulate pathogenic processes. MiRNAs regulate 30% of protein-coding genes in the gene expression-governing neuronal network. Three different subtypes of stroke are unveiled through miRNA micro array analysis like large artery, small artery and cardio embolic stroke. There are 836 miRNAs have listed in stroke patient's blood samples. Out of these, let-7f, miRNA-126, miRNA-1259, miRNA-142-3p, miRNA-15b, miRNA-186, miRNA-519e and miRNA-786-5p were expressed poorly, but let-7e, miRNA-1184, miRNA-1246, miRNA-1261, miRNA-1275, miRNA-1285, miRNA-1290, miRNA-181a, miRNA-25*, miRNA-513a-5p, miRNA-550, miRNA-602, miRNA-665, miRNA-891a, miRNA-933, miRNA-923 and miRNA-939 miRNAs are expressed high [80] .

The down regulation of miRNA-29c leads to de-repression of its target DNA methyltransferase 3a is promoting ischemic brain damage. Highly expressing miRNA-29c in PC12 cells are down regulating in adult rats after focal ischemia and oxygen-glucose deprivation (OGD) respectively. Pandi and his group have been well documented that pre miRNA-29c prevents OGD-induced cell death [66]. MiRNA-497 works as a proapoptotic regulator and targets the expression of anti-apoptotic genes like Bcl-2 [101]. Knockdown of miRNAs, miRNA-497 and miRNA-15a are considered to be beneficial as they contribute to the post ischemic brain death by targeting BcL-2. Whereas miRNA-21 up-regulation prevents the translation of Fas ligand and thereby decrease post ischemic apoptosis [66]. MiRNA-29c is considered a neuroprotector because it plays a vital role in adult brain and cellular functions. MiRNA-9, miRNA-124a, miRNA-124b, miRNA-135, miRNA-153, miRNA-183 and miRNA-219 are brain specific miRNAs in mouse and affect the mammalian neuronal process too [73]. Both in vivo and in vitro experiments displayed that miRNA-29b-2, miRNA-339-5p, miRNA-19b and 341 are up-regulated in response to CI [20]. These studies clearly show that during CI or stroke, the miRNAs are significantly contributed in disease pathogenesis.

6. MiRNA in diagnosis and their Clinical applications

Recent studies have shown that circulating miRNAs have the potential of early detection and prognosis of many diseases, including cardiovascular diseases, cancer, diabetes, rheumatoid arthritis, and kidney diseases. The currently available proteins based biomarkers for identifying cardiac diseases are providing false-positive results that lead to wrong diagnosis and treatment. Therefore, early detections of cardiac functional or damage-related biomarkers are needed for proper diagnosis and to reduce the death rates in humans. Studies from clinical samples raised the possibility of considering miRNAs as a useful biomarker to define disease states. Studies have shown that miRNAs appear to be the most important biomarker for acute and prognostic disease assessment as well as therapeutic agents for cardiovascular diseases. It is believed that miRNAs could be used as highly sensitive early diagnostic biomarkers and therapeutic targets for many cardiovascular diseases such as atherosclerosis, acute myocardial infarction, heart failure, hypertension and stroke as well as cancer, and was reviewed and published recently [26, 71]. In heart failure patients, miRNA-423-5p is highly expressed in the blood irrespective of age and gender and might be a sensitive biomarker for heart diseases [83]. Moreover, plasma miRNA-423-5p is elevated in dilated cardiomyopathy patients and has a positive correlation with the N-terminal pro-brain natriuretic peptide [24]. A recent study was shown that the serum of coronary artery disease patients had reduced circulating miRNA-126 and miRNA-145 [27]. It has been shown that miRNA-208 up regulated and miRNA-1 and miRNA-133a/b were down regulated in cardiac tissue samples from MI patients when compared to normal controls [8]. Therefore, miRNAs represent important biomarker for the identification and prognosis of different diseases. And the use of miRNAs in the translational field is increasing as they can be detected easily by quantitative polymerase chain reaction (qPCR), which is relatively inexpensive and sensitive to even low amounts due to robust amplification of signal.

7. Challenges in miRNA therapy:

It is important to identify the gene targets and the signaling pathways responsible for their cardiovascular effects in order to develop new miRNA based therapeutics. Also, inhibition of miRNAs by antisense strategies or pharmacological approaches can serve as an alternate, safe method. The preclinical and clinical studies we discussed above indicate that miRNAs playing an important role in disease prognosis and pathogenesis. MiRNA mimics and antagomir can target these mRNAs that are responsible for disease phenotype can have profound effects on different diseases, supporting enthusiasm for further discovery of miRNAs as novel drug candidates. However, in general, several challenges and questions remain to be answered in the developments of miRNA-based therapy. To data, most of the in vivo miRNA studies have been focused on site-specific phenotypic effects, which might ignore the off-target effects in other tissues. Thus, studies are needed to focus on the in vivo miRNA effects on systemic approach instead of site-specific approaches. Another important challenge is to identify appropriate translational dosing regiments in order to get the lowest doses with highest efficiency and minimal side effects.

8. Summary

The role of miRNAs in regulating the major genes of different cardiovascular disorders is summarized in Fig. 2-6 to make us understand the key target molecules for each miRNA that regulates. With this information, one can easily formulate a better therapeutic strategy for cardiovascular diseases. Taken together, these recent pieces of evidence show that miRNAs play powerful roles in cardiovascular systems and are sure to open the door to be previously unappreciated medical therapies.

9. Future perspectives

It is now known that miRNAs have some role in the context of diseases. It regulates many valuable genes in the pathways and is also involved in the regulation of epigenetic modifications. But it is important to understand the exact mechanisms how they are involved in the initiation and in the progression of the diseases. Moreover, it is necessary to identify the early phase and late phase miRNAs during the disease progression. The early phase miRNAs could serve as the important marker specific for the disease that can be targeted in the therapy. However, since late phase miRNAs remain in the body for a longer time, they may not be disease specific. Also, miRNAs could be more readily used as therapy in case of chronic diseases like diabetes or neurological disorders than acute diseases like MI or stroke because the availability of the specific miRNAs will be higher in the chronic diseases. Additionally, the use of miRNA in therapy will be more successful if it is used in systemic disorders rather than organ specific diseases. The miRNA serve as an important biomarker for diseases as it has some advantages over other candidates like proteins and metabolites [36].

10. Conclusion

The research of miRNAs in cardiovascular diseases is a fairly recent area of study with a promising future. MiRNAs role in identifying the prognosis, diagnosis and changes associated with different types of diseases, including cardiovascular disease is truly exciting. Even though encouraging outcomes in the miRNA research from animal models, still several technical and practical difficulties need to be addressed before translation to clinical practice. Once the challenges and the problems are solved in the field of miRNA-based therapy, it will be a potential candidate to compete with the protein inhibitors in the market.

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Abbreviations Used

ATH	Atherosclerosis
ALDH2	Aldehyde dehydrogenase 2
BMP	Bone morphogenetic protein

Bcl2	B-cell lymphoma
CAD	Coronary artery diseases
CDC42	Cell division cycle 42
CPCs	Cardiac progenitor cells
CM	Cardiomyopathy
CCL2	Chemokine (C-C motif) ligand 2
CircRNAs	Circular RNAs
CTGF	Connective tissue growth factor
Cx40	Connexin 40
DCM	Diabetic cardiomyopathy
DGCR8	DiGeorge Critical Region 8)
DLK1	Delta-like 1 homology
Dyrk	Dual specificity Tyrosine Regulated Kinase
EC	Endothelial cell
ECM	Extra cellular matrix
Erk	Extracellar signal- regulated kinase
eNOS	Endothelial nitric oxide synthase
FGF	Fibroblast growth factor
FOXO3	Forkhead box O-3
Grb2	Growth factor receptor-bound protein 2
GATA4	Gata binding protein 4
HGF	Hepatocyte growth factor
HSP70	Heat shock protein 70
HSF1	Heat shock factor 1
HUR	Human antigen R
IGFR1	Insulin growth factor 1 receptor
ICAM	Intercellular adhesion molecule
LDLs	Low-density lipoproteins
MMP	Matrix metalloproteinase

mRNA	messenger RNA
MiRNA	Micro RNA
MAPK	Mitogen-activated protein kinases
MCP1	Monocyte chemo attractant protein-1
MEF2A	Myocyte enhancer factor 2A
MI	Myocardial infarction
NOS2	Nitric oxide synthase 2
ncRNA	Noncoding RNA
NF-KB	Nuclear factor kappa-B
NR3C2	Nuclear receptor subfamily 3 group C member 2
PAD	Peripheral artery diseases
PPARγ	Peroxisome proliferator-activated receptor gamma
PTBP1	Polypyrimidine tract-binding protein 1
PTEN	Phosphatase and tensin homolog
PiRNA	Piwi-interacting RNAs
PI3K	Phosphatidylinositol-3-kinase
PH	Pulmonary hypertension
RAA	Renin-angiotensin-aldosterone
rRNA	Ribosomal RNA
Rho	Ras homology gene family
RISC	RNA inducing silencing complex
scaRNAs	Small cajal body specific RNAs
SMA-α	Smooth muscle actin-alpha
snoRNAs	Small nucleolar RNAs
STK35	Serine/threonine-protein kinase 35
tRNA	Transfer RNA
TRBP	Transactivation response RNA binding protein
TGFβ	Transforming growth factor beta
TNNI3K	Cardiac troponin-I3 interacting kinase 3

VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells

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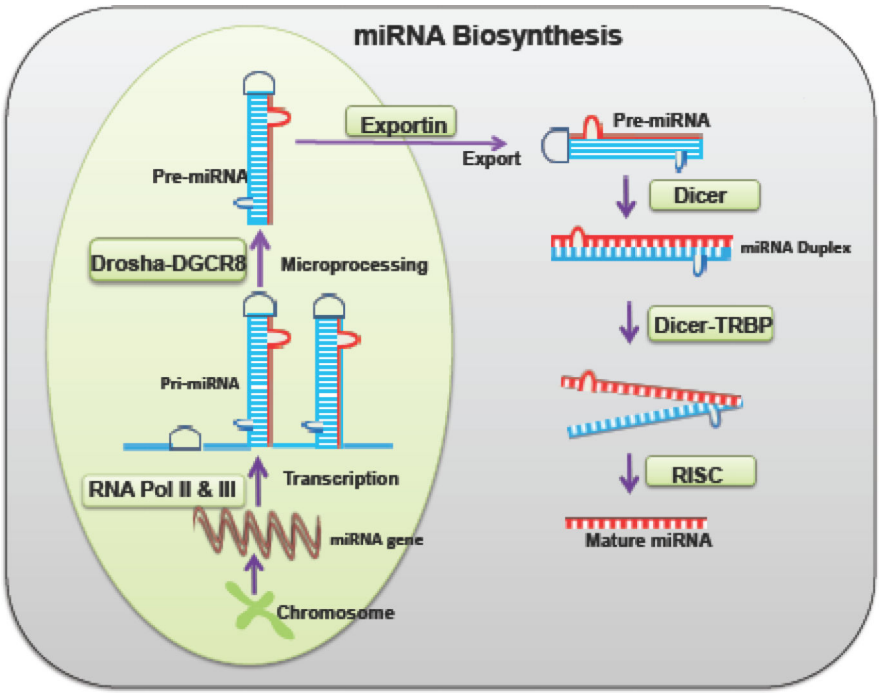

Figure 1. The common miRNA biosynthesis pathway

pri-miRNA- primary miRNA; pol II & III- polymerase II or III; DGCR8 -DiGeorge Critical Region 8; TRBP – Transactivation response RNA binding protein; RISC- RNA-induced silencing complex.

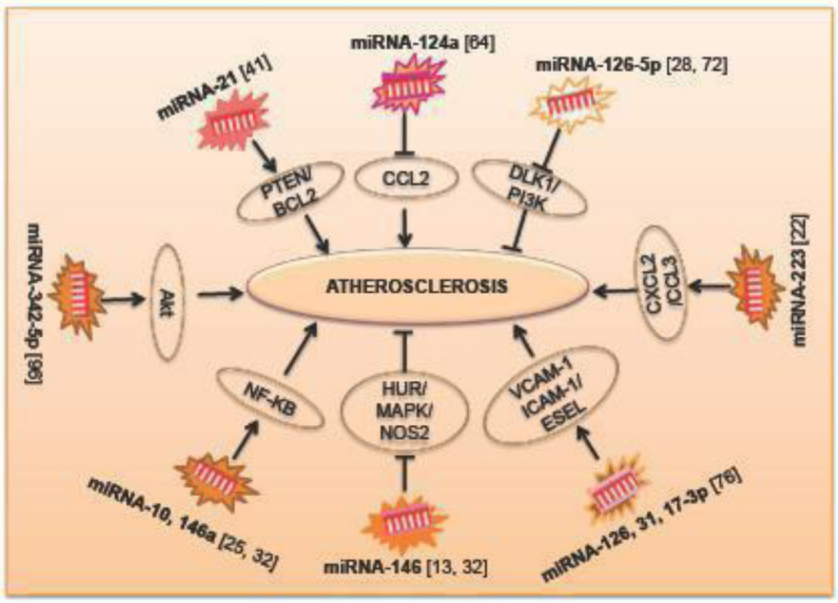

Figure 2. The role of miRNAs involved in atherosclerosis

PTEN - Phosphatase and tensin homolog; Bcl2 - B-cell lymphoma; CCL2 - Chemokine (C-C motif) ligand 2; DLK1- Delta-like 1 homology; PI3K- phosphatidylinositol-3-kinase; CCL2- Chemokine (C-C motif) ligand 2; CXCL2- Chemokine (C-X-C) ligand 2; VCAM - Vascular cell adhesion molecule; ICAM – Inter-cellular adhesion molecule; HUR – Human antigen R; MAPK - Mitogen-activated protein kinases; NOS2 - Nitric oxide synthase 2; NF-KB – Nuclear factor kappa-B.

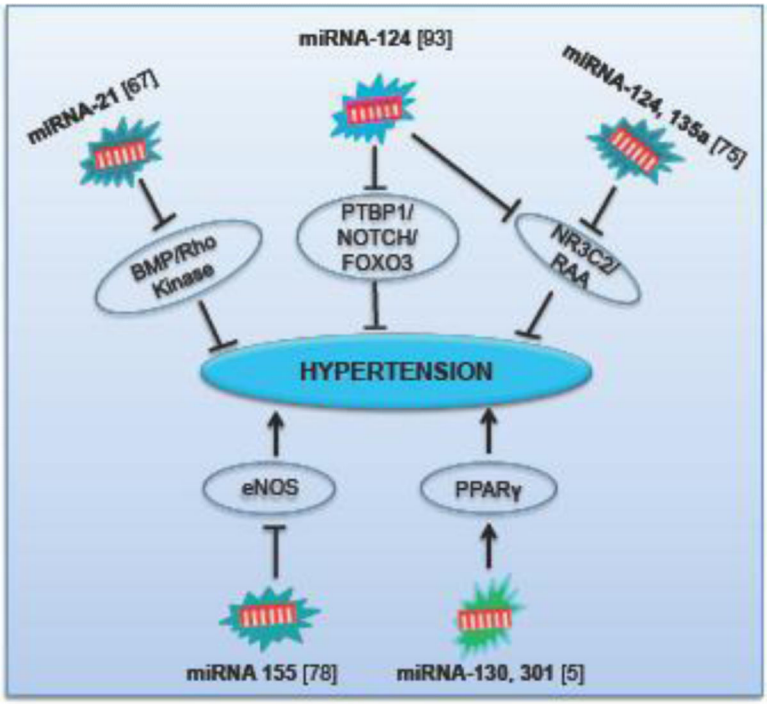

Figure 3. The miRNA-mediated pathways involved during hypertension

PPARγ – Peroxisome proliferator-activated receptor gamma; eNOS- Endothelial nitric oxide synthase; BMP - Bone morphogenetic protein; Rho – Ras homology gene family; PTBP1 – Polypyrimidine tract-binding protein 1; FOXO3 – Forkhead box O-3; NR3C2 – Nuclear receptor subfamily 3 group C member 2; RAA - Renin-angiotensin-aldosterone.

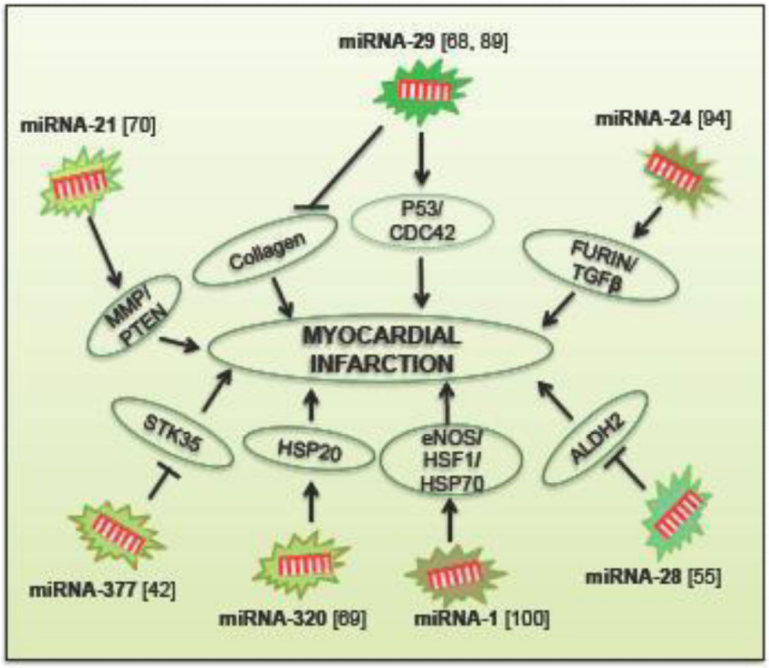

Figure 4. The role of miRNAs in pathogenesis of MI

ALDH2 – Aldehyde dehydrogenase 2; eNOS- Endothelial nitric oxide synthase; HSF1 – Heat shock factor 1; HSP70 – Heat shock protein 70; STK35 – Serine/threonine-protein kinase 35; MMP - Matrix metalloproteinase; PTEN - Phosphatase and tensin homolog; CDC42 – Cell division cycle 42; TGFβ - Transforming growth factor beta.

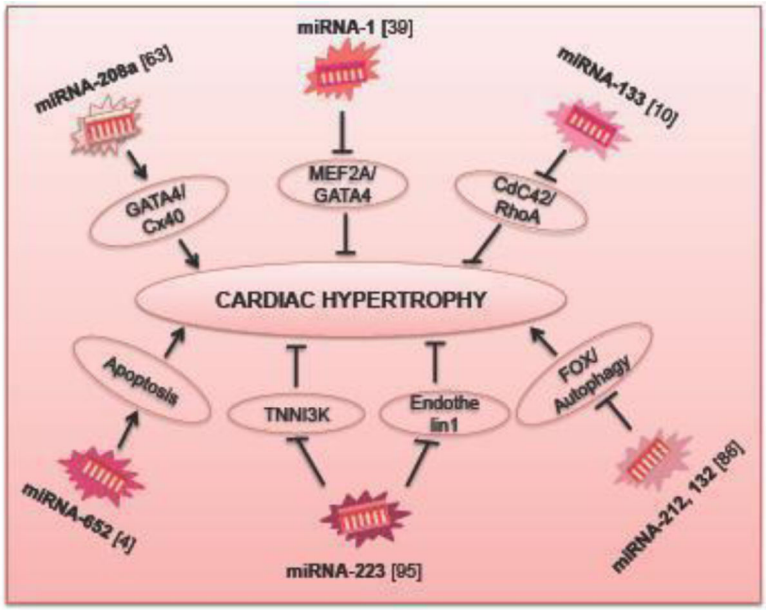

Figure 5. The role of miRNAs in cardiac hypertrophy

TNNI3K – Cardiac troponin-I3 interacting kinase 3; Cx40 – Connexin 40; MEF2A – Myocyte enhancer factor 2A; CDC42 – Cell division cycle 42; RhoA – Ras homology gene family member A; FOX - Forkhead box O.

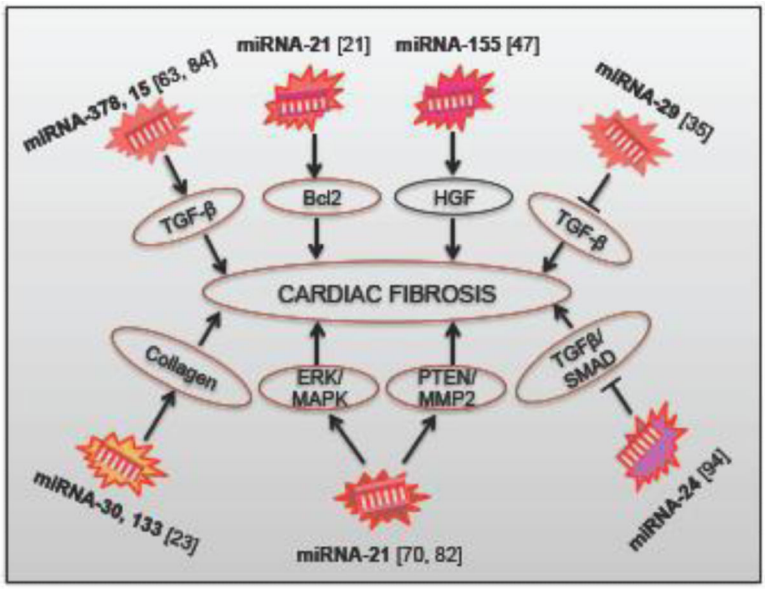

Figure 6. The role of miRNAs in the development of cardiac fibrosis

HGF - Hepatocyte growth factor; Bcl2 - B-cell lymphoma; TGFβ - Transforming growth factor beta; Erk – Extracellular signal- regulated kinase; MAPK - Mitogen-activated protein kinases; - Matrix metalloproteinase; PTEN - Phosphatase and tensin homolog.