5 RNA Binding Proteins and Non-coding RNA's in Cardiovascular Diseases

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Abstract

Cardiovascular disease (CVD) is the leading cause of mortality as well as morbidity worldwide. The disease has been reported to be chronic in nature and the symptoms of the disease worsen progressively over a long period of time. Inspite of noteworthy achievements have been made in the therapy of CVD yet the available drugs are associated with various undesirable factors including drug toxicity, complexity, resistance and many more. The versatility of RNAs makes them crucial therapeutics candidate for many human diseases. Deeper understanding of RNA biology, exploring new classes of RNA that possess therapeutic potential will help in its successful translation to the clinic. Understanding the mode of action of various RNAs such as miRNA, RNA binding proteins and siRNA in CVD will help in improved therapeutics among patients. Multiple strategies are being planned to determine the future potential of miRNAs to treat a disease. This review embodies the recent work done in the field of miRNA and its role in cardiovascular disease as diagnostic biomarker as well as therapeutic agents. In addition the review highlights the future of miRNAs as a potential therapeutic target and need of designing micronome that may reveal potential predictive targets of miRNA-mRNA interaction.

Keywords

Cardiovascular disease (CVD) · RNA Binding Proteins (RBPs) · Aptamer · microRNA

1 Introduction

Cardiovascular diseases (CVDs) have been found to be the leading cause of mortality around the globe. More than 80% of deaths are as a result of CVD, Ischemic heart disease and stroke. On an average, 235 per 100,000 deaths were reported to be due to the CVDs worldwide. In India the number is higher in comparison to other countries. The Global Burden of Disease has been estimated to be 272 per 100,000 population in India [1]. Among Indians, early disease onset, accelerated build-up and increased fatality have been observed. Numerous factors have been reported to be involved in the pathogenesis of the disease. These factors include both modifiable and non-modifiable factors such as genetic factors, lifestyle, obesity, excessive tobacco use, low fruit

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and vegetable intake etc. Moreover, optimal therapy is not being received by the individuals from lower socioeconomic backgrounds frequently which have led to poorer outcomes. In the vicinity of poorer outcomes, such epidemic diseases can be counteracted by developing the strategies that includes formulation and effective implementation of evidence-based policy, reinforcement of health systems, as well as emphasis on prevention, early detection, along with treatment with the use of both conventional and innovative techniques. Most of such strategies are being tested in undergoing community-based studies. Among these studies, non-coding RNAs such as miRNA, aptamers and RNA binding proteins (RBPs) are also being explored for early detection of disease and good outcome among patients with cardiovascular disease [2].

The eukaryotic genome is comprised of both protein coding and non-protein coding DNA. Although there has been much agreement that a small fraction of these genomes has important biological functions, but it is still debatable that how the rest of the genome is contributing to the body. Hangauer et al. [3] demonstrated the fact that 85% of the human genome is actively transcribed into non-coding RNAs [3]. Due to the ambiguity, much of the speculation is centred that low level of DNA is transcribed into actual coding RNA yet the other non-coding RNA is arbitrarily assigned with various names such as micro RNA, silencing RNA, Long non coding RNA etc. RNA has become a spotlight of attention for developing novel therapeutic schemes and hence variety of therapeutic strategies is being coming into the picture that includes RNA interference, use of aptamers and role of microRNA (miRNA) that can alter the complex gene expression patterns [4]. The versatility of RNAs makes them crucial therapeutics candidate for many human diseases. Deeper understanding of RNA biology, exploring new classes of RNA that possess therapeutic potential will help in its successful translation to the clinic. Understanding the mode of action of various RNAs including long non-coding RNAs (lncRNAs), miRNA, siRNA, etc. in CVD will help in improved therapeutics among patients [5].

It is due to the fact that RNA offers various advantages in disease management as it can be edited and modified in its various forms such as secondary and tertiary structures. Although scientists are in process of manufacturing RNA-targeting therapies using variety of endogenous gene silencing regulators, Small interfering RNAs (Si RNAs), aptamers and microRNA for cardiovascular diseases yet the development of a novel, risk free therapeutic strategy is a major challenge and need of the hour in cardiovascular medicine [6]. Moreover, it has been observed that multiple cell types are being comprised by cardiovascular system which helps to amend the phenotypic response to any acute or chronic injury. The cellular phenotypic changes are controlled by various proteins such as RNA binding proteins (RBPs) and non coding RNAs such as miRNA etc. [7] which ultimately determine cardiovascular health and disease. While performing phenotypic conversions, RBPs are known to establish an impact on mRNA fates which is further responsible for mediating transcriptional/post-transcriptional modification. Similarly, it is well documented that an individual non coding RNA has capability to influence hundreds of transcripts and ultimately to affect complex programs of gene expression and thereby affecting the overall genotypic or phenotypic expressions of a cell [8, 9]. Moreover, it has been predicted that miRNA is a key player in regulating various cellular processes including cardiovascular development. It is pertinent to mention that in recent years RNA therapeutic strategies such as RNA binding proteins, miRNA, aptamers etc. are upcoming and witnessing huge progress in the field of cardiovascular diseases [10, 11]. The present manuscript has been compiled to summarize various approaches of RNA binding proteins and non-coding RNA in prognosis, diagnosis and therapeutics of cardiovascular diseases.

2 RNA and Its World of Therapeutics

Non coding DNA accounts for 98.5% of human genome. This non-coding DNA is transcribed to a wide range of functional RNA species widely called non coding RNAs [12]. These are classified into three different classes called small around19–25 nucleotides, intermediate-sized around 20–200 nucleotides and long around 200 nucleotides. It has been observed that the actual number of the non-coding RNA within the genome is unknown but the structure as well as function of them is being revealed using various bioinformatics software [13]. Many of the non-coding RNAs are not validated for their function and considered to be product of spurious transcription but as per current studies these are found to be highly potential biomarkers for CVD. In addition these so called spurious transcripts are emerging as next frontiers in the drug discovery.

According to the central dogma, different types of RNA passively convert the information encoded in the DNA to polypeptides via replication followed by transcription and translation respectively [14]. For such functions, RNA is not alone rather different types of RNA associate them with diversity of proteins from the site of transcription i.e. nucleus to the outreaches i.e. cytoplasm. These proteins are classified under the category of RNA binding proteins that are helpful in performing various tasks such as transport, localization, translation and stability of mRNAs or other types of RNAs. In addition, these are the key factors to play important role in communication of crucial information to the translation machinery for critical surveillance of any kind of mutations. Hence RBPs possess the entire responsibility to shape the gene expression of a cell at multiple centres. In addition, it is well known fact that RBPs are keen factors in development, in various physical and chemical changes as well in context to development of heart. According to different studies, it has been demonstrated that RBPs have been placed in some specific stages of heart development and are involved in almost all stages of cardiogenesis such as formation, morphogenesis and maturation of heart [15]. The deeper insights into the function of RBPs may give rise to some specific targets that may provide attractive, novel targets for the prognosis, diagnosis and treatment of various heart diseases.

3 RNA Binding Proteins and Their Characteristics

RNA-binding proteins (RBPs) are very important interaction partners for all cellular RNAs. These proteins have been reported to regulate RNA processing at numerous levels including alternative splicing, mRNA stability, mRNA localisation and translation efficiency. The activities of RBPs in nucleus and cytoplasm are regulated through their cellular compartmentalisation. In the nuclear compartment, they function as splicing regulators often during development. In the cytoplasm, RBPs function in the regulation of mRNA localisation, mRNA stability and regulates the translational efficiency of mRNA. RNAs associate with RBPs to form dynamic ribonucleoprotein particles (RNPs) for execution of RNA function [16, 17]. Nascent pre-mRNA are covered with myriad of RBPs that collectively form RNPs. Previously, it has been reported that human genome encodes more than 700 RBPs. These proteins interact with the target mRNAs at 3′- and 5′- untranslated regions, intronic and exonic regions. Numerous sequencing-based RBP foot-printing studies have reported intricate and combinatorial interactions between RNA and RBPs [18]. Some of the general feature of RNA binding proteins are compiled and shown in Fig. 5.1.

4 RNA Binding Proteins in Cardiovascular Diseases

The function of RBPs can be disrupted in the disease. The competition for access of target mRNA by RBPs is determined by their expression level in a healthy and diseased individual. These can be either primary cause of the disease or a

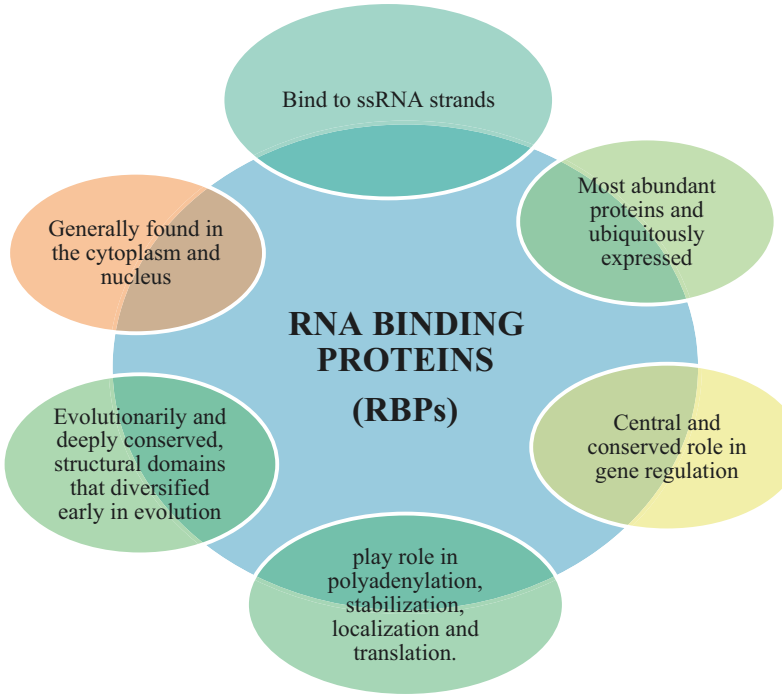

Fig. 5.1 Schematic representation of characteristics of RNA binding proteins

consequence. RBPs including quaking, HuR, muscle blind and SRSF1 have been found to be the major key players in cardiovascular disease. Homozygous alterations in these RBPs are reported to be associated with cardiac and vascular complications. These are crucial for maintaining mRNA transcript abundance and its translation into mature proteins.

Various studies have demonstrated the importance and co-ordinating role for RBPs in foetal, juvenile, and adult hearts. In addition, these studies have also demonstrated that how altered RBP levels can impact cardiac function in health and disease. During diseased conditions the expression of RBPs is varied that leads to defective splicing and causes translation of defective protein. Splicing defects can lead to heart dysfunction. Major RBPs that are associated with spliceosome and cardiovascular disease risk include Troponin T, SERCA2a/b and CETP [19, 20]. In addition, it as been observed that Celf1 and Muscleblind1 (MBNL) are associated with postnatal splicing for the effective organization of transverse tubules and calcium handling. However, Serine/Arginine rich splicing factor (SRSF1) has been found to guide the splicing pattern for maintaining electrical conductivity among cardiomyocytes during juvenile to adult transition. Another RBP of interest is RBM20 which is highly expressed in human heart. Even single nucleotide polymorphism (SNP) in exonic region of RBM20 results in increased risk of DCM due to altered expression of this RBP. It affects ion homeostasis, sarcomere organization and diastolic function including titin, tropomyosin I, PDZ and LIM domain 5. A variety of RNA binding proteins are engaged in different role and are helpful in the alleviation of CVD [21]. The availability of various RNA binding proteins and their role in CVD is compiled in Table 5.1.

RBPs have been reported to associate in repair of damaged vessels during vascular injury. For

Table 5.1 Various RNA binding proteins and their role in CVD

RBPs	Disease	Diagnostic criteria
Muscleblind1 (MBNL1)	Cardiomyocytes	Regulation of voltage gated channels responsible for cellular expression and splicing of the SCN5A, a voltage-gated sodium channel [22]
Poly (rc) binding protein (PCBP2)	Hypertrophy of cardiomyocytes	Inhibit hypertrophy of cardiomyocytes which is induced due to angiotensin II as it is responsible for degradation of GPR56 mRNA degradation [23, 24]
CIRP	Cardiac diseases	Enhances the translation of essential ion channel subunits Loss of CIRP results in defective voltage-gated potassium channel function and diminished bioelectric activity in mammalian hearts [25].
SRSF2	Cardiomyopathy	Extensive fibrosis, myofibril disarray, dilated cardiomyopathy evident after 5 weeks, decreased ventricle muscle contractility due to loss of this RBPs

the initiation of repair, RBPs co-ordinate important splicing events of mRNA of SM-myosin heavy chain, myosin light chain kinase, smoothelin, tropomyosin, metavinculin, calponin, and caldesmon. The actual procedure of repair used by these RBPs is largely unknown. Moreover, the impact of other RBPs has been found on expression of eNOS, enzyme involved in the synthesis of Nitric oxide (NO) by endothelial cells (EC) which triggers vasoconstriction. Experimental studies have been carried out to investigate the function of RBPs in human ECs. RBPs impact eNOS biology through hnRNP L, a protein that co-ordinates eNOS pre-mRNA alternative splicing that results in generation of a truncated, dominant negative eNOS isoform [26, 27]. Although evidences indicate that these alternative truncated eNOS isoforms affect NO production but the pathophysiological relevance in context with EC function in patients with CVD is unknown till date.

In addition, RNA-binding proteins have also been implicated in the posttranscriptional regulation of various other vital EC-derived factors, including VEGF, endoglin and HIF1a [28]. An isoform of RBP76/DRBP76/NF90 interacts with the 30 UTR of the VEGF mRNA and enhances VEGF production by human ECs whereas alterations in SRSF1 levels in senescent ECs alters splicing of VEGF and endoglin pre-mRNA [29, 30].

5 Micro RNAs and Its Biogenesis and General Properties

miRNA are considered to be potential post-transcriptional regulators and plays a vital role in gene expression. On an average, nearly 1000 miRNAs are encoded in the human genome and these are originated from various non-coding DNA/RNA regions. Depending upon the genomic location as well as gene structure, miRNA are classified as intergenic, intronic and exonic miRNA. According to this, more than 50% of the miRNA are located in the intergenic regions. The intergenic miRNA may exist either as single gene or may be as cluster of genes under the control of one promoter whereas intronic miRNA are located in introns of annotated genes that may be encoded from coding or non-coding genes. Exonic RNAs are originated from the overlapping region across an exon or intron of non-coding genes.

The biogenesis of miRNA involves various steps that convert a pre mature pre-miRNA to a mature miRNA. There is involvement of various

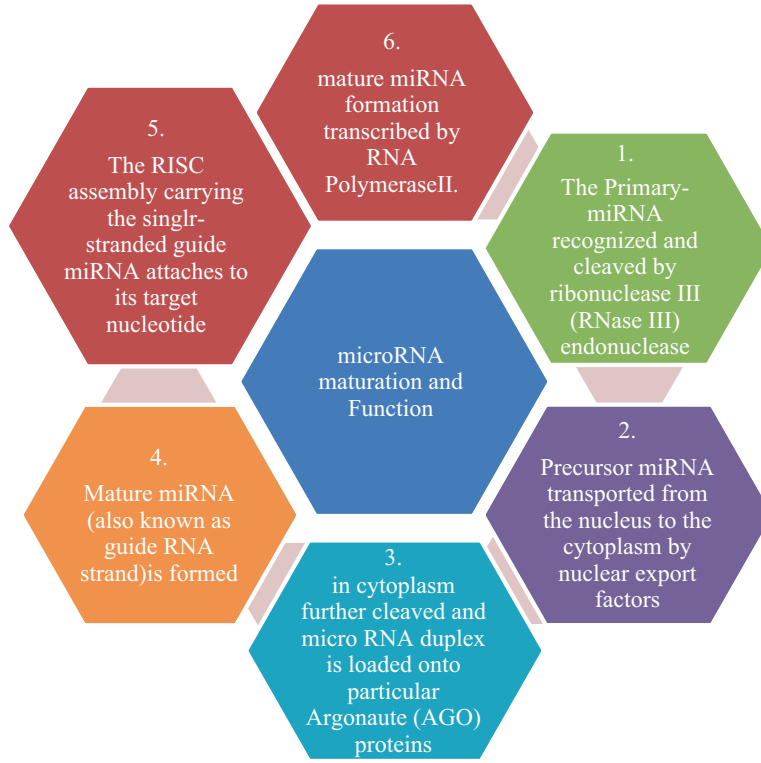

Fig. 5.2 Representation of biogenesis of miRNA

RNAs, RNA binding proteins and enzymes as well. The step by step process of miRNA biogenesis is shown in Fig. 5.2.

Structurally, miRNA are endogeneous, a class of highly abundant RNAs and are short nucleotides of length 19–22 nucleotides approximately. miRNA are endogenous but an exogenous miRNA can also be introduced into a cell by a viral vector that encodes pre-miRNA or by other synthetic vehicle carrying synthetic pre-miRNA or miRNA [31, 32]. The general properties of the miRNA are shown in Table 5.2.

6 miRNA in Cardiovascular Diseases

Recent advances in the field of miRNA have revealed its importance in numerous human diseases including coronary heart disease [33]. Deletion of specific region of dgcr8 gene, required of miRNA production in cardiomycotes results in ventricular malfunction and premature lethality [34]. Another miRNA, miRNA-21 (miR-21) has been found to be involved in patho-

Table 5.2 Representation of general properties of miRNA

Features	Property of miRNA
Prior to processing	Originated from a precursor miRNA (pre-miRNA) that contains 70–100 nucleotides with hairp in structure
Structure	19–25 nucleotide RNA duplex with 2 nucleotides 3′ overhang
Complementary	Partially complementary to mRNA, typically targets the untranslated mRNA regions at 3′ end
mRNA target	Multiple targets varying from 1–100 at a same time
Mechanism of gene regulation	Translational repression of mRNA, rarely endonucleolytic cleavage of mRNA, degradation of mRNA, mRNA de-adenylation and mRNA sequestration
Clinical applications	Potential biomarkers, diagnostic tools, therapeutic agent and drug target

genesis of cardiac fibrosis by upregulating the ERK-MAP kinase signalling pathway in cardiac fibroblasts [35]. Repression of miR21 significantly improves cardiac outcome. In addition, miRNA has also been reported to be involved in cardiac fibrosis [36]. Increased levels of miR21 have been found to mitigate fibrosis as they reduce collagen production. Similarly, decreased levels of miR-29 after MI induced production and deposition of collagen fibers. miRNAs are crucial for other regenerative processes in the heart [37]. Collectively, miRNA or anti-miR delivery has great therapeutic potential for a variety of diseases [38]. miRNAs based therapeutics in cardiovascular diseases can be used via different strategies. A reverse association of some miRNA and good outcome has been detected in patients with cardiovascular disease. It has been found that a decoy against miR-24 to reverse its inhibition on angiogenesis improves cardiac function in a mouse MI model [39]. Embryonic stem cells that overexpress miR-1 (ESCs) improved stem cell differentiation into cardiomyocytes and reduced levels of apoptosis following injection in the infarcted heart. miR-126 is expressed in mesenchymal stem cells and it improves angiogenesis and overall cardiac function in infracted myocardium. Further, treatment of infarcted heart cells with lentiviruses encoding miR-1/133/208/499 enables direct in vivo conversion of cardiac fibroblasts into cardiomyocyte-like cells in the infarcted heart [10]. A variety of miRNA are engaged in different role and are helpful in the alleviation of CVD either acting as diagnostic biomarker, therapeutic agents or as drug targets. The availability of various miRNA as biomarker reported in various studies is compiled in Table 5.3.

7 Commonly Expressed miRNA in Cardiovascular Diseases

miR-21 is first mammalian RNA identified and is one of the most commonly studied miRNA in a CVD. It is abundant in vessel wall and differentially expressed upon shear and mechanical stress to the vessel. Among humans, it is expressed in podocytes, dendritic cells and CD14+ monocytes [47] of normal cells rather highly expressed in various cancers and CVD [48] and human atherosclerotic plaque [49]. It is dynamically regulated in various pathological processes i.e. cell sur-

Table 5.3 Role of various miRNA as biomarker in cardiovascular diseases

miRNA	Disease	Diagnostic criteria
miR-122	Hyperlipidemia	Serves as a primary regulator of lipid biosynthesis. Aberrant levels is an indication of coronary artery disease (CAD) [40, 41]
miR-126, miR-9	Hypertension, CHF (cardiac heart failure), stroke	Regulation in vascular integrity and angiogenesis indicates disease [42, 43]
miR-143/145	Hypertension, CAD, stroke	Macrophage differentiation and polarized activation processes indicates disease [10]
miR-1254, miR-423, miR-30d	Chronic heart failure (CHF)	Increased level of miR-1254 represents the disease whereas miR-423 and miR-30d levels are decreased in CHF
miR-21, miR-210, miR-423, miR-1, miR-26b	Heart failure (HF)	Increased level of miR-21, 210, 423,1 and 26b represents the heart failure.
miR-1306, miR-30d, miR-126, miR-423, miR-18a	Acute heart failure (AHF)	Increased level of miR-1306 and mi-30d represents the disease whereas miR-126, miR-423 and miR-18a levels are decreased in acute heart failure
miR-30c, miR-146a	Heart failure with preserved ejection fraction	Decreased level of miR-1306 and mi-30d represents the heart failure with preserved ejection fraction disease
miR-26a	Cardiovascular repair; acute coronary syndromes	Up regulation in the patients' plasma [44]

(continued)

Table 5.3 (continued)

miRNA	Disease	Diagnostic criteria
miR-16, miR-27a, miR-101, and miR-150	Left ventricular contractility (LV)	Downregulation of miR-101 or miR-150 and upregulation of miR-16 or miR-27a correlate with higher risk of impaired LV contractility [45]
miR-1, miR-134, miR-186, miR-208, miR-223, and miR-499	Angina pectoris	Up regulation in serum samples [46]

vival, apoptosis and cell invasiveness [50]. Studies have reported that overexpression of miRNA21 in endothelial cells inhibits the expression of Peroxisome Proliferator-Activated Receptor-alpha (PPARα) that further results in increased expressions of VCAM-1 and MCP-1 [51]. Hydrogen peroxide and lipopolysaccharides alter the expression of various miRNAs, including miRNA21 in endothelial cells [52].

In addition, miR-155 is a "immuno-miR," with a pivotal role in both innate and adaptive immunity. It is second most commonly studied miRNA in atherosclerosis and hypertension. It is significantly expressed in hematopoietic stem cells and promotes B cell-related immunoglobulin production, T cell proliferation in response to antigen as well as cytokine production [53]. Various studies confirm that mineralocorticoid plays an important role in stimulation of hypertension. It has been observed that high serum levels of miR-155 in response to mineralocorticoid are available in the patient exhibiting greater reduction in systolic blood pressure. In contrast, levels of miR-155 were detected to be dramatically low in aorta of aged white mouse. In a cross sectional study of 932 Chinese patients, a negative co-relation was observed among plasma levels of miR-155 and severity of coronary atherosclerosis [54]. Moreover, miR-155 is found to be pro-inflammatory and predictive of worst outcome in patients with atherosclerosis.

miR-146 is another "immuno-miR" that primarily functions in innate immunity and negatively regulate the production of pro-inflammatory cytokines (Perry et al. 2008). As per a study of 50 patients, elevated expression of miR-146a, miR-146b and miR-21 was observed in plaque boarded arteries in comparison to normal vessels [50]. Moreover, it acts a potential mediator through which Apo E suppresses myeloid cell inflammation i.e. NF- κB activation [55]. Apo E is associated with normal physiologic removal of circulating triglyceride-rich particles e.g., VLDL. A strong anti-inflammatory and atheroprotective role of miR-146 family has been evidenced in various studies.

miR-143 and miR-145 form a classical cluster on chromosome 5 as these two are present in close proximity. Although miR-143 and miR-145 are available as cluster but expression level of miR-143 was found to be more than miR-145 due to an unknown mechanism. In a cohort (cohort that exhibited hyper-homocysteinemia (Hhcy) without carotid atherosclerosis, Hhcy with carotid atherosclerosis, and carotid atherosclerosis (without Hhcy) study, higher expression of miR143/145 was observed in patients with Hhcy in comparison with healthy controls [33]. Moreover, as per Santovito et al. 2013, miR-145 was up-regulated in carotid atheromas from hypertensive patients in comparison of carotid atherosclerosis without hypertension [56].

Another prototypical immune miR i.e. miR-223 is highly expressed in myeloid cells. Downregulation of this miR is required for monocyte-to-macrophage differentiation [57] but miR-223 was found to be elevated in the visceral adipose of obese humans in the absence of hyperlipidemia and hypertension [57, 58]. A positive correlation was observed in increased miR-223 levels and the incidence of acute ischemic stroke [58]. Increased expression of miR-223 maybe because of hypomethylation of the miR-223 promoter and an increased hypomethylation of promoter region of miR223 was observed in atherosclerotic cerebral infarction patients. Apart above explained examples, a variety of miRNA are engaged in in the alleviation of CVD either as therapeutic agents or as drug targets. The availability of various miRNA as therapeutic agents and drug targets are compiled in Table 5.4.

Table 5.4 Role of various miRNA as therapeutic agent in cardiovascular diseases

miRNA	Therapeutic agent	Mechanism of action
miR-121	Hyperlipidemia	Reduces plasma cholesterol levels by 1. Repressing mRNA targets by binding to other regions including 5′ UTRs or protein-coding exons 2. Imperfect base pairing to the 3′ untranslated regions (3′ UTR) of messenger RNAs (mRNAs) thereby inducing repression of the target mRNA [59, 60]
miR-33	Atherosclerosis	Raised HDL and induced regression of atherosclerotic plaques by using 2′ F/MOE-modified anti-sense oligonucleotide; anti-miR-33 lentivirus [61, 62]
miR-34a	Myocardial infarction	Improve systolic pressure and increase angiogenesis and Akt activity with the use of LNA-anti-miR-34a [63]
miR-208	Obesity, diabetes, metabolic syndrome	Provides resistance to high-fat diet-induced obesity, improves systemic insulin sensitivity and glucose tolerance by imperfect base paring with the use of MGN-9103 (LNA modified anti-sense oligonucleotide) [64]
miR-29	Atrial fibrillation	Deregulation of miR-29 followed by targeting of mRNAs encoding fibrosis-promoting proteins by regulating genes involved in cardiac fibrosis and apoptosis [65]
miR-133 miR-30	Cardiac fibrosis	Direct interaction of both′ UTR of CTGF and down-regulate its expression followed by decreased production of collagen
miR-30	Myocardial infraction	Increased expression in MI and decreased expression in cardiac hypertrophy. In MI, it regulates several ion channel genes including gap junction protein alpha 1 (GJA1) that encode connexin 43, calcium channel beta-2 (CACNB2) etc.
miR-195	Cardiac hypertrophy	Up-regulated during cardiac hypertrophy. It regulates sodium channel (SCN)5A that encodes cardiac Na^+ channel etc.

8 Ongoing Clinical Trials in the Field of miRNA as Their Potential Role in CVD

Micro RNAs are considered as potential biomarkers, drug targets and novel therapeutic agents in cardiovascular disease. Their diagnostic value has been evaluated in various studies and hence these are emerging as novel drug targets, therapeutic targets with respect to coronary artery disease (CAD) and myocardial infarction (MI). As per current state of art, a number of clinical trials are being conducted on variety of miRNA of potential use. The first promising in vitro results are raising hope for future clinical application. A list of ongoing clinical trials in the field of cardiovascular diseases using various miRNA targets has been compiled in Table 5.5.

9 FDA Approved miRNA Drugs

Though the field of miRNA and its role in various diseases is not yet fully unfolded but the researchers are desperate and making critical steps for seeking approval for newly manufactured/to be manufactured new miRNA based medicines from

Table 5.5 Representation of list of ongoing clinical trials in the field of cardiovascular diseases using various miRNA targets [66]

Identification number	Recruiting status	Type of study	Conditions of diseases	Treatment	No of enrolments	Study completion date
NCT03792607	Recruiting	Observational	Type 2 diabetes mellitus Cardiovascular diseases	Mi RNA and Methylome	35	June 14, 2020
NCT03635255	Recruiting	Observational	Adverse cardiovascular	Mi RNA	450	September 21, 2020
NCT03395041	Recruiting	Observational	Coronary stenosis Periodontal diseases Acute coronary syndrome Non-ST elevation Myocardial infarction Unstable angina acute myocardial infarction Atherosclerosis Atheromatous plaques	Cardiac imaging tests	100	June 1, 2021
NCT03391908	Recruiting	Observational	Coronary stenosis Periodontal diseases Acute coronary syndrome Non-ST elevation Myocardial infarction Unstable angina acute myocardial infarction Atherosclerosis Atheromatous plaques	Cardiac imaging tests	100	January 2021
NCT03875495	Recruiting	Interventional (clinical trial)	Multiple myeloma	Temferon	9	March 2023
NCT03474614	Recruiting	Interventional (clinical trial)	Cerebral cavernous malformations	Propranolol	20	August 30, 2020
NCT03430583	Recruiting	Observational	Single ventricle heart disease	MZ101	100	December 31, 2020
NCT02267200	Completed	Observational	Hypertension,Pulmonary		100	September 2016
NCT02176395	Recruiting	Interventional (clinical trial)	Acute stroke	Danhong Placebo	46	June 2019

Table 5.6 List of miRNA based FDA approved drugs to be used for cardiovascular diseases

miRNA	Disease	Detection approach	References
miRNA -208a/b, miR-499	Acute myocardial infarction (AMI) and myocardial injury	Microarray and real time PCR	[66]
miRNA423-5p	Heart failure	Microarray and real time PCR	[67]
miR-328	Atrial fibrillation (adverse electrical remodelling)	Microarray and real time PCR	[68]
miR-1	AMI	Real time PCR	[69]
miR-26a	Cardiovascular repair, acute coronary syndromes	Real time PCR	[70]
miR-16, miR-27a, miR-101 and miR-150	Left ventricular contractility	Real time PCR	[5]
miR- 133	AMI and coronary artery stenosis	Real time PCR	[42]
miR-126, miR-17/ miR-92a, miR-155	Coronary artery disease	Microarray and real time PCR	[71]
miR-126	Congestive heart failure	Real time PCR	[46]
miR-203, miR-223, miR-499, miR-1, miR-134, miR-186	AMI and angina pectons	Deep sequencing	[48]
miR-21/590-5p family, miR-126, miR-451	Coronary artery disease	Microarray and real time PCR	[72]

US Food and Drug Administration (FDA). MiRNA has already made its way in the treatment of number of diseases and due to the positive outcomes various miRNA based drugs are entering to the market after seeking FDA approval. First anti-cancer miRNA-based drug, MRX-34 (a liposome-based miR-34 mimic) developed by Mirna Therapeutics came to the clinic in 2013 for the treatment of hepatocellular carcinoma (mirnarx.com; NCT01829971). The FDA approval for few of the miRNA has led the field to the new heights and it seems more promising that miRNA may contribute the disease alleviation by acting as a diagnostic as well as therapeutic modality. Few of such FDA approved drugs are enlisted in Table 5.6.

10 Future Prospects

miRNA and RNA binding proteins are two complex components of gene expression. Discovery of novel miRNAs and their utility in disease diagnosis and prognosis is highly appreciable that has been resulted as component of large and simultaneous research work of various branches such as molecular biology, biotechnology, bioinformatics, clinical-trial design, epidemiology, statistics as well as health-care economics. A number of miRNA biomarkers have been presented in various studies and these are emerging due to their attractive advantages over other molecular therapeutics such as small size along with conserved sequences and their stability in the body fluids. Such advantages and current achievements in the field of RNA are portraying a promising future for miRNA-based therapeutics in disease diagnosis and disease prevention as well. Although a huge amount of data has been gathered and evidencing the use of RNA in therapeutics but still the scientists has scratched the surface of the complex gene expression. Much more is yet to be done in the future with respect to miRNA and elucidating its potential. For implementation of this approach few new strategies needs to be designed for their effective delivery as well as several obstacles including their stability, renal clearance, off-target effects, inefficient endocytosis by target cells or the immunogenicity of delivery vehicles, need to be overcome. The RNA therapeutics have been depicted using various preclinical studies involving small animals rather involvement of large animals and patients will further explore their efficacy in future. In addition, development and improvement of RNA-based therapeutics requires robust design of the

RNA agents to avoid adverse effects and optimal delivery strategies to maximize their benefits.

Moreover, miRNA is targeting the mRNA which is structure specific as well as sequence specific. According to various bioinformatics based software, multiple databases are there which stores predicted microRNA to mRNA target relationships. These relationships are computed using diverse algorithms. Prediction databases generally compare the in vitro data to the data generated by bioinformatics tools which ultimately results in microRNA to mRNA transcript interactome generally referred as micronome. Now a days, micronome is developed to study the involvement of miRNA in well known signalling pathways and its role in diseases as well. The development of miRNA based micronome in Cardiovascular diseases will significantly improve the understanding of their involvement and the generation of novel therapeutic as well as drug targets in the field of RNA therapeutics in cardiovascular diseases.

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