Pathological microRNAs in acute cardiovascular diseases and microRNA therapeutics

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ABSTRACT

Cardiovascular diseases are one of the leading causes of morbidity and mortality. In recent researches, it is demonstrated that microRNAs (miRNAs) are expressed extensively in cardiovascular system and regulate gene expression in various cardiovascular diseases. Here, we are giving overview on number of miRNAs involved in pathophysiology of various cardiovascular diseases, and diagnostic and therapeutic potentials of miRNAs in these diseases. MiRNAs are a group of small non-coding mRNAs with approximately 18–22 nucleotides in length that regulate gene expression post transcriptionally. MiRNAs are regulated in various cardiovascular diseases like hypertension, congestive heart failure, congenital heart defects, coronary artery disease and stroke. Some of these miRNAs also act as potential biomarker of these cardiovascular diseases. Inhibition of these miRNAs via different approaches like chemically modified antisense oligonucleotide, antagonirs, and locked nucleic acids serves as effective approaches for inactivating pathological miRNAs. Clinical trials are being conducted on therapeutic and diagnostic potentials of miRNAs. However, extensive researches are required to explore the therapeutic and diagnostic values of miRNAs as successful as classical approaches.

1. Introduction

The heart is among the most susceptible organs to birth defects. About 1% of newborns suffer with heart malformation[1]. Moreover, one of the leading causes of morbidity and mortality in adults is cardiovascular disease[1,2]. According to the report of Centers for Disease Control and Prevention and the National Health and Nutrition Examination Survey III, about 47% of death is contributed to cardiovascular diseases. Cardiovascular diseases include hypertension, coronary artery disease (CAD), congestive heart failure (CHF), congenital cardiovascular defects and stroke. The physiological and pathological process of heart brings alteration in gene expression[3]. MicroRNAs (miRNAs) have recently emerged to exhibit powerful and unexpected role in various cardiovascular diseases[4,5]. MiRNAs are a group of small non-coding mRNAs with approximately 22 nucleotides[6]. MiRNAs are encoded with different types of genes and the ones having major portions are called intergenic miRNAs and intragenic miRNAs. RNA polymerase II is responsible for transcription of miRNA and forms long primary miRNAs (pri-miRNAs)[6,7]. These pri-miRNAs are processed by nuclear RNAase endonuclease III (Drosha) which forms precursor miRNAs (pre-miRNA) containing 60–100 nucleotides. Drosha is associated with DGCR8 (co-factor of Drosha) and the combination of Drosha and DGCR8 is called “microprocessor”. It may be indicated that these two play a significant role in miRNA processing[8]. The pre-miRNAs formed from intergenic miRNAs are moved out of nucleus to cytoplasm with help of exportin-5. In cytoplasm, RNAase endonuclease III (Dicer) further carries out processing of pre-miRNA to generate mature miRNAs which contain around 22 nucleotide units[9].

Development of heart begins with the formation of two endocardial tubes. Fusion of these two endocardial tubes leads to formation of primitive heart tube that septates into four chambers and paired arterial trunks to form adult heart[10]. Different
miRNAs are involved at different stages of cardiac development. For example, miR-218 promotes heart field fusion and transforms cardiac crescent to linear heart tube, miR-138 promotes heart looping and atrioventricular canal development from linear heart tube, miR-1, miR-133 and miR-17-92 clusters carry out embryonic heart maturation and septation, and miR-15 promotes post-natal heart growth and maturation[14]. Cardiac muscle is enriched with miR-1, and it accounts for approximately 40% of all miRNAs in myocardium[15]. In vertebrates, miR-1 and miR-133 originate from common bicusistronic transcript[16], and clusters of both i.e. miR-1-1/133a-2 and miR-1-2/133a-1 are highly expressed in myocardium[16]. MiR-15, miR-133, miR-199, miR-590 are involved in cardiomyocyte differentiation. MiR-133, miR-1, miR-499 and miR-208 are involved in stem and progenitor cell differentiation. MiR-133, miR-1, miR-499 and miR-208 are involved in direct reprogramming i.e. fibroblast to myocytes[15]. Cardiac progenitor cell differentiation is enhanced by expression of miR-499[15]. Myocardial progenitor differentiation is also found to be enhanced by miR-17-92 clusters[16]. Similarly, miR-138 is involved in patterning of chambers and valve region[17]. The importance of miRNAs in cardiogenesis is also revealed by germline deletion of miR-1-2 and miR-126 resulting in ventricular septal defects and vascular leakage, respectively[18]. MiR-21 appears to be upregulated in hypertension and miR-126 levels. Levels of both these miRNAs were lowered in hypertensive patients compared to healthy control[20,21].

In another research, it was found that 46 miRNAs were expressed in hypertensive patients compared with healthy individuals, among which 27 miRNAs were further detected (Table 1). Among these 27 miRNAs, 9 miRNAs were upregulated i.e. human cytomegalovirus-miR-UL112, miR-605, miR-623, miR-let-7e, miR-516b, miR-600, kshv-miR-K12-6-3p, miR-602 and miR-1252. About 18 miRNAs were down-regulated: miR-296-5p, miR-133b, miR-625, miR-1236, miR-518b, miR-1227, miR-615-5p, miR-18b, miR-1249, miR-324-3p, ebv-miR-BART17-3p, ebv-miR-BART19-5p, kshv-miR-K12-10a, kshv-miR-K12-10b, miR-4865p, miR-30d, miR-664 and miR-634 21. MiR-let-7a and miR-206 are known to be involved in vascular remodeling in hypertension[22].

Exercise is a highly recommended lifestyle for hypertensive patients. In research, it was found that exercise may alter the expression of specific miRNAs targeting RAS genes. Results in the same research demonstrated that decrease in miR-143 expression enhanced cardioprotective genes and miR-27 in heart which was an inhibitor of angiotensin converting enzymes[23].

Some miRNAs contribute to vascular remodeling which perpetuates hypertension[24], like miR-130a, which inhibits the growth arrest-specific homeobox and regulates vascular smooth muscle cells contributing to vascular remodeling in hypertension[25].

Pulmonary arterial hypertension which is characterized by elevated pulmonary artery pressure to levels in systemic circulation is also not away from alteration in some specific miRNAs. In a published research, it was shown that miR-22 and miR-30 levels were decreased whereas miR-322 and miR-451 were increased during the development of pulmonary arterial hypertension in hypoxic and monocrotaline model[26,27]. MiR-30 is known to play a role in extracellular matrix

## Table 1

<table>
<thead>
<tr>
<th>MiRNAs in cardiovascular diseases.</th>
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<tr>
<td><strong>MiRNAs upregulated</strong></td>
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<td><strong>Hypertension</strong></td>
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<td>Human cytomegalovirus-miR-UL112, miR-605, miR-623, miR-let-7e, miR-516b, miR-600, kshv-miR-K12-6-3p, miR-602 miR-1252, miR-27, miR-322, miR-451</td>
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<td><strong>Congenital heart disease</strong></td>
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<td>MiR-423-5p, miR-320a, miR-22, miR-92b, miR-122, miR-129-3p, miR-3155, miR-3175, miR-583, miR-568, miR-30d, miR-200a-star, miR-1979, miR-371-3p, miR-155-star, miR-5025p, miR-100</td>
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<td>**miR-296-5p, miR-133b, miR-625, miR-1236, miR-518b, miR-1227, miR-615-5p, miR-18b, miR-1249, miR-324-3p, ebv-miR-BART17-3p, ebv-miR-BART19-5p, kshv-miR-K12-10a, kshv-miR-K12-10b, miR-4865p, miR-30d, miR-664, miR-634, miR-9, miR-126, miR-143, miR-22, miR-30 Has-let-7e-5p, Has-miR-155-5p, Has-miR-222-3p, Has-miR-433, Has-miR-487b miR-421</td>
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remodeling in heart and hence is expressed in experimental model of pulmonary hypertension[27]. MiR-759 is also known to be involved in chronic thromboembolic pulmonary hypertension[28].

3. MiRNAs in CAD

Circulating miRNAs are biomarkers of CAD. Some miRNAs are increased while some are decreased. Endotheliually expressed miR-126, miR-92a, miR-17, miR-145, miR-155, miR-208 and miR-133a are decreased in CAD[29]. In a research, it was demonstrated that reduction of endotheliually expressed miRNAs may be attributed to uptake into atherosclerotic lesions within the vasculature of CAD patients[26,30]. Smooth muscle-enriched miR-145 was also reduced in patients with CAD. Cardiac muscle-enriched miRNAs, miR-133 and miR-208a, were increased in patients with CAD[30]. Patients with CAD have increased levels of miR-221 and miR-222 in endothelial progenitor cells (EPCs), and these miRNAs bring about mobilization of EPCs[26,30].

Apart from direct regulating condition of CAD, miRNAs are involved indirectly as well. For example, it was shown in study that oxidative stress defences in human EPCs are regulated by miR-21. The authors induced dysfunctioning in human EPCs by using asymmetrical dimethylarginine, an endogenous nitric oxide synthetase inhibitor, and they found that miRNA-21 was up regulated which led to extracellular signal regulated kinases-mitogen activated protein kinase-dependent reactive oxygen species production and EPC migratory defect, and also reduced NO bioavailability[31]. That leads to endothelial dysfunction with increased risk of cardiovascular disease with established CAD[22]. Antagonism of miR-21 improves dysfunctional angiogenic progenitor cells in CAD patients[33]. These findings suggested that antagonism of miR-21 can be considered as potential therapeutical target in CAD patients[30,33].

4. MiRNAs in congenital heart disease (CHD)

One of the most occurring congenital anomalies in newborns is CHD affecting about 1.35 million infants worldwide[34]. CHDs include ventricular septal defect (VSD), atrial septal defect, patent duc tus arteriosus, tetralogy of Fallot, pulmonary valve atresia, coarctation of the aorta and tricuspid atresia[35-38]. VSD is one of the most common CHDs. In a research, 36 miRNAs were found in patients with VSDs. Among these, 8 miRNAs were validated to be expressed between VSD samples and control sample. Among these eight miRNAs, seven were downregulated: has-let-7e-5p, has-miR-155-5p, has-miR-222-3p, has-miR-433, has-miR-487b and one upregulated: has-miR-498[39].

Tetralogy of Fallot is a type of CHD which involves four anatomical abnormalities of heart[39], i.e. pulmonary infundibular stenosis, overriding aorta, ventricular septal defect and right ventricular hypertrophy[40]. MiR-421 was found to be highly expressed in right ventricle of infants with tetralogy of Fallot. In a research, it was concluded that dysregulation of miR-421 in infant heart tissue had negative impact of SOX-4, a key regulator of Notch and Wnt pathway[40]. Activities of Notch and Wnt pathway are closely intertwined during embryonic development[41]. Hence, dysregulation of miR-421 contributes to congenital defects[40].

In a research, 52 target gene of 16 miRNAs, targets of hsa-miR-650 and targets of hsa-miR-650 showed the highest degree of association with CHDs[42].

HOX gene clusters are involved in cardiac septation to valve formation in different species. MiR-196a is expressed in HOX gene clusters in mammals and was found to upregulate HOXb8 in context of limb development. Furthermore, level of miR-196a was found to be increased in fetal heart sample as compared to adult one[43].

5. MiRNAs in CHF

In a research, 186 miRNAs were screened in serum of heart failure patients. Among these 186 miRNAs, four miRNAs i.e. miR-423-5p, miR-320a, miR-22 and miR-92b, were found to be increased in serum of heart failure patients. In the same research, significant association was revealed between miRNAs and prognostic parameters like elevated serum brain natriuretic peptide level, a wide QRS, and dilatation of the left ventricle and left atrium[44].

In a study, plasma from 12 CHF patients were compared with 12 healthy subjects by using microarray method, which revealed expression of 108 miRNAs in CHF patients. Among these 108 miRNAs, miR-423-5p was highly expressed in CHF. The endothelial-enriched miR-126 was negatively correlated with heart failure in patients. Also, decreased levels of miR-107, miR-139 and miR-142-5p were displayed in heart failure conditions. Interestingly, liver specific miRNA, miR-122, was reported to elevate in heart failure patients[45].

Changes in remodeling process, like left ventricular hypertrophy and dilatation, take place in patients with CHF. In a study, relationship between expression of miRNAs and electro gram parameters related to left ventricular mass index (LVMI) was investigated in patients with CHF. Results in this study demonstrated that 29 miRNAs were altered in CHF patients. Among these 29 miRNAs, three miRNAs, i.e. miR-182, miR-200a-star and miR-568, were found to have inverse correlation with LVMI, and two miRNAs i.e. miR-155 and miR-595 were found to have direct correlation with LVMI[46].

Another published research by using microarray profiling method in CHF patients showed upregulation of 18 miRNAs: miR-21, miR-4278, miR-650, miR744star, miR-516-5p, miR-1292, miR-182, miR-1228, miR-595, miR-663b, miR1296, miR-1825, miR-299-3p, miR-662, miR-122-star, miR-3148, miR-518e-star, and miR-2054, and downregulation of 11 miRNAs: miR-129-3p, miR-3155, miR-3175, miR-583, miR-568, miR-30d, miR-200a-star, miR-1979, miR-371-3p, miR-155-star, and miR-5025p[36].

Endo et al. focused his study on miR-210 which can be biomarker of CHF, since it is known to be induced by hypoxia. He concluded in his study that increase in plasma level of miR-210 represented discrepancy between the pump function of heart and oxygen demand in peripheral tissue and hence it can be a new biomarker for chronic heart failure[47].
A transgenic approach revealed that myocardial overexpression of miR-195 in mice was sufficient to induce pathological cardiac growth and heart failure. The expression of miR-100 is increased in the failing heart. Unlike miR-100, miR-92 is downregulated in heart failure.[48]

With the help of three mouse models of heart failure: adrenergic receptor transgenic mice, C57BL/6 mice undergone transverse aortic constriction, and C57BL/6 mice treated with isopropanol, Vettori et al. demonstrated that miR-21 was upregulated in heart failure.[49]

6. MiRNAs in stroke

Stroke is responsible for 10% of deaths worldwide and is one of the leading causes of disability. MiRNAs are involved in stroke risk factors including hypertension, atherosclerosis, atrial fibrillation, diabetes and dyslipidemia.[50]

MiRNAs like miR-21, miR-221 and miR-145 are known to be associated with cardiovascular system. On the basis of this, levels of these three miRNAs were evaluated in patients with stroke. Regression models containing serum miRNA level and risk factor were used for the prediction for stroke. Results in this study demonstrated that miR-21 and miR-222 were novel biomarkers of stroke but miR-145 were not.[51]

By real-time PCR technique, levels of miRNAs were quantified in 197 patients with ischemic stroke at interval of 24 h, 1 week, 4 weeks and 48 weeks. For control, 50 healthy volunteers were selected. Results showed that circulating levels of miR-301 and miR-126 were downregulated whereas let-7b was upregulated in ischemic stroke patients until 24 weeks. However, levels of these miRNAs were normalized 48 weeks after the symptom onset. Further, the researchers concluded that miR-30a, miR-126 and let-7b acted as biomarkers of ischemic stroke.[52]

In a study, it was reported that panel of 32 miRNAs was identified in stroke subtypes based on stroke patients’ blood miRNAs profile. Consistent upregulation of miR-125b-2, miR-27a, miR-422a, miR-488 and miR-627 was found during ischemic stroke. Thus, it was concluded that these miRNAs served with diagnostic value and reflected onset of ischemic stroke.[53]

Some miRNAs like miR-298, miR-155, and miR-362-3p may be altered more than 2 folds. Elevation of some miRNAs or miRNA mimics has been used as a factor by many scientists to treat experimental stroke. For example, lenti-miR-424 treatment decreased ischemic stroke. Similarly, insertion of miR-17-92 increased cell proliferation in stroke. MiR-223 has a therapeutic role in stroke.[54]

In a study, plasma levels of miR-107 and glutamate were elevated proportionally in patients with ischemic stroke. Hence, miR-107 plasma level may act as a biomarker for monitoring excitotoxicity with ischemic stroke patients.[55]

7. Therapeutic and diagnostic potentials of miRNAs

MiRNAs play a significant role in growth and development of heart. Also, miRNAs are expressed extensively in normal as well as diseased heart. Hence, miRNAs may serve as a potential diagnostic tool for monitoring cardiovascular diseases.[56] MiRNA targets differ from classical approaches of drug development. Since, classical approaches have high specificity for single targets whereas single miRNAs have multiple targets, and modulating the expression of single miRNA can influence an entire gene network.[57][58] Other challenges of using miRNAs as diagnostic and therapeutic tool are modes of delivery, specificity, toxicity, reversibility and regulation.[59]

Recently, many miRNAs serve to be biomarkers of cardiovascular diseases. For example, the circulating miR-1 is significantly higher in patients with acute myocardial infarction.[60] Similarly, plasma concentration of miR-423-5p is higher in heart failure patients but not in healthy people.[61] MiRNAs not only serve as diagnostic markers, but their expression can also be modulated by using anti- or mimic miRNAs in pathological conditions. For example, miR-21 mimic is demonstrated to impart the same neuroprotection as miR-21.[62]

Inhibition of miRNAs serves as effective technique for inactivating pathological miRNAs. Chemically modified antisense oligonucleotide and antagonirs are extensively used tools to modify miRNAs, and it is found to be an effective therapeutic approach for treatment of certain disease.[61] For example, infusion of an antagonist-145 that targets miR-145, decreases cortical infarcts.[63] Similarly, locked nucleic acids (LNAs) (analogs of RNAs) are also used for inhibition of miRNAs. For hepatitis-C treatment, a LNAs-based anti-miRNA was developed which specifically acted against liver specific miR-122, and this anti-miRNA can also be utilized in cardiovascular disease, since miR-122 also regulates plasma cholesterol level.[64]

Global miRNA research market is one of the most valued markets nowadays. According to BCC research report 2014, the global market for miRNAs research tools was valued at 478.8 million dollars in 2013 and is expected to reach 1 billion dollars in 2019.[65] Several companies involved in miRNA therapeutics are listed in Table 2.[66][67]

8. Conclusions

The review provides evidence that miRNAs play an important role in growth and development of heart as well as in different pathological conditions of heart. Several miRNAs are altered and act as biomarkers of these conditions while antagonism of some miRNAs via different techniques like LNAs and antisense oligonucleotide is required for prevention of these pathological threats. However, shortcomings are needed to be resolved in understanding miRNAs-based regulation of gene expression. For example, as mentioned earlier that single miRNA has multiple targets, hence, it is necessary but quite challenging to find out the exact mechanism and pathway followed by these miRNAs. Therefore, it is necessary to define roles of individual miRNAs and its important target, in different settings based on cell types and pathological implication. One more hurdle that needs consideration, is in-vivo delivery of miRNAs-based therapeutics. Thus, extensive researches on miR-
commercial therapy promise great future strategies aimed at treatment and prevention of cardiovascular diseases. However, many aspects in miRNAs-based diagnostic and therapeutic tool are still awaiting discovery to make it as successful as conventional therapy.

**Conflict of interest statement**

The authors report no conflict of interest.

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