HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Journal of Acute Disease

journal homepage: www.jadweb.org

Review article http://dx.doi.org/10.1016/j.joad.2015.08.001

Pathological microRNAs in acute cardiovascular diseases and microRNA therapeutics

Syed Salman Ali[1*], Chandra Kala[1], Mohd Abid[1], Nabeel Ahmad[2], Uma Shankar Sharma[3], Najam Ali Khan[1]

[1]*School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24) Moradabad, 244102, Uttar Pradesh, India*

[2]*Department of Biotechnology, IFTM University, Lodhipur Rajput, Delhi Road (NH-24) Moradabad, 244102, Uttar Pradesh, India*

[3]*Sir Madanlal Institute of Pharmacy, Alampur Hauz, Agra Road, Etawah, 206001, Uttar Pradesh, India*

ARTICLE INFO

Article history:
Received 3 May 2015
Received in revised form 5 May 2015
Accepted 2 Aug 2015
Available online 9 Oct 2015

Keywords:
Non-protein coding RNAs
Antisense oligonucleotide
Hypertension

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, antagomirs, 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

*Corresponding author: Syed Salman Ali, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad, 244102, Uttar Pradesh, India.
Tel: +91 8273540113
E-mail: salmanali.ali32@gmail.com
Peer review under responsibility of Hainan Medical College.

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[11]. Cardiac muscle is enriched with miR-1, and it accounts for approximately 40% of all miRNAs in myocardium[12]. In vertebrates, miR-1 and miR-133 originate from common biscistronic transcript[13], and clusters of both *i.e*. miR-1-1/133a-2 and miR-1-2/133a-1 are highly expressed in myocardium[14]. MiR-15, miR-133, miR-199, miR-590 are involved in cardiomyocyte proliferation. MiR-133, miR-1, and miR-499 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[11]. 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 expressed in valvular endothelium and is necessary for development of atrioventricular valve[19]. MiR-208a was found to be important in proper development of the cardiac conduction system[15].

Due to involvement of miRNAs at various levels of cardiac development, up regulation and down regulation of miRNAs via various mechanisms are known to be involved in cardiovascular diseases. This review emphasizes the involvement of different miRNAs in cardiovascular disease.

2. MiRNAs in hypertension

Several researches showed that miRNAs were known to influence the condition of hypertension directly or indirectly. In a research, 60 hypertensive patients and 29 healthy individuals were studied for assessment of miRNAs-9 and miRNAs-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 downregulated: 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 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[25,26]. MiR-30 is known to play a role in extracellular matrix

Table 1
MiRNAs in cardiovascular diseases.

Cardiovascular disease	MiRNAs upregulated	MiRNAs downregulated
Hypertension	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	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
Congenital heart disease	Has-miR-498, miR-196a	Has-let-7e-5p, has-miR-155-5p, has-miR-222-3p, has-miR-433, has-miR-487b miR-421
CHF	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	MiR-107, miR-139, miR-142-5p, miR-126, 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, miR-2054, miR-92
Stroke	Let-7b, miR-125b-2, miR-27a, miR-422a, miR-488, miR-627, miR-107	MiR-301, miR-126

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. Endothelially 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 endothelially 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-dependant 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[32]. 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 therapeutic 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 ductus 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[36].

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[34].

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[42].

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[43].

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[44].

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 cardio 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[45].

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[46].

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 miR423-5p 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 antagomirs 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 antagomir-145 that targets miR-145, decreases cortical infarcts[62]. 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[63].

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[64]. Several companies involved in miRNA therapeutics are listed in Table 2[65–74].

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-

Table 2
Commercial development in miRNA therapeutics.

Name of company	Product	MicroRNA target	Implication	Stage of development	Reference
Santaris Pharma	Miravirsen (SPC3649)	Inhibitor of miR-122	Hepatitis C	Phase II	[65]
Regulus therapeutics	RG-101	Inhibitor of miR-122	Hepatitis C	Expect to initiate Phase II	[66]
	RG-012	Inhibitor of miR-21	Alport syndrome	Expect to initiate Phase I	[67]
	RG-125	Inhibitor of miR-103/107	Non-alcoholic steatohepatitis with type 2 diabetes/prediabetes	Expect to initiate Phase I	[68]
	Not specified	Inhibits miR-133a/b	Atherosclerosis	Preclinical stage	[69]
	Not specified	Inhibits miR-10b	Suppression of lung metastasis from breast tumours, inhibition of neuroblastoma	Preclinical stage	[69]
	Not specified	Inhibition of miR-350-5p	Inhibition of neuroblastoma	Preclinical stage	[69]
Marina Biotech	MRX34	Mimic of miR-34	Haematological malignancy	Phase I ongoing, dose of Phase II decided	[70,71]
MiRNA Therapeutics	MRX34	Mimic of miR-34	Primary liver cancers and solid cancer with liver metastasis	Phase I	[72]
	MiR-Rxlet-7	Let-7 mimic	Not specified	Preclinical stage	[72]
	MiR-Rx-06	Mimic of miR-16	Not specified	*In-vivo* formulation	[72]
	MiR-Rx-06	Undisclosed	Not specified	*In-vivo* formulation	[72]
	MiR-Rx07	Undisclosed	Not specified	*In-vivo* formulation	[72]
Rosetta Genomics	MiR-34a mimetic	MiR 34a and p53	Cancer and liver infection	Preclinical stage	[73,74]
MiRagen therapeutics	Not specified	Inhibition of miR-155	Haematological malignancies	IND enabling	
	Not specified	Inhibition of miR-155	Amyotrophic lateral sclerosis	IND enabling	
	Not specified	Inhibition of miR-29	Cutaneous and pulmonary fibrosis	IND enabling	
	Not specified	Inhibition of miR-92a	Peripheral artery disease	Preclinical stage	[69]
	MGN-9103	Inhibition of miR-208	Chronic heart failure	Preclinical stage	[69]
	MGN-1374	Inhibition of miR-15 and miR-195	Postnatal cell cycle arrest in heart generation after myocardial infarction	Preclinical stage	[69]
	MGN-4893	Inhibition of miR-451	Polycythemia vera	Preclinical stage	[69]

based 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.

References

[1] Latronico MV, Catalucci D, Condorelli G. Emerging role of microRNAs in cardiovascular biology. *Circ Res* 2007; **101**(12): 1225-36.

[2] Yang B, Lu Y, Wang Z. Control of cardiac excitability by microRNAs. *Cardiovasc Res* 2008; **79**(4): 571-80.

[3] American Heart Association. *2000 heart and stroke statistical update*. Dallas (TX): American Heart Association; 2000.

[4] El-Armouche A, Schwoerer AP, Neuber C, Emmons J, Biermann D, Christalla T, et al. Correction: common microRNA signatures in cardiac hypertrophic and atrophic remodeling induced by changes in hemodynamic load. *PLoS One* 2011; http://dx.doi.org/10.1371/annotation/45ab2d79-0ce9-4832-a0d1-5274b5a1ddaa.

[5] van Rooij E, Marshall WS, Olson EN. Toward microRNA-based therapeutics for heart disease: the sense in antisense. *Circ Res* 2008; **103**(9): 919-28.

[6] Gambari R, Fabbri E, Borgatti M, Lampronti I, Finotti A, Brognara E, et al. Targeting microRNAs involved in human diseases: a novel approach for modification of gene expression and drug development. *Biochem Pharmacol* 2011; **82**(10): 1416-29.

[7] Lynn FC. Meta-regulation: microRNA regulation of glucose and lipid metabolism. *Trends Endocrinol Metab* 2009; **20**(9): 452-9.

[8] Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, et al. Molecular basis for the recognition of primary microRNAs by Drosha-DGCR8 complex. *Cell* 2006; **125**(5): 887-901.

[9] Vermeulen A, Behlen L, Reynolds A, Wolfson A, Marshall WS, Karpilow J, et al. The contributions of dsRNA structure to Dicer specificity and efficiency. *RNA* 2005; **11**(5): 674-82.

[10] Moorman A, Webb S, Brown NA, Lamers W, Anderson RH. Development of the heart: (1) formation of cardiac chambers and arterial trunks. *Heart* 2003; **89**(7): 806-14.

[11] Williams AH, Valdez G, Moresi V, Qi X, McAnally J, Elliott JL, et al. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. *Science* 2009; **326**(5959): 1549-54.

[12] Fuller AM, Qian L. MiRiad roles for microRNAs in cardiac development and regeneration. *Cells* 2014; **3**(3): 724-50.

[13] Liu N, Olson EN. MicroRNA regulatory networks in cardiovascular development. *Dev Cell* 2010; **18**(4): 510-25.

[14] Porrello ER. MicroRNAs in cardiac development and regeneration. *Clin Sci (Lond)* 2013; **125**(4): 151-66.

[15] Ostadal B, Dhalla NS. *Cardiac adaptations. Molecular mechanisms (advances in biochemistry in health and disease)*. Vol. 4. New York: Springer; 2013.
[16] Wang J, Greene SB, Bonilla-Claudio M, Tao Y, Zhang J, Bai Y, et al. Bmp signalling regulates myocardial differentiation from cardiac progenitors through a microRNA-mediated mechanism. *Dev Cell* 2010; **19**(6): 903-12.
[17] Cordes KR, Srivastava D. MicroRNA regulation of cardiovascular development. *Circ Res* 2009; **104**(6): 724-32.
[18] Bonet F, Hernandez-Torres F, Esteban FJ, Aranega A, Franco D. Comparative analyses of microRNA microarrays during cardiogenesis: functional perspectives. *Microarrays* 2013; **2**(2): 81-96.
[19] Kolpa HJ, Peal DS, Lynch SN, Giokas AC, Ghatak S, Misra S, et al. MiR-21 represses Pdcd4 during cardiac valvulogenesis. *Development* 2013; **140**(10): 2172-80.
[20] Kontaraki JE, Marketou ME, Zacharis EA, Parthenakis FI, Vardas PE. MicroRNA-9 and microRNA-126 expression levels in patients with essential hypertension: potential markers of target-organ damage. *J Am Soc Hypertens* 2014; **8**(6): 368-75.
[21] Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM, et al. Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. *Circulation* 2011; **124**(2): 175-84.
[22] Da Costa Martins PA, De Windt LJ. MicroRNAs in control of cardiac hypertrophy. *Cardiovasc Res* 2012; **93**(4): 563-72.
[23] Fernandes T, Hashimoto NY, Magalhães FC, Fernandes FB, Casarini DE, Carmona AK, et al. Aerobic exercise training-induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin II, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7). *Hypertension* 2011; **58**(2): 182-9.
[24] Renna NF, de Las Heras N, Miatello RM. Pathophysiology of vascular remodeling in hypertension. *Int J Hypertens* 2013; **2013**: 808353.
[25] Wu WH, Hu CP, Chen XP, Zhang WF, Li XW, Xiong XM, et al. MicroRNAs-130a mediates proliferation of vascular smooth muscle cells in hypertension. *Am J Hypertens* 2011; **24**(10): 1087-93.
[26] Jamaluddin MS, Weakley SM, Zhang L, Kougias P, Lin PH, Yao Q, et al. MiRNAs: roles and clinical applications in vascular disease. *Expert Rev Mol Diagn* 2011; **11**(1): 79-89.
[27] Caruso P, MacLean MR, Khanin R, McClure J, Soon E, Southgate M, et al. Dynamic changes in lung microRNA profiles during development of pulmonary hypertension due to chronic hypoxia and monocrotaline. *Arterioscler Thromb Vasc Biol* 2010; **30**(4): 716-23.
[28] Chen Z, Nakajima T, Tanabe N, Hinohara K, Sakao S, Kasahara Y, et al. Susceptibility to chronic thromboembolic pulmonary hypertension may be conferred by miR-759 via its targeted interaction with polymorphic fibrinogen α gene. *Hum Genet* 2010; **128**(4): 443-52.
[29] Contu R, Latronico MV, Condorelli G. Circulating microRNAs as potential biomarkers of coronary artery disease: a promise to be fulfilled? *Circ Res* 2010; **107**(5): 573-4.
[30] Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, et al. Circulating microRNAs in patients with coronary artery disease. *Circ Res* 2010; **107**(5): 677-84.
[31] Fleissner F, Jazbutyte V, Fiedler J, Gupta SK, Yin X, Xu Q, et al. Short communication: asymmetric dimethylarginine impairs angiogenic progenitor cell function in patients with coronary artery disease through a microRNA-21-dependent mechanism. *Circ Res* 2010; **107**(1): 138-43.
[32] Böger RH, Sullivan LM, Schwedhelm E, Wang TJ, Maas R, Benjamin EJ, et al. Plasma asymmetric dimethylarginine and incidence of cardiovascular disease and death in the community. *Circulation* 2009; **119**(12): 1592-600.
[33] Quintavalle M, Condorelli G, Elia L. Arterial remodeling and atherosclerosis: miRNAs involvement. *Vascul Pharmacol* 2011; **55**(4): 106-10.
[34] Xing HJ, Li YJ, Ma QM, Wang AM, Wang JL, Sun M, et al. Identification of microRNAs present in congenital heart disease associated copy number variants. *Eur Rev Med Pharmacol Sci* 2013; **17**(15): 2114-20.
[35] Gao Y, Huang GY. Advances in the etiology of congenital heart disease and epidemiological studies. *Chin J Evid Based Pediatr* 2008; **3**: 213-22.
[36] Li D, Ji L, Liu L, Liu Y, Hou H, Yu K, et al. Characterization of circulating miRNA expression in patients with a ventricular septal defect. *PLoS One* 2014; **9**(8): e106318.
[37] Breitbart RE, Fyler DC. Tetralogy of Fallot. In: Keane JF, Lock JE, Fyler DC, editors. *Nadas' pediatric cardiology*. Philadelphia: Saunders Elsevier; 2006.
[38] Gatzoulis MA, Webb GD, Daubeney PEF. *Diagnosis and management of adult congenital heart disease*. Edinburgh: Churchill Livingstone; 2003.
[39] Anderson RH, Tynan M. Tetralogy of Fallot-a centennial review. *Int J Cardiol* 1988; **21**(3): 219-32.
[40] Bittel DC, Kibiryeva N, Marshall JA, O'Brien JE. MicroRNA-421 dysregulation is associated with tetralogy of Fallot. *Cells* 2014; **3**(3): 713-23.
[41] Hayward P, Kalmar T, Arias AM. Wnt/Notch signalling and information processing during development. *Development* 2008; **135**(3): 411-24.
[42] Wang ZG. *MicroRNAs and cardiovascular disease*. Oak Park: Bentham Science Publishers; 2010.
[43] Goren Y, Kushnir M, Zafrir B, Tabak S, Lewis BS, Amir O. Serum levels of microRNAs in patients with heart failure. *Eur J Heart Fail* 2012; **14**(2): 147-54.
[44] van Empel VP, De Windt LJ, da Costa Martins PA. Circulating miRNAs: reflecting or affecting cardiovascular disease? *Curr Hypertens Rep* 2012; **14**(6): 498-509.
[45] Cakmak HA, Barman HA, Ikitimur B, Coskunpinar E, Oltulu YM, Can G, et al. The assessment of relationship between dysregulated microRNAs and left ventricular mass and mass index in systolic heart failure. *J Am Coll Cardiol* 2013; **62**(18 Suppl 2): C17.
[46] Cakmak HA, Barman HA, Coskunpinar E, Oltulu YM, Ikitimur B, Can G, et al. The diagnostic importance of microRNAs in congestive heart failure. *J Am Coll Cardiol* 2013; **62**(18 Suppl 2): C17-8.
[47] Endo K, Naito Y, Ji X, Nakanishi M, Noguchi T, Goto Y, et al. MicroRNA 210 as a biomarker for congestive heart failure. *Biol Pharm Bull* 2013; **36**(1): 48-54.
[48] Wang N, Zhou Z, Liao X, Zhang T. Role of microRNAs in cardiac hypertrophy and heart failure. *IUBMB Life* 2009; **61**(6): 566-71.
[49] Vettori S, Gay S, Distler O. Role of microRNAs in fibrosis. *Open Rheumatol J* 2012; **6**: 130-9.
[50] Koutsis G, Siasos G, Spengos K. The emerging role of microRNA in stroke. *Curr Top Med Chem* 2013; **13**(13): 1573-88.
[51] Tsai PC, Liao YC, Wang YS, Lin HF, Lin RT, Juo SH. Serum microRNA-21 and microRNA-221 as potential biomarkers for cerebrovascular disease. *J Vasc Res* 2013; **50**(4): 346-54.
[52] Long G, Wang F, Li H, Yin Z, Sandip C, Lou Y, et al. Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans. *BMC Neurol* 2013; **13**: 178.
[53] Sepramaniam S, Tan JR, Tan KS, DeSilva DA, Tavintharan S, Woon FP, et al. Circulating microRNAs as biomarkers of acute stroke. *Int J Mol Sci* 2014; **15**(1): 1418-32.
[54] Li Y, Liu Y, Wang Z, Hou H, Lin Y, Jiang Y. MicroRNA: not far from clinical application in ischemic stroke. *Int Schol Res Not Stroke* 2013; http://dx.doi.org/10.1155/2013/858945.
[55] Yang ZB, Zhang Z, Li TB, Lou Z, Li SY, Yang H, et al. Up-regulation of brain-enriched miR-107 promotes excitatory neurotoxicity through down-regulation of glutamate transporter-1 expression following ischaemic stroke. *Clin Sci (Lond)* 2014; **127**(12): 679-89.
[56] van Rooij E, Sutherland LB, Liu N, Williams AH, McAnally J, Gerard RD, et al. A signature pattern of stress-responsive

microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc Natl Acad Sci USA* 2006; **103**(48): 18255-60.

[57] van Rooij E, Olson EN. MicroRNAs: powerful new regulators of heart disease and provocative therapeutic targets. *J Clin Invest* 2007; **117**(9): 2369-76.

[58] Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, et al. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. *Biochem Biophys Res Commun* 2010; **391**(1): 73-7.

[59] Tijsen AJ, Creemers EE, Moerland PD, de Windt LJ, van der Wal AC, Kok WE, et al. MiR423-5p as a circulating biomarker for heart failure. *Circ Res* 2010; **106**(6): 1035-9.

[60] Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of microRNA in vascular neointimal lesion formation. *Circ Res* 2007; **100**(11): 1579-88.

[61] Davis S, Propp S, Freier SM, Jones LE, Serra MJ, Kinberger G, et al. Potent inhibition of microRNA *in vivo* without degradation. *Nucleic Acids Res* 2009; **37**(1): 70-7.

[62] Dharap A, Bowen K, Place R, Li LC, Vemuganti R. Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome. *J Cereb Blood Flow Metab* 2009; **29**(4): 675-87.

[63] Torres AG, Fabani MM, Vigorito E, Gait MJ. MicroRNA fate upon targeting with anti-miRNA oligonucleotides as revealed by an improved Northern-blot-based method for miRNA detection. *RNA* 2011; **17**(5): 933-43.

[64] BCC Research LLC. MicroRNA research tools, diagnostics and therapeutics: global markets. Report code: BIO115B. Wellesley: BCC Research LLC; 2014. [Online] Available from: http://www.bccresearch.com/market-research/biotechnology/microRNA-research-tools-diagnostics-therapeutics-report-bio115b.html [Accessed on 24th March, 2015]

[65] Lindow M, Kauppinen S. Discovering the first microRNA-targeted drug. *J Cell Biol* 2012; **199**(3): 407-12.

[66] Bhat B, Neben S, Tay J, Liu K, Chau N, Hogan D, et al. RG-101, a GaINAC-conjugated anti-miR employing a unique mechanism of action by targeting host factor MicroRNA-122 (miR-122), demonstrates potent activity and reduction of HCV in preclinical studies. *Hepatology* 2013; **58**(6): 1393A.

[67] Regulus Therapeutics Inc. Regulus receives orphan drug designation for RG-012, a microRNA therapeutic for the treatment of Alport syndrome. San Diego: Regulus Therapeutics Inc.; 2014. [Online] Available from: http://www.regulusrx.com/regulus-receives-orphan-drug-designation-for-rg-012-a-microrna-therapeutic-for-the-treatment-of-alport-syndrome-2/ [Accessed on 30th March, 2015]

[68] Christoph. RG-125 (AZD4076), a microRNA therapeutic targeting microRNA-103/107 for the treatment of NASH in patients with type 2 diabetes/Pre-diabetic, selected as clinical candidate by AstraZeneca. Regulus Therapeutics Inc. [Online]. Available from: http://mirnablog.com/a-microrna-therapeutic-targeting-microrna-103107-for-the-treatment-of-nash-in-patients-with-type-2-diabetespre-diabetes-selected-as-clinical-candidate-by-astrazeneca/ [Accessed on 5th April, 2015]

[69] Hydbring P, Badalian-Very G. Clinical application of microRNAs. *F1000Res* 2013; http://dx.doi.org/10.12688/f1000research.2-136.v3.

[70] Marina Biotech. Marina Biotech announces that licensee miRNA therapeutics enrolls first patientin hematologic malignancy cohort of on-going trial. Boston: Marina Biotech, Inc.; 2014. [Online] Available from: http://www.marinabio.com/files/2314/0856/2070/14-06-12_-_Marina_Biotech_Announces_That_Licensee_Mirna_Therapeutics_Enrolls_First_Patient_in_Hematologic_Malignancy_Cohort_of_On-Going_Trial.pdf [Accessed on 5th April, 2015]

[71] BioSpace. Marina Biotech reports that partner MiRNA therapeutics presented interim phase 1 data on its first-in-class microRNA-34 mimic. Colorado: BioSpace; 2014. [Online] Available from: http://www.biospace.com/News/marina-biotech-inc-reports-that-partner-mirna/355635 [Accessed on 4th April, 2015]

[72] MiRNA Therapeutics. Product development pipeline. Austin: MiRNA Therapeutics; 2014. [Online] Available from: http://www.mirnarx.com/pipeline/mirna-pipeline.html [Accessed on 6th April, 2015]

[73] Rosetta Genomics. Research programme: microRNA-targeted therapeutics-Rosetta Genomics. Jersey: Rosetta Genomics; 2013. [Online] Available from: http://adisinsight.springer.com/drugs/800028935 [Accessed on 26th April, 2015]

[74] Wahid F, Shehzad A, Khan T, Kim YY. MicroRNAs: synthesis, mechanism, function, and recent clinical trials. *Biochim Biophys Acta* 2010; **1803**(11): 1231-43.