MicroRNAs as solutions are producing big power for heart regeneration

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Cardiovascular diseases (CVDs) have become a predominant cause of mankind death globally. In order to overcome the limited capacity of adult mammalian heart to repair for cardiac injury, promising progress has been made in uncovering molecular mechanisms that will promote cardiac regeneration. Over the past decade, microRNAs (miRNAs), that can inhibit mRNA translation and promote mRNA degradation, have been demonstrated essential to cardiogenesis, cardiac pathology and regeneration. These studies imply that miRNAs may be employed as potential therapeutic targets for patients with various CVDs. In this review, we discuss the current observations attesting to the pivotal roles miRNAs played in heart morphogenesis, and the possibility of employing miRNAs to change cells fate and enhance cardiac regeneration.


Introduction

Heart disease is a worldwide leading cause of human morbidity and mortality [1], which represents a major global health concern. According to statistics at 2012, there are more than 17 million people died of cardiovascular diseases (CVDs), accounting for one third of world deaths, which rank first among all causes of death, especially, in elderly people over the age of 50 [2]. Mammalian cardiomyocytes have extremely limited regenerative capacity to efficiently repair injury after postnatal 7 days by comparison with amphibians and fish [3-6]. Recently, a growing body of research suggests that non-coding RNAs (ncRNAs) have emerged as critical components of various biological processes [7]. Among the ncRNAs, microRNAs (miRNAs), are small (21 to 24 nucleotide long) endogenous single-stranded RNAs that function via post-transcriptional gene silencing in diverse organisms and organs [8, 9]. MiRNAs are typically transcribed by RNA polymerase II as primary miRNA (pri-miRNA) in the nucleus, and then processed to generate approximate 70 nucleotide long pre-miRNA with stem-loop secondary structure by RNase III-like enzyme Drosha [10, 11]. The pre-miRNA is then transported to cytoplasm, cleaved by RNase III-like enzyme Dicer [12], subsequently incorporated as mature miRNA and loaded into the RNA-induced silencing complex (RISC) [13], ultimately directed to their target genes by a perfect base-pairing between the miRNA “seed sequence” and the complementary sequence of the 3’UTR of target mRNA [14, 15]. MiRNAs typically alter gene expression on the post-transcriptional level via promoting the degradation of mRNA or via suppressing the translation process of protein from mRNA [16]. Furthermore, previous studies showed that miRNAs are abundant in heart and play critical roles in the cardiogenic regulatory network, heart development, cardiology, and cardiac regeneration [13, 17]. In this review, we examine the recent investigations into the functions of miRNAs in cardiac development and cardiovascular diseases, as well as in cardiac regeneration for therapeutic purposes.
Role of miRNAs in heart development

The heart is the first formed organ during vertebrate embryonic development, and is composed of different cell types, including: cardiomyocytes, endocardial cells, epicardial cells, vascular cells, fibroblasts, and cells of the conduction system. A decade of studies have uncovered that myogenic transcription factors MEF2, NK2, and transcription factors GATA, Tbx, and Hand were integrated in cardiogenic network to regulate heart morphogenesis. For mammalian, the heart structure will sharply change after birth for adapting to the blood circulation and functional requirements from adult heart, accompanied with the loss of its most proliferative capacity shortly after birth.

Previous studies have indicated that miRNAs contribute to the biological processes in neonatal, postnatal, and adult heart. For example, cardiac-specific knockout of Dicer or Drosha caused diluted cardiomyopathy and cardiac dysfunction, supporting the significance of miRNAs during cardiogenesis. In addition, miRNAs also control the heart development through participating the regulatory networks. Previous study found that miR-1 is largely abundant in cardiac muscle, which is tightly controlled by transcription factors MEF2, indicating a common regulatory axis that controls cardiac development. Zhao et al. showed that targeted deletion of miRNA-1-2 could modulate transcription factors Gata6, Handl to affect cardiogenesis, cardiac conduction, and cell-cycle in mice. Overexpression of miR-133a under the heart-specific β-MHC promoter in embryonic cardiomyocytes caused embryonic lethality by inhibiting its proliferation, indicating that miR-133a is a negative factors for cardiogenesis. Loss-of-function of miR-218 impaired the migration of cardiac progenitor cells during the heart tube formation in zebrafish. In addition, profiling experiments identified the miR-15 family which consists of miR-15a, miR-15b, miR-16-1, miR-16-2, miR-195, and miR-497, and further studies revealed that the miR-15 family negatively modulated cardiomyocyte proliferation and triggered embryonic mitotic arrest of cardiomyocytes. Besides, miR-18, miR-20, miR-23b, miR-24, miR-26a, miR-30c, miR-143, miR-200a/b, miR-295, and miR-335 exhibit up-regulation in mouse stem cells and cardiomyocytes implying their potential roles during heart development. Although recent studies have provided some insight into the mechanisms by which miRNAs regulate cardiogenesis, much still remains poorly understood and warrants further research.

MiRNAs and cardiovascular diseases

Encouragingly, recent growing evidences suggested that microRNAs play critical roles in initiation and progression of CVDs, including myocardial infarction (MI), heart failure, hypertension, angiogenesis, coronary artery disease, atrial fibrillation, cardiomyopathy, stroke, and dyslipidaemia. In myocardial infarction (MI) patients, miR-26a and miR-191 were reduced compared with healthy controls, whereas miR-1, miR-208b, miR-499, miR-19a, miR-34a, miR-497, miR-133, miR-663b, miR1291, miR328, miR-134, and miR-423 were significantly up-regulated, those miRNAs might prove to be the new biomarkers for MI and a predictor of the risk of MI. Moreover, additional work has shown vital regulation of miRNAs in the development of MI disease. Wang et al. found that miR-103/107 participates in the regulation of MI by directly binding to long non-coding RNA (lncRNA) H19 and mediates FADD expression and necrosis in both in vitro and in vivo animal models. Further, due to the research of Liu’s team, miR-150 was proved to act as vital regulator in cardioprotective effects against MI-induced injury by inhibiting monocytes accumulation. Besides, Li et al. demonstrated that miR-99a plays a cardioprotective role in post-infarction left ventricular (LV) remodeling and its overexpression can improve cardiac function and survival ration in MI animal model by mediating Mtor/P70/S6K signaling.

On the other hand, miRNAs have become one of most favorable molecular targets in regulation of heart failure. MiR-340-5p can regulate heart failure by targeting gene dystrophin. Sang et al. reported that the expression of miR-133a was reduced in chronic heart failure patients, and it can improve the cardiac function and fibrosis via inhibiting AKT kinase in heart failure animal model. Overexpression of miR-30d led to cardiomyocyte growth and protected against apoptosis by targeting the motogen-associated kinase 4, which provide its paramount functional role as novel marker for cardiac resynchronization (CRT) response in heart failure. While Su et al. demonstrated that miR-221 plays an essential role in autophagy balance and cardiac remodeling by mediating the p27/CDK2/mTOR signaling pathway, implicating miR-221 as a potential therapeutic target in heart failure. Furthermore, recent reports also demonstrated multiple miRNAs involving in the development of heart failure, while provided new biomarkers in diagnosis and therapeutics of heart diseases.

Particularly, our research found that a variety of miRNAs are closely related with heart diseases. Dr. Wang Kun demonstrated that miR-23a is involved in the regulation of cardiac hypertrophy by modulating the translation activity of Foxo3a 3’UTR. Moreover, the mice with knock down of miR-23a exhibited exaggerated cardiac hypertrophy in response to stimulation. These findings indicated miR-23a as a potential therapeutic target for heart diseases. Intriguingly,
our present work demonstrated the accumulation of oxidized miRNAs was involved in the ischemic heart. Specially, the oxidized miR-184 was proved to participate in the onset and development of cardiac infraction by regulating apoptosis through misrecognition of Bcl-XL and Bcl-w [47], suggesting that miRNAs oxidation is a common feature in the clinically myocardial injury. And regulation of miRNAs oxidation might provide a new promising therapeutic regimen for heart disorders.

Additionally, several studies have highlighted the significant involvement of miRNAs in hypertension, coronary artery disease (CAD), and stroke. As reported recently, miR-27b was dramatically up-regulated in pulmonary arterial hypertension (PAH) [48]. Subsequently, it was proved to play pivotal role in endothelial function and NO release through regulating expression of PPAR. Moreover, miR-126, miR-30c, miR-96, miR-130/131, miR-505, and miR-208 have been identified to participate in the regulation of hypertension [49-55]. Tang’s team argued that miR-206 expression was dramatically increased in patients with coronary artery disease compared with healthy donors. More importantly, miR-206 mediates PI3K/Akt/eNOS signal pathway and down-regulates angiogenesis which contribute to the pathophysiology of coronary artery disease [56].

**MiRNAs in cardiac regeneration**

Mammalian cardiomyocytes would retain very limited proliferation or regenerative ability 7 days after postnatal [57], whereas cardiac regeneration occurs through the direct division of cardiomyocytes all the life in the lower vertebrates such as in zebrafish [58]. Despite some evidence showed slight renewal after injury, the adult mammalian heart is not sufficient to response to injury and cell loss due to its limited regenerative capacity. During the past decade, strategies for cardiac regeneration have made important progress, which mainly focus on three concepts, including: induction of terminally differentiated cardiomyocyte into proliferation, progenitor cell transplantation, and reprogramming of cardiac fibroblasts to functional cardiomyocytes [16]. Exciting studies in the past few years indicated that miRNAs play critical roles in applying the three therapies for heart repair.

A single miRNA may mediate their effects to the cardiac regeneration through targeting multiple genes, or a pathway involving in cardiomyocyte proliferation could be coordinately regulated by multiple miRNAs. Due to the function of the miR-15 family in the negative regulation of cell proliferation during cardiac development, treatment with locked nucleic acid based anti-miR15 increased proliferation of adult mouse hearts and enhanced cardiac function after injury [59], suggesting members of miR-15 family may be applied as potential targets for individuals with heart failure. Another recent study identified that miR-17-92 cluster acted as a key component in protecting against cardiac ischemic injury through inducing proliferation [60]. Compared with controls, overexpression of the miR-17-92 cluster was sufficient to enhance cardiomyocyte proliferation in healthy hearts or the hearts after myocardial infarction surgery [60]. Furthermore, Eulalio and colleagues identified two miRNAs, hsa-miR-590 and hsa-miR-199a, which elicited almost complete cardiac regeneration following myocardial infarction in adult animals transduced with adeno-associated virus serotype (AAV) 9- hsa-miR-590 and AAV9-hsa-miR-199a [61].

Cardiomyocytes derive from embryonic stem cells (ESCs) [13, 62], and some cardiac stem cells nicher in the postnatal heart are identified as cardiac progenitors (CPs) that harbor the ability to differentiate into various cardiac cell types and regenerate heart [22, 63, 64]. However, resident CPs modulate their differentiation into various cardiac cell lineages following a clonal different pathway from ESCs, and the mechanisms are unknown [22, 65]. Further miRNA profiling studies identified some linked miRNAs in human and mouse adult CPs that are distinctive from CPs of other periods [66], indicating the potential of miRNAs to modulate CP cells fate for cardiac diseases. Moreover, miR-499 was observed to impact on the processes of CP-derived cardiomyocytes to integrate into the heart in vivo [22, 66, 67].

The exciting explosion that mouse adult fibroblasts could be reverted to pluripotent status by adding four transcription factors Oct3/4, Sox2, c-Myc, and KLF4, was performed by Shinya Yamanaka in the cell reprogramming field [68]. These induced pluripotent stem cells (iPSCs) contain potential to differentiate into any somatic cell type. Most recently, Ieda et al. and Qian et al. demonstrated that three cardiac-specific transcriptional factors Gata4, Mef2c, and Tbx5 were able to direct reprogram murine cardiac fibroblast into functional cardiomyocytes both in vitro and in vivo [69, 70]. In addition, this performed reprogramming experiment imparted remarkable functional improvements in mouse MI models [70]. However, this current reprogramming technology using virally encoded transcription factors has the risk of insertional mutagenesis [71]. In order to increase the safety of reprogrammed cells, a mixture of miR-1, miR-133, miR-208, and miR-499 was investigated to directly convert cardiac fibroblast into cardiomyocyte-like cells, when transiently transfected cardiac fibroblasts in vitro [72]. Further studies are required to confirm the tremendous power of miRNAs to sufficiently trigger cardiac reprogramming.

**Future perspectives**
The newly characterized small regulators, miRNAs, have emerged as critical components in multiple cardiac biological processes, including heart development, cardiac remodeling, cardiovascular diseases, and cardiomyocyte regeneration. Moreover, recent study reported that miRNAs might be packaged within intercellular signaling organelles - exosomes or microvesicles, and then circulated in body fluids for cell-to-cell communications [73]. Based on the fact that mimics or inhibitors of miRNAs can be mass produced and transfected cells with low toxicity in vivo, miRNAs stand as prime tools of therapeutic applications for heart repair. Therefore, understanding the functions of miRNAs under various cardiac conditions and miRNA-mRNA interactions among different signaling cascades, as well as new advanced methodologies such as single-cell sequencing, will herald novel regenerative therapies for patients with CVDs. Moreover, current progresses about miRNAs application in cardiac regeneration are mainly made in mouse model, which remain to be demonstrated in human patients.

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References


