

Heart regeneration after myocardial infarction: the role of microRNAs

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Abstract: The curative therapy after myocardial infarction (MI) is to regenerate new functional myocardial tissue: the formation of new contractile cardiomyocytes and the revascularization in the injured region. Although, it is admitted that adult mammal heart can regenerate during physiological ageing and after heart injuries. Its regenerative capacity is very limited and could not compensate the loss of an amount of functional tissue after MI. Therefore, stimulation of the regenerative capacity of the human heart could be the primary option to restore cardiac function after MI. MicroRNAs (miRNAs) are small noncoding RNAs that implicated in the gene expression. Several miRNAs have been shown to control important processes that contribute to the consequences of MI. MiRNAs play an important role during heart development and regulate cardiomyocytes proliferation and revascularization after MI. New data provide a proof of concept for identifying and activating conserved molecular programs to regenerate the damaged heart. In this review, we focus on the role of miRNAs in heart development and regeneration after MI.

Keywords: miRNA; heart development; heart regeneration; myocardial infarction (MI)

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Introduction

Cardiovascular diseases (CVDs) continue to be a major cause of morbidity and mortality worldwide. Despite undeniable progress in cardiology and cardiac surgery, many patients progress to heart failure after acute myocardial infarction (AMI). A major reason is that AMI induces the loss of a given amount of functional myocardial tissue, including cardiomyocytes (CMs), endothelial cells (ECs), smooth muscle cells (SMCs). All of these cell types contribute to structural, biochemical, and electrical properties of the functional heart. Unlike lower vertebrates that have a high cardiac regeneration rate, the mammal heart was for a long time considered as post-mitotic organ with no capacity to regenerate (1). The various treatments aimed to delay the onset of heart failure or to limit the consequences of CVDs, do not have the ability to replace the damaged cardiac cells and regenerate new functional myocardial tissue. The curative therapies of injured heart need both the formation of new contractile CMs and the revascularization in the injured region in order to supply sufficient oxygen and nutrition for the new-formed CMs. In this review, we discuss: (I) the heart regeneration after myocardial infarction (MI); (II) the role of microRNA (miRNA) in heart development and regeneration after MI; and (III) the therapeutic potential of modulating the expression of the miRNAs. MiRNAs are endogenous, small, highly conserved noncoding RNA molecules that have

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emerged as fundamental posttranscriptional regulators of gene expression.

Heart regeneration after MI

CMs renewal after MI

Most of non-mammalian animals, such as zebrafish, can regenerate the heart after myocardial injury throughout life. Multiple studies have demonstrated that zebrafish can fully restore its heart in 30 days after up to a 20% loss of ventricular tissue. This regenerative process is primarily driven by preexisting CMs proliferation (2). In mammals, CMs undergo extensive proliferation during embryogenesis and fetal development. Shortly after birth, many CMs withdraw from the proliferative cell cycle (3). Beginning at postnatal day 5 in rodents, the majority of CMs undergo a final round of DNA replication and karyokinesis in the absence of cytokinesis, which results in binucleation of approximately 90% of CMs by the second week of postnatal life (4). Binucleated CMs are thought to be incapable of division, as polyploidy generally indicates terminal differentiation. Now many studies demonstrated that new CMs are generated in human hearts during physiological ageing and after heart injuries (5-7)

The source of new CMs has been attributed to: (I) division of existing CMs (8,9); (II) progenitors residing with in the heart (10); (III) exogenous niches, such as bone marrow (11).

Studies in mice indicate that mammals retain regenerative ability after birth for only a short period (<7 days). During this period, hearts can regenerate after amputation of the ventricular apex (12) and after ischemic MI (13) through proliferation of preexisting CMs. Adult mammalian CMs can slowly self-renew at a rate of 1% of total CMs per year, declining with age. This renewal results in the exchange of ~50% of adult CMs in a human life span (5). Therefore, the induction of CMs proliferation might contribute to therapeutic cardiac regeneration.

Recent studies suggest that adult CMs can reenter the cell cycle and proliferate in response to certain mitogens such as fibroblast growth factor (FGF) (14), periostin (15), neuregulin (16). It appears that this proliferative potential is restricted to a small proportion of mononucleated CMs. The stimulation of CM proliferation as a new therapeutic strategy to induce cardiac regeneration seems still to be a challenge.

Another issue for new-formed CMs after injury is the

differentiation of endogenous precursor cells. A pool of tissue-specific resident cardiac stem cells, known as cardiac progenitor cells (CPCs), was identified in the adult human myocardium. The characterization of these cells remains a difficult task, due to the lack of a highly specific marker. The c-kit, stem cell antigen-1 (Sca-1) and islet-1 (nuclear transcription factor) have been proposed (17). The participation of the endogenous CPCs to heart regeneration in physiological conditions is controversial (7,18). After injury, CPCs can be activated and may differentiae into new CMs with different stimuli, such as FGF-2 (basic FGF) (19), thymosin β (20), prostaglandin E2 (21), human stem cell factor (22), stromal-cell derived factor 1 (SDF1) (23) or brain natriuretic peptide (BNP) (10,24). These results suggest the use of CPCs might be another therapeutically issue for AMI.

Revascularization after MI

Functional regeneration of cardiac tissue, which has inherently high metabolic activity, requires CMs to have a sufficient blood supply. Therefore, the growth of new blood vessels, termed angiogenesis, is essential for the process. The precise mechanisms of revascularization following injury are not well defined.

Results from non-cardiac injury models (for example, using zebrafish fin regeneration) suggest that neovascularization during regeneration is dependent on classical angiogenic signaling mechanisms that involve various endogenous factors such as vascular endothelial growth factor receptor 2 (VEGFR2) (25), hypoxia-inducible factor 1 (HIF1- α) (26) and SDF1 α (27). In rodents, activation of HIF1- α pathway improved cardiac function after MI and microvascular density in the peri-infarct area after MI (28-31). Numerous factors are expressed in the ischemic heart where they promote angiogenesis, such as VEGF, FGF. Preclinical studies showed exciting results based on protein or gene transfer delivery of these growth factors after MI, but so far, clinical trials showed mixed results in patients with AMI (32).

There is evidence to support both local proliferation of ECs and a contribution from a remote stem cell source after MI. The program of coronary vascular formation is directed by epicardium, which acts as a source of both trophic factors and progenitor cells (33). Epicardium-derived cells (EPDCs) invade the underlying myocardium and undergo epithelial-to-mesenchymal transition (EMT), which rise pericytes, SMCs and fibroblasts. The precise contribution of EPDCs to the ECs continues to be debated (34). The epicardium is

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quiescent in the adult heart but is reactivated and expands in response to injury (35).

In mice, reactivation of EPDC following MI demonstrates a capacity for tri-lineage differentiation into the major cardiovascular cell types (CM, SMC, and EC) (20,36,37). Studies showed that the stimulation of T β 4 (peptide thymosin beta 4) or with modified RNA encoding VEGF-A supported neovascularization after MI through regulation of EPDC activation and subsequent differentiation into SMCs and ECs (37,38).

However, the main origin of ECs of neovessels in the injured heart was not clear. A study in 2014 that included fate mapping with Col1a2-CreER transgenic mice to label the fibroblast reported that after MI, ~35% of fibroblastderived cells expressed the EC marker vascular endothelial (VE)-cadherin (39). A recent study used six different mouse lines expressing Cre driven by fibroblast marker, including the same Col1a2-CreER transgenic mice to trace the fate of fibroblasts after MI. They demonstrated that no fibroblasts gave rise to ECs after MI. ECs of neovessels in the injured heart derive from pre-existing EC proliferation (40). To develop novel revascularization strategies in ischemic heart disease and identify the source of neovascular ECs are important steps. More studies are needed to clarify these questions.

These data point to the possibility of a novel therapeutic strategy: stimulation of the regenerative capacity of the human heart. Cardiac regeneration research is evaluating two major avenues: (I) exogenous cell transplantation and (II) stimulation of endogenous regenerative processes. The therapeutic application of various types of cells, including progenitor cells derived from adult heart, bone marrow, or adipose tissue, has been shown to have cardioprotective effects in experimental models of AMI and in some clinical trials [reviewed in (41)]. However, the establishment of successful cell therapies is challenging owing to poor homing and survival of the implanted cells, and a limited cardiac differentiation capacity of most types of adult progenitor cells. Therefore, a therapeutic ideal- relative to cell transplantation-would be to stimulate the resident source by different agents. In this field, the critical role of miRNAs is demonstrated. Recent studies have given evidences that miRNAs play an important role in cardiac development and reparation after injury.

miRNA

Recently, a novel batch of endogenous small non-coding

regulatory RNAs: miRNAs have received attention in the development of cancer and regeneration of tissue, miRNAs bind complementary sequences in target mRNAs, resulting in selective degradation or selective inhibition of their translation. miRNA is a class of natural, endogenously expressed single-stranded, small (~22 nucleotide) noncoding RNAs. The transcription of miRNAs depends on their location within the genome. MiRNAs can be located in introns of coding genes or noncoding genes or in exons. Then the miRNAs transcription depends on the host gene.

miRNA biogenesis had been detailed in review (42). Briefly, the process of miRNAs biogenesis begins when the hairpin primary miRNA (pri-miRNA) is transcribed. The maturation of miRNAs is mediated by the two RNase III endonuclease Dicer and Drosha. In the first step, the microprocessor complex composed of Drosha and DGCR8 mediates the nuclear processing of the primiRNA into stem-loop precursors of approximately 60-70 nucleotides (pre-miRNA). The nuclear export of the precursor is subsequently mediated by exportin-5 in a Ran-GTP dependent manner. In the second step, Dicer cleaves the pre-miRNA in the cytoplasm into the mature ~22 nucleotide miRNA, which incorporates as single-stranded RNA into the RNA-induced silencing complex (RISC). This complex directs the miRNA to the target mRNA, which leads either to translational repression or degradation of the target mRNA.

miRNAs are important modulators of all cellular pathways, especially cell fate determination, differentiation, proliferation, programmed cell death, and other processes during embryogenesis and in adult life.

Interestingly, miRNAs have recently been discovered in extracellular vesicles, in particular exosomes. Exosomes are endogenous nanovesicles of 30–100 nm in diameter. They are secreted by multiple cell types, including cardiac cell types (CMs, ECs, fibroblasts and resident stem cells), into extracellular space after fusion with the plasma membrane. The results of a number of studies suggest that exosomes derived from cardiac cells are responsible for cell-to-cell communication in the heart under both physiological and in pathological conditions. It has been shown that exosomal miRNAs can target mRNAs in recipient cells. This recent concept adds a new level of complexity to the role of miRNAs in CVDs (43).

miRNA in cardiac development

The critical role of miRNAs in cardiac development was

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first shown through loss of function mutations in the miRNA-processing enzyme, such as Dicer or DGCR8. A knockout of Dicer precluded the processing of premiRNAs into their mature form in cardiac progenitors and led to defects in heart development as well as embryonic lethality (44). Mice lacking DGCR8 in muscle tissue die prematurely with signs of heart failure and dilated cardiomyopathy (45).

MiR-1 and miR-133 families

Muscle-specific miRNAs, especially the miR-1 family and the miR-133 family, play important roles in muscle growth and differentiation. The miR-1 family and the miR-133 family express in the developing heart, and increased expression was found in neonatal mouse hearts (46). Myocyte enhancer factor 2 (MEF2) and serum response factor (SRF) cooperatively regulate the expression of two bicistronic miRNA clusters encoding miR-133a-1/miR-1-2 and miR-133a-2/miR-1-1 in cardiac and skeletal muscle (47,48). Mice lacking either miR-133a-1 or miR-133a-2 are normal, whereas deletion of both miRNAs causes lethal ventricular-septal defects (VSDs) in approximately half of double-mutant embryos or neonates; miR-133a doublemutant mice that survive to adulthood succumb to dilated cardiomyopathy and heart failure (49). Mice lacking miR-1-2 have a spectrum of abnormalities, including VSDs in a subset that suffer early lethality, cardiac rhythm disturbances in those that survive, and a striking myocyte cell-cycle abnormality that leads to hyperplasia of the heart with nuclear division persisting postnatally (44). Many genes involved during cardiogenesis were upregulated in the miR-1-2 mutants mice, such as Hrt2/Hey2 and Hand1.

MiR-15 family

In mammals, enlargement of the heart during embryonic development is primarily dependent on the increase in CM number through CM proliferation. The miR-15 family, including miR-15a, miR-15b, miR-16, miR-195, and miR-497, targets cell-cycle genes such as checkpoint kinase 1 (*Cbk1*), who is involved in many functions during DNA repair and mitosis. Inhibition of miR-15 family was associated with the de-repression of a number of cell cycle genes in the heart and a persistence of CM mitosis beyond the normal development window of cell cycle arrest (13). Premature overexpression of miR-195 in the embryonic heart was associated with ventricular hypoplasia and

ventricular septal defects in β -myosin heavy chain-miR-195 transgenic mice (13).

MyomiRs group

A number of myosin genes coexpress miRNAs, named myomiRs (miR-208a, miR-208b, and miR-499) as introns with vital purposes in cell differentiation, including the direction of cardiac myosin gene expression. *Mby6* (α -myosin heavy chain) coexpresses miR-208a, *Mby7* (β -myosin heavy chain) coexpresses miR-208b, and *Mby7b* (myosin heavy chain 7b) coexpresses miR-499. MiR-208a was shown to be required for cardiac growth and expression of the β -myosin heavy chain protein, the primary contractile protein of the heart (50). Both miR-208a and miR-208b, members of the miR-208 family, target *Tbrap1* (thyroid hormone receptor-associated protein 1) and myostatin, important negative regulators of muscle growth. MiR-208a and miR-208b are differentially expressed during heart development parallel to the expression of their respective host genes, *Mby6* and *Myb7* (50).

MiR-138 and miR-143 families

Organ patterning during embryonic development requires precise temporal and spatial regulation of protein activity. Some miRNAs inhibit protein expression and are essential for normal cardiac morphogenesis. The miR-138 family is required to establish appropriate chamber-specific gene expression patterns. They repress chondroitin sulfate proteoglycan core protein 2 (*cspg2*) and atrio-ventricular canal-specific transcripts in the developing ventricle via regulation of a network of development signals. This contributes to CM maturation and helps to establish the distinct identity of cardiac structures (51).

In zebrafish, miR-143 is required for chamber morphogenesis through direct repression of add3 (adducin3), which encodes an F-actin capping protein. Knockdown of miR-143 or disruption of the miR-143-add3 interaction inhibits ventricular CM F-actin remodeling, which blocks their normal growth and elongation and leads to ventricular collapse and decreased contractility (52). In addition, the expression of zebrafish miR-143 is dependent on heartbeat. Knocking-down miR-143 results in de-repression of retinoic acid signaling, and produces abnormalities in the outflow tracts and ventricles (53).

MiR-218

A highly conserved miRNA, miR-218, is encoded



Figure 1 The major miRNAs implicated in cardiac development. MiR-1/miR-133 family are critical regulators of cardiac development, which are involved in muscle growth and differentiation. MiR-15 family targets cell cycle genes and negatively regulates cell proliferation and induces embryonic cardiomyocyte mitotic arrest. MyomiRs, miR-208a and miR-208b, coexpressing with *Mby6* and *Mby7*, mediate myosin heavy chain (Mhy) isoforms, switching during fetal and adult stage. miR-138, miR-143 and miR-218 control cardiac patterning. MiR-216 implicate in the vascularization during development.

intronically in *slit2* and *slit3* and negatively regulates *Robo1* and *Robo2*. Knockdown experiments indicate that *Slit2*, *Robo1* and miR-218 are required for the formation of the linear heart tube in zebrafish (54). Besides, the miR-218 family is part of a regulatory circuit through tbx5, a transcription factor that mediates vertebrate cardiac development, which controls heart morphogenesis. Tbs5 overexpression affects heart development in humans and mice, resulting in heart-looping defects and chamber abnormalities (55,56)

Other miRNAs

Some other miRNAs, such as miR-126, were enriched in ECs derived from mouse embryonic stem (ES) cells and in developing mouse embryos. Knockdown of miR-126 in zebrafish and in mice resulted in loss of vascular integrity and hemorrhage during embryonic development (57,58). MiR-126 functioned in part by directly repressing negative regulators of the VEGF pathway, including the SPRED1 (Sprouty-related protein) and PIK3R2/p85-β (phosphoinositol-3 kinase regulatory subunit 2).

These articles illustrate the vital, yet not fully understood, role of miRNAs in cardiac development (*Figure 1*).

miRNA after MI

The participation of miRNA in cardiac development suggested a role of miRNAs on heart regeneration, because cardiac development is a period of high CM proliferation and vascularization. Several miRNAs have been shown to control processes that contribute to the cardiac reparation after MI (*Figure 2*).

miRNA in cardiomyocyte proliferation after MI

Role of miR-15 family

Studies showed some miRNAs could modulate cell cycle and play important roles in cell reentry and CMs proliferation. MiRNAs expression profiling of hearts of neonatal mice in this period showed that the miR-15 family, plays important roles during heart development, was expressed at low levels just after birth, and upregulated at the time when CMs lost their capacity to proliferate. Knockdown of the miR-15 family in neonatal mice with locked nucleic acid (LNA)-modified anti-miRNAs was associated with an increased number of mitotic CMs (13). Although these data imply that postnatal inhibition of the miR-15 family increases CM mitotic entry and progression. Few CMs staining positive for Aurora B kinase at postnatal

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Figure 2 MicroRNAs implicated in heart regeneration after myocardial infarction by regulating cardiomyocytes proliferation and angiogenesis. MiR-222, miR-17~92 cluster and miR-590/miR-199a induce cardiomyocytes proliferation, whereas miR-15, miR-1/miR-133 family as well as miR-99/100 and Let-7a/c inhibit proliferation of cardiomyocytes. MiR-15, miR-26a, miR-24 and miR-92a are antiangiogenic via inhibition of endothelial-cell function. miR-210 induces angiogenesis by inhibiting antiangiogenic factors. SRF, serum response factor; BMP, bone morphogenic protein; HOPX, homeodomain-only protein homeobox.

12 days, suggesting that loss of miR-15 family function was not sufficient to drive complete mitotic progression through cytokinesis. Inhibition of the miR-15 family from an early postnatal age until adulthood increases myocyte proliferation in the adult heart and improves left ventricular systolic function after adult MI (9).

Role of miR-99/100 and Let-7a/c

As we previously reported, adult zebrafish heart regeneration occurs by the spontaneous dedifferentiation of resident CMs as a response to injury (2). Dedifferentiated CMs were shown to re-enter the cell cycle and accounted for all newly formed cells repairing the heart. MiR-99/100 and Let-7a/c and their protein targets fnt β (beta subunit

of farnesyl-transferase) and smarca5 (SWI/SNF-related matrix associated actin-dependent regulator of chromatin subfamily a, member 5) were shown to be critical regulators of CM dedifferentiation and heart regeneration in zebrafish. Proliferation and myocardial regeneration were suggested to be mediated through their targets fnt β and smarca5.

The expression of miR-99/100 and Let-7a/c was low during zebrafish heart development and was downregulated after amputation of ventricular apex, concomitantly with high levels of fnt β and smarca5 (59). However, adult mice heart, in contrast to zebrafish, failed to regenerate their heart due to a failure in downregulating these two mRNAs after MI. Overexpression of either protein, fnt β or smarca5, in primary murine CMs *in vitro* resulted CM dedifferentiation and proliferation. Intracardiac delivery of an adenoviral vector encoding anti-miR-99/100 and anti-Let-7a/c significantly improved heart function 15 days after MI (59).

Role of miR-1 and miR-133 families

MiR-1 family and miR-133 family are also critical mediators of cell proliferation in cardiac muscle. In zebrafish, induced transgenic elevation of miR-133 levels after injury inhibited myocardial regeneration, while transgenic miR-133 depletion enhanced CM proliferation (60). Transgenic overexpression of miR-1 or miR-133a in the developing heart leads to a decreased pool of proliferating ventricular CMs and thin-walled ventricles (47,49). MiR-1 inhibits CM proliferation by repressing expression of the Hand2 (heart and neural crest derivatives-expressed protein 2) (47). The target of miR-133 was attributed to SRF and cyclin D2 (cellcycle regulators G1/S-specific cyclin-D2) (49). Both these targets were involved in the cell cycle control. A recent study showed overexpression of cyclin-dependent kinase 1 (CDK1), CDK4, cyclin B1, and cyclin D1 efficiently induced cell division in post-mitotic mouse, rat and human CMs (61).

Role of miR-222

MiR-222 was shown to regulate cell proliferation and differentiation in vascular SMCs and some cancers (62,63) through in part targeting the cell-cycle inhibitor p27 (cyclin-dependent kinase inhibitor). In heart, miR-222 is necessary for exercise-induced cardiac proliferation due to its role in inhibiting p27, homeodomain-interacting protein kinase 1 (HIPK1) and homeobox containing 1 (HMBOX1) (64). Mice with inducible CMs miR-222 expression were resistant to adverse cardiac remodeling and dysfunction after ischemic injury with an increase of proliferating CMs (64).

Roles of miR-17~92 cluster

MiR-17~92 cluster, consisting of miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a, has been shown to be involve in the cardiac development. Mice deficient for miR-17~92 died shortly after birth lung hypoplasia and a ventricular septal defect. MiR-17~92 cluster has also been shown to be important for CM proliferation (65). Overexpression of miR-17~92 cluster induced CM proliferation in embryonic, neonatal and adult hearts. Moreover, overexpression of miR-17~92 cluster protected the heart from MI-induced injury in mice (66,67). PTEN (phosphatase and tensin homolog), a negative regulator of survival and proliferation, is one of the miR-17~92 cluster functional targets and is downregulated by miR-19a and miR-19b (66). Similarly, overexpression of the miR-302~367 cluster, which is a paralogs of miR-17~92 cluster (65), is sufficient to improve regeneration following adult MI; which acts via repression of the Hippo pathway (68).

Role of other miRNAs

In a high throughput overexpression approach in which 875 miRNAs were tested, at least 40 miRNAs strongly increased both DNA synthesis and cytokinesis in neonatal mouse and rat CMs. Two miRNAs in particular, miR-590 and miR-199a, were shown to promote cell-cycle re-entry of adult CMs *ex vivo* and to promote CM proliferation in both neonatal and adults rat CMs (69). Both, these miRNAs inhibit the expression of many negative regulators of the cell cycle, Homer protein homologue 1 (Homer1) and homeodomain-only protein homeobox (HOPX) (70). Injection of adeno-associated virus expressing miR-590 and miR-199a in the peri-infarcted area induced proliferation of CM and stimulated cardiac regeneration after MI in adult mice (69).

In a recent study, the authors compared miRNA profiles in CM between postnatal day 0 (P0) and day 10 (P10) using miRNA arrays, and found that 21 miRNAs were upregulated at P10, whereas 11 were downregulated. Among them, miR-31a-5p was identified as being able to promote CM proliferation. Neonatal rats injected with miR-31a-5p antagomir at day 0 for 3 days exhibited reduced expression of CM proliferation markers. *RhoBTB1* was identified as a target gene of miR-31a-5p (71).

miRNA in revascularization after MI

Several miRNAs have been described to affect angiogenesis after MI. Initial studies of the miRNAs biogenesis enzymes Drosha and Dicer evidenced a crucial need for a balanced miRNAs expression to sustain EC integrity and angiogenic properties (72-74).

Importance of miR-17~92 cluster

The miR-17~92 cluster is also highly expressed in human ECs. As we reported this cluster consists of miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a. miR-92a and controls angiogenesis and neovascularization after AMI (75). MiR-92a was shown to inhibit endothelial-cell migration and sprouting in cell-culture studies. New pharmacological area is the use of antagomiRs. Antagomirs known as anti-miRs are a class of chemically engineered

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oligonucleotides that prevent other molecules from binding to a desired site on an mRNA molecule. *In vivo*, pharmacological inhibition of miR-92a using antagomiRs increased capillary density and improved heart function after AMI in mice (75,76). In a large-animal study, catheter-based delivery of anti-miR-92a reduced infarct size associated with a significant upregulation of capillary density (77). The targets by which miR-92a elicits its effects include integrin α 5, which prevents endothelial-cell apoptosis and is important for vessel maturation (75). In addition, several vasculoprotective genes are repressed by miR-92a, such as *KLF2* and *SIRT1* (78).

Importance of miR-23/24/27 cluster: role of miR-24

MiR-24 belongs to miR-23/24/27 cluster, who is involved in the regulation of angiogenesis during vascular disorders and ischemic heart disease (79). MiR-24 is enriched in cardiac ECs and considerably upregulated after cardiac ischemia. Inhibition of miR-24 improved neovascularization after AMI in mice. AntagomiRs directed against miR-24 or local adenovirus-mediated miR-24 decoy delivery improved the recovery after AMI via actions on endothelial nitric oxide synthase (eNOS), GATA-2 (endothelial transcription factor), and PAK4 (serine/threonine-protein kinase) (80,81).

Importance of miR-26a

MiR-26a is commonly dysregulated in diverse cancers and CVDs, involving in various biological processes, such as proliferation, migration, invasion, angiogenesis, and metabolism by targeting multiple mRNAs. MiR-26a is also increased after AMI and inhibits angiogenesis *in vitro* and *in vivo*. Ectopic expression of miR-26a markedly induced EC cycle arrest and inhibited EC migration, sprouting angiogenesis, whereas blockade of miR-26a had the opposite effects. Overexpression inhibited formation of the caudal vein plexus in zebrafish, and exercise-induced angiogenesis was blocked in mice. Administration of a LNA-based antimiR induced robust angiogenesis; reduced myocardial infarct size, and improve heart function. Mechanistic studies demonstrated that miR-26a inhibits the bone morphogenic protein (BMP)/SMAD1 signaling pathway in ECs (82).

Importance of miR-15 family

The miR-15 family not only regulated cell cycle genes during heart development and reduced CM proliferation, but also inhibited angiogenesis, via targeting of the wellcharacterized proangiogenic and endothelial survival factors: VEGF-A, FGF2 and Akt-3 (RAC- γ serine/threonineprotein kinase). On the basis of these detrimental effects on CM proliferation and vascularization, inhibition of miR-15 might be an attractive strategy to improve recovery after ischaemia preventing postischaemic heart failure (83,84).

Importance of miR-210

Recently, several studies have indicated the possibility role of miRNAs, especially miR-210, in the physiopathology of CVDs; miR-210 inducing effects on CMs and the vasculature. This miRNA is induced by hypoxia (85) and upregulated after AMI (86). *In vitro*, miR-210 overexpression reduced cell death. *In vivo, overexpression* by intramyocardial injection of minicircle vectors carrying the miR-210 precursor improved cardiac function and angiogenesis after AMI in mice (86). Minicircle DNA vectors consist of a circular expression cassette devoid of the bacterial plasmid DNA backbone; they provide several advantages including sustained transgene expression in quiescent cells/tissues. The potential targets for miR-210 are *Efan3* and *Ptp1b*, involved in inhibition of angiogenesis and induction of apoptosis, respectively.

Conclusions and prospects

In conclusion, the end goal of complete regeneration of the infarcted heart included CM renewal, neovascularization and scar reduction. The emerging biology of endogenous regeneration is exposing a multiplicity of therapeutic targets, such as small-molecule stimulator, miRNAs or cell transplantation. Numerous roles for miRNAs in cardiac development and cardiac regeneration after MI have been identified. Several miRNAs might be attractive candidates or targets to improve recovery after MI. The inhibition or overexpression of several miRNAs after MI was shown to limit tissue injury, stimulate CM proliferation and improve neovascularization.

With advances in our understanding, new opportunities exist to manipulate miRNAs for tissue regenerative therapies in the future. However, many challenges are posed by the complexities of miRNA signaling networks and the precise spatiotemporal resolution in various diseases. For example, each miRNA targets many potential mRNAs. Identification of those mRNAs that are relevant to cardiac regeneration, especially to CM proliferation or angiogenesis, is a challenge. Further studies designed to ameliorate current therapeutic methodologies are required to develop new therapies.

Several therapeutic approaches that interfere with

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the molecular pathways that are involved in CVDs have shown the potential to delay and reverse cardiac dysfunction (87). In particular, modulation of cardiac metabolism, gene expression, pharmacological therapy, and miRNAs represents encouraging strategies. With the current advancements of miRNAs functions based on pathophysiological context and cell type in cardiovascular disease, we foresee new therapeutic applications for the treatment of MI in the relatively near future.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi. org/10.21037/ncri.2018.04.08). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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