Targeting microRNAs to promote cardiac repair and cardiomyocyte proliferation as a potential regenerative therapeutic approach

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Abstract

MicroRNAs (miRNAs) have emerged as important modulators of cardiovascular biology, repair, and regeneration. miRNAs posttranscriptionally regulate the expression of a network of proteins and consequently, cardiovascular development. miRNA targeting has been shown to: (i) improve the cardiac repair capacity of patient-derived circulating or bone marrow–derived mononuclear cells (BMCs); (ii) induce pluripotent stem cells or direct cardiac reprogramming; and (iii) promote cardiomyocyte proliferation after cardiac injury. Several groups, including our group, have recently targeted miR-126 or miR-34a to improve the cardiac repair potential of circulating mononuclear cells or BMCs from patients with ischemic cardiomyopathy. Moreover, targeting of several miRNAs induced reprogramming of mouse and human cells to pluripotency, and initial experimental studies suggest that fibroblasts can be directly converted into cardiomyocytes in vivo by targeting miRNAs–a process termed direct reprogramming. Recent data suggest a marked early postnatal cardiomyocyte proliferation in response to myocardial injury. Cardiomyocyte proliferation still occurs in adults, although at a very low rate (0.5% to 1% per year). These observations have stimulated the interest in investigating therapeutic strategies to promote cardiomyocyte reentry into the cell cycle to compensate for cardiomyocyte loss after myocardial infarction. Experimental studies have shown that miR-1, miR-133a, miR-29a, and the miR-15 family repress cardiomyocyte proliferation, whereas miR-199a and miR-590 promote cardiomyocyte proliferation in injured murine hearts. Therapeutic targeting of miRNAs is being intensely examined to promote cardiac repair and regeneration, a development aided by high-throughput functional screening assays. Large animal data suggest that targeting miRNAs can improve cardiac function after injury, indicating a potential future applicability for this approach to enhance cardiac repair and regeneration in the clinical setting. ■ *Heart Metab*. 2014;65:9-14

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Abbreviations

BMC: bone marrow mononuclear cells; **CHF:** chronic heart failure; **EOC:** early angiogenic outgrowth cell; **ESC:** embryonic stem cell; **iPSC:** inducible pluripotent stem cell; **miRNA:** microRNA; **RISC:** RNA-induced silencing complex

Heart failure is a leading cause of mortality and morbidity. Interventional and surgical revascularization, pharmacological treatment, and devices, such as cardiac resynchronization therapies, are strategies to improve cardiac function and improve symptoms and outcomes.[1] However, the prognosis of patients with heart failure remains poor, and the frequent underlying cause, myocardial cell loss, has not been successfully addressed. Although a very limited number of cardiac cells harbor a potential to reenter the cell cycle[2] or stimulate cardiomyocytes toward a proliferative state, these mechanisms cannot compensate for the acute or slowly proceeding cardiomyocyte loss occurring after acute myocardial infarction or in chronic heart failure (CHF), a progressive disease.

Therefore, therapeutic interventions to enhance cardiac regeneration directly are being intensely investigated as a potential novel therapeutic strategy. MicroRNAs (miRNAs) regulate cardiac developmental processes, cardiomyocyte proliferation, and viability by posttranscriptionally modulating messenger RNA (mRNA) translation into proteins. As miRNAs can be targeted using anti-miRNAs or miRNA mimics, exogenous modification of miRNA levels emerges as an interesting novel therapeutic strategy to enhance cardiac repair and regeneration. This review will focus on miRNAs involved in cardiac repair, cardiac lineage commitment, and regulation of cardiomyocyte proliferation.

MicroRNA biogenesis

miRNAs are small (≈19 to 22 nucleotides long), single-stranded, noncoding RNAs. The primary transcript is transcribed by RNA polymerase,[3] which is then processed by Drosha. The precursor miRNA (pre-miRNA) is transported from the nucleus to the cytoplasm, where it is frequently cleaved by Dicer.[3] One strand of the unwound, double-stranded miRNA helix is preferentially integrated in the RNA-induced silencing complex (RISC) associating with other RNA-regulating proteins (eg, the Argonaute family).[3] Within the RISC, miRNAs repress protein expression by interacting with partially complementary mRNAs sequences (3'-untranslated regions), which leads to degradation or translational repression of the targeted mRNA.[3] In humans, more than 1500 miRNAs have been identified. miRNAs target several genes to hundreds of genes for potent regulation of biological processes, and in addition, several miRNAs can collectively target one mRNA.[4] These combinatorial interactions allow for a complex fine-tuning of regulatory processes, including cardiac repair, lineage commitment, proliferation, and survival of cardiomyocytes.

Targeting microRNAs as a strategy to prime circulating or bone marrow–derived mononuclear cells to improve their cardiac repair capacity

Cell-based therapies, using different autologous patient-derived cell populations, are being intensely examined as a potential strategy to improve cardiac function after injury *(Figure 1)*.[5] However, in several clinical trials of cardiac cell–based therapies, the functional and clinical benefits have been modest, which is potentially related to the impaired cardiac repair capacity of adult patient–derived cell populations. In this context, several groups, including ours, have demonstrated a dysregulation of miRNA expression in early angiogenic outgrowth cells (EOCs) of patients with CHF.[6] Notably, overexpression of miR-126, which is reduced in EOCs from patients with CHF, enhanced the in vivo cardiac repair capacity.[6] Another approach is to prevent apoptosis of transplanted cells, a process thought to substantially decrease the cardiac repair capacity after cell transplantation due to low survival of transplanted cells. Expression of miR-34a, a proapoptotic miRNA, was increased in bone marrow mononuclear cells (BMCs) from patients with myocardial infarction.[7] Pretreatment of BMCs with miR-34a inhibitors improved their capacity to restore cardiac function in a murine infarct model.[7] Hu et al applied a cocktail consisting of miR-21, miR-24, and miR-221 to cardiac progenitor cells, and showed an increase in their survival after cardiac transplantation in an experimental myocardial infarct model, which resulted in a better preserved left ventricular function.[8] Bim, an apoptosis inducer, was repressed by these three miRNAs,[8] demonstrating that multiple miRNAs can synergistically repress one target.

Hence, miRNAs have the potential to improve the

impaired cardiac repair capacity of adult stem cells and progenitor cells.[8] In addition, miRNA modulation of adult stem cells and progenitor cells may serve as a strategy to enhance cardiac repair processes in cell-based therapies.

Targeting microRNAs to promote cardiomyocyte lineage commitment in pluripotent stem cells

Stimulation of cardiomyocyte lineage commitment has been studied in embryonic stem cells (ESCs) and inducible pluripotent stem cells (iPSCs) as a potential strategy to promote regeneration of the myocardium *(Figure 1)*. Human ESC-derived cardiomyocytes enhanced cardiac function in a rat myocardial infarction model.[9] Recently, transplantation of human ESC-derived cardiomyocytes has been shown to integrate and survive after transplantation in nonhuman primates in

an experimental myocardial infarct model, resulting in remuscularization of substantial amounts of the infarcted monkey heart, albeit with an occurrence of nonfatal ventricular arrhythmias.[10] Dynamic regulation of miRNAs is involved in differentiation of ESCs toward a cardiomyocyte fate. In vitro studies have shown that miR-1, together with miR-499, are upregulated during the differentiation of human ESC (hESCs) and cardiac progenitor cells toward cardiomyocytes and overexpression of these miRNAs enhances differentiation toward a cardiomyocyte fate.[11,12]

miR-1 mediates developmental cardiomyocyte differentiation and its role in cardiomyocyte differentiation of hESCs in vivo was recently analyzed.[13] Transplantation of hESCs treated with miR-1 mimics improved cardiac function and increased the number of donor-derived cardiomyocytes.[13] Of interest, a decrease in cardiac apoptosis was detected in these
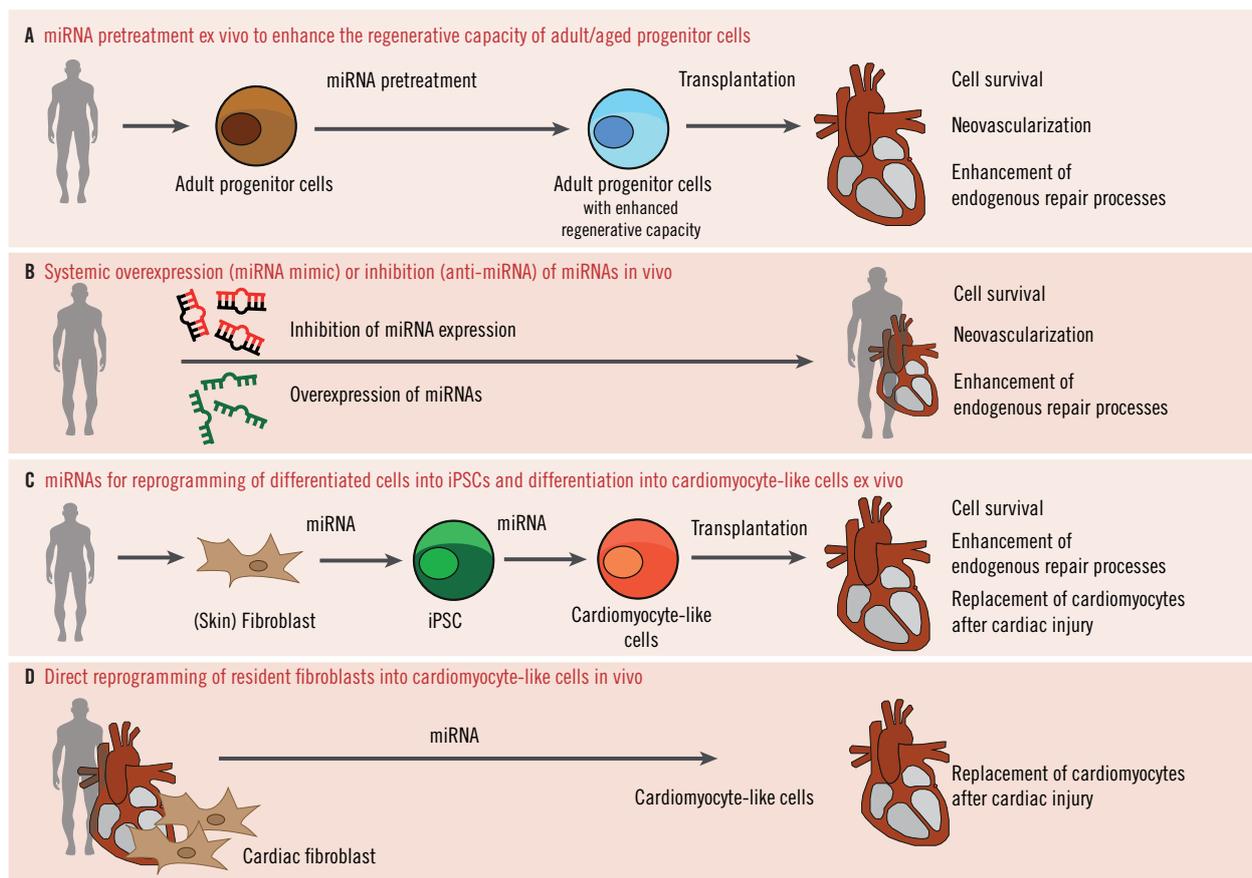
Fig. 1 *Potential therapeutic approaches targeting miRNAs to enhance cardiac repair processes and regeneration.*
miRNA-based therapies have been shown to efficiently enhance mechanisms involved in cardiac repair and regeneration in experimental models. **A.** *Isolated aged and adult progenitor cells frequently show an impaired cardiac repair potential, partly due to dysregulation of miRNAs. Priming of these cells by targeting miRNAs may result in an enhanced cardiac repair capacity.* **B.** *Direct inhibition of proteins involved in cell survival, apoptosis, and proliferation of cardiomyocytes and endothelial cells by local or systemic direct targeting of miRNAs may enhance cardiac repair and regenerative processes.* **C.** *Conversion of skin fibroblasts into iPSCs can be achieved by transfection with miRNAs. Thereafter, iPSCs can be differentiated toward a cardiomyocyte fate and used for cardiac regeneration after transplantation.* **D.** *miRNAs have the potential to directly convert cardiac fibroblasts into cardiomyocyte-like cells in vivo. The efficacy of such approaches in human cells needs to be further investigated.*
Abbreviations: *iPSCs, inducible pluripotent stem cells; miRNAs, microRNAs.*

mice,[13] suggesting that miR-1 not only facilitates cardiomyocyte differentiation, but also protects resident cardiomyocytes within the hostile milieu after cardiac injury. The role of miRNAs to enhance the cardiomyocyte lineage commitment of iPSCs has to be further explored in future studies.

Together, these studies reveal important roles of miRNAs for cardiac lineage commitment in stem cells. However, profiling of miRNAs involved in this differentiation process revealed complex spatiotemporal expression of different miRNAs.[14]

Targeting microRNAs to stimulate heart repair by direct cardiac reprogramming

Apart from governing ESC/iPSCs toward cardiomyocytes, another potential option for regenerating the heart is transdifferentiation of resident cardiac host cells directly into cardiomyocytes *(Figure 2)*. This process, termed direct reprogramming, circumvents the step of dedifferentiation into pluripotent stem cells and pursues reprogramming directly from endogenous noncardiomyocytes (eg, cardiac fibroblasts) toward functional cardiomyocytes. Qian et al recently reported the feasibility of direct reprogramming of mouse fibroblasts into cardiomyocytes.[15] Transduction of fibroblasts derived from mouse hearts and skin with three cardiac transcription factors (Gata4, Mef2c, Tbx5) directly induced cardiomyocyte-like cells with expression of cardiomyocyte-specific structures and promoters. These findings were successfully translated into a murine myocardial infarction model by using viral transfection of cardiac transcription factors.[15,16] Interestingly, Jayawardena et al[17] extended these observations by using miRNAs involved in cardiac muscle development. Overexpression of miR-1, miR-133, miR-208, and miR-499 in mouse cardiac fibroblasts directed these

cells toward cardiomyocytes with expression of cardiomyocyte markers and functions in vitro. Moreover, after an experimentally induced myocardial infarction, intramyocardial injection of this set of miRNAs converted cardiac fibroblasts into cardiomyocyte-like cells, which was shown by genetic tracing methods.[17] Recently, Nam et al[18] reported direct reprogramming of human fibroblasts, which are more resistant to reprogramming techniques, into cardiomyocyte-like cells. Use of cardiac-specific transcription factors (Gata4, Mef2c, Tbx5, and Hand2) alone were sufficient to reprogram mouse fibroblasts,[16] but not human fibroblasts.[18] Addition of myocardin (Myocd) effectively induced cardiac gene expression. Furthermore, the use of four transcription factors (Gata4, Hand2, Tbx5, and Myocd) together with miR-1 and miR-133 further improved reprogramming efficiency toward a cardiomyocyte phenotype.[18] Cardiac troponin T could be detected in 19% of transfected human fibroblasts. However, calcium transients and beating cells were rarely observed, indicating that together with the lack of upregulation of some cardiac genes, a full cardiomyocyte differentiation could not be achieved, yet.

Targeting microRNAs to stimulate cardiomyocyte proliferation in injured hearts

For many years, cardiomyocyte proliferation was thought to be a prerequisite in embryogenesis and for lower vertebrates *(Figure 2)*. However, elegant studies have now shown that cardiomyocyte proliferation still occurs in adults, although at a low level ($\approx$1% turnover rate/year).[2] Experimental studies have reported proliferation of cardiomyocytes after surgical injury in neonatal mice.[19] These observations indicate postnatal regeneration of the heart and the possibility of adult cardiomyocytes to reenter the cell cycle, also in an unstimulated state. Spatiotemporal expression of miRNAs plays an important role in cardiac muscle development; therefore, it may be used as a therapeutic strategy to stimulate endogenous regenerative processes leading to duplication of resident cardiomyocytes. Cardiac deletion of enzymes required for the biogenesis of miRNAs resulted in dilated cardiomyopathy and premature lethality,[20,21] indicating a pivotal role of miRNAs to modulate proliferative and apoptotic processes of cardiomyocytes.

Several miRNAs are involved in cardiac growth and function by either enhancing or decreasing mitotic pro-
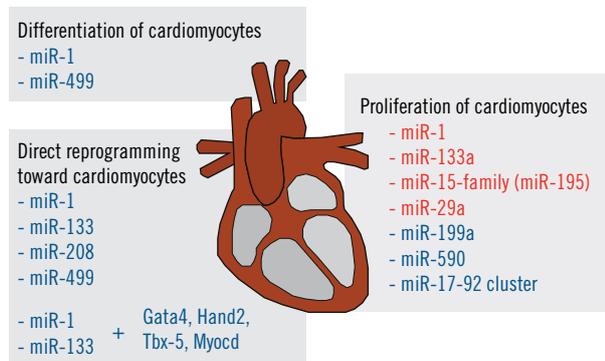

Differentiation of cardiomyocytes
- miR-1
- miR-499

Direct reprogramming toward cardiomyocytes
- miR-1
- miR-133
- miR-208
- miR-499

- miR-1
- miR-133
+ Gata4, Hand2, Tbx-5, Myocd

Proliferation of cardiomyocytes
- miR-1
- miR-133a
- miR-15-family (miR-195)
- miR-29a
- miR-199a
- miR-590
- miR-17-92 cluster

Fig. 2 *miRNAs involved in the regulation of cardiomyocyte differentiation and proliferation.*
Abbreviation: *miRNAs, microRNAs.*

cesses. miR-1 is a skeletal and cardiac muscle specific miRNA consisting of two miRNAs, miR-1-1 and miR-1-2, which are encoded by two genes.[22] Mice lacking miR-1-2 die early due to ventricular septal defects.[22] Mutant adult mice with normal cardiac function revealed hyperplasia, which is partly due to increased expression of proteins involved in cardiac morphogenesis and development, such as Hand2.[22] In contrast, cardiac-specific overexpression of miR-1 leads to decreased ventricular cardiomyocyte proliferation.[23]

miR-133a, which is cotranscribed as a bicistronic construct with miR-1, is involved in cardiac development. Deletion of miR-133a-1 and miR-133a-2 causes lethal ventricular septal defects in embryonic and neonatal stages and dilated cardiomyopathy in surviving adult mice.[24] In these double-knockout mice, disorganization of sarcomeres and increased proliferation and apoptosis of cardiomyocytes were detected. Consistently, cell cycle genes were upregulated in double-knockout mice. In contrast, diminished cardiomyocyte proliferation was observed in miR-133 transgenic mice.[24] These observations indicate that miR-133a is pivotal for withdrawal from the cardiomyocyte cell cycle and full differentiation into cardiomyocytes.

The transient regenerative capacity in postnatal murine hearts[19] was used to detect upregulated and downregulated miRNAs using a microarray approach.[25] miR-195, a member of the miR-15 family, is highly upregulated in mouse hearts between days 1 and 10 after birth. Therapeutic inhibition of the miR-15 family with anti-miRNAs in neonatal mice increased cardiomyocyte proliferation by enhancement of cell cycle genes.[25] Porrello et al[26] further investigated the impact of miR-15 on cardiac regeneration after cardiac injury in postnatal mice. Myocardial infarction in pups at day 1 functionally and fully resolves through day 21.[26] Cardiac-specific overexpression of miR-195 in these mice impaired the cardiac regenerative capacity with extensive fibrosis in the infarcted area and diminished proliferating cardiomyocytes,[26] consistent with the antiproliferative effects of the miR-15 family. Furthermore, pretreatment of postnatal mice with anti-miR-15 improved left ventricular function after induction of myocardial infarction in adult mice.[26]

A similar approach using a miRNA array was also conducted in cardiomyocytes of rats to decipher alterations in miRNA expression on postnatal days 2 and 28.[27] miR-29a expression increased extensively from day 2 to day 28 and was investigated for its antiproliferative capacity. Indeed, treatment of neonatal cardiomyocytes with miR-29a inhibitors significantly increased cardiomyocyte proliferation. Direct targets include cyclin D2, a cell cycle regulator.[27]

These studies investigated miRNAs with antiproliferative effects on cardiomyocytes. In contrast, an elegant study reported that miRNAs can also induce proliferation of cardiomyocytes.[28] To detect miRNAs involved in cardiomyocyte proliferation, a functional high-throughput screening was performed. Neonatal cardiomyocytes were transfected with a miRNA library consisting of 875 miRNAs.[28] Remarkably, 204 miRNAs increased neonatal cardiomyocyte proliferation in vitro. Two pro-proliferative miRNAs, miR-199a and miR-590, were further used for in vivo experiments. Overexpression of these miRNAs in neonatal rats revealed a thicker myocardium and increased cardiomyocyte proliferation. Moreover, intramyocardial overexpression of miR-199a and miR-590 in adult mice undergoing myocardial infarction induced cardiomyocyte proliferation in the peri-infarct area, reduced infarct size, and improved left ventricular function.[28]

The miR-17-92 miRNA cluster is involved in proliferative processes. Cardiac specific deletion of miR-17-92 leads to decreased cardiomyocyte proliferation in postnatal hearts.[29] Consistently, overexpression of miR-17-92 in embryonic and postnatal cardiomyocytes increased their proliferative capacity, culminating in a thickened myocardium due to hyperplasia. Intriguingly, induced cardiac expression of miR-17-92 in adult mice, where the proliferative capacity of cardiomyocytes is diminished, resulted in an increased cardiomyocyte proliferation. In addition, cardiac overexpression of miR-17-92 preserved cardiac function after myocardial infarction.[29] These studies indicate that reentry of cardiomyocytes into the cell cycle can be induced by administration of pro-proliferative miRNAs.

Conclusion

miRNAs are powerful mediators of the cardiac repair capacity in adult cell populations, cardiomyocyte lineage commitment in pluripotent stem cells, and cardiomyocyte cell cycle regulation. Therefore, therapeutic miRNA targeting to enhance cardiac repair, either by pretreatment of patient-derived cells used for cell-based therapies or direct local or systemic application, is a highly interesting potential therapeutic option to promote cardiac regeneration. Efficient inhibition of

deleterious miRNAs in large animal models by using locked nucleic acid (LNA)-modified antisense miRNA has now been reported[30,31] and a clinical phase 2 trial using LNA-modified antisense miRNA in patients with chronic hepatitis C successfully decreased viral load without toxic side effects.[32] Whereas anti-miRNA treatment seems to be effective, stable overexpression using miRNA mimic constructs is more challenging and needs an optimized delivery system.[33] Of note, miRNAs do not exclusively mediate their functions within the cell compartment, but are also actively or passively secreted. Therefore, pleiotropic effects may further enhance the therapeutic potential after miRNA delivery.

Moreover, a cell-specific delivery of miRNAs may be desirable because one miRNA can induce opposite effects in different cell types. The understanding of intercellular communication of miRNAs and their respective targets and functions in each cell type may contribute to an optimization of delivery strategies for miRNA-targeted therapies. ∎

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