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EDITORIAL COMMENT

Route 411 to Cardiac Regeneration*

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Each year, millions of people suffer from myocardial infarctions (MIs), resulting in the loss of billions of cardiomyocytes (CMs). The limited proliferation capacity of adult CMs results in a limited self-healing capacity of the human heart, and in the replacement of dead CMs by fibrotic scar tissue after MI. Although formation of scar tissue prevents rupture of the ventricular wall, remodeling of the remaining myocardium often leads to development of heart failure. Because the loss of CMs and the resulting loss of function are irreversible, effective cardiac regeneration strategies could prevent heart failure in millions of patients. In the last 2 decades, many strategies have been tested to obtain scarless myocardial repair: for example, transplantation of stem or progenitor cells; reprogramming of fibroblasts; or stimulation of proliferation of surviving CMs (**Figure 1**).[1]

Different approaches have been used to induce proliferation of CMs, including the administration of signaling proteins (eg, neuregulin-1 or periostin), overexpression of cell cycle genes (eg, cyclins), overexpression of transcription factors (eg, *Tbx20* or *Gata4*), and activation of intracellular signaling pathways (eg, Hippo, Notch, or ERBB signaling pathways).[2] A number of studies demonstrated that micro-RNAs (miRNAs) can regulate cell cycle re-entry of CMs as well. miRNAs are important in cardiac development and in normal cardiac physiology and can be altered in cardiac disease.[3] Because miRNAs typically regulate multiple genes simultaneously, they can be useful to release the cell cycle arrest and to reactivate cell division in adult CMs. Because miRNAs can be activated by mimics or inhibited by antagomirs, they also have promising therapeutic potential.[3]

In this issue of *JACC: Basic to Translational Science*, Nugroho et al[4] identified miR-411 as a potent inducer of CM proliferation. They showed that miR-411 induced CM regeneration and improved cardiac function after MI through activation of the Hippo/YAP pathway. These findings built on previous observations that miR-411 expression is higher in the developing embryonic heart than in the adult heart and that miR-411 regulates proliferation of cancer cells.[4]

As a first step, Nugroho et al[4] found native miR-411 expression to be significantly higher in primary neonatal rat CMs than in other neonatal non-CM cardiac cell types as well as adult CMs, suggesting a role for miR-411 in CM proliferation. In vitro, transfection of CMs with miR-411 mimics not only induced DNA synthesis and mitosis, but also protected CMs from hydrogen peroxide–induced oxidative stress. In vivo, miR-411 mimics were injected at the site of injury in a mouse model of MI, resulting in decreased scar formation, reduced myocardial hypertrophy, and improved cardiac function after MI. Similar to the in vitro results, miR-411 increased CM proliferation and decreased CM apoptosis in the in vivo MI model. Nugroho et al[4] made a commendable effort to gain mechanistic insights into the signaling pathways underlying the effects of miR-411. They identified activation of YAP, the main downstream effector of the Hippo pathway, as the main mechanism of miR-411–induced CM proliferation.[4]

The study by Nugroho et al[4] is an excellent addition to previous studies that have demonstrated that miRNAs are involved in cardiac regeneration and that miRNAs can induce CM proliferation. After birth, gene expression programs regulating CM proliferation start to lose importance relative to gene expression programs regulating CM differentiation, leading to a dramatic decrease in proliferative capacity of

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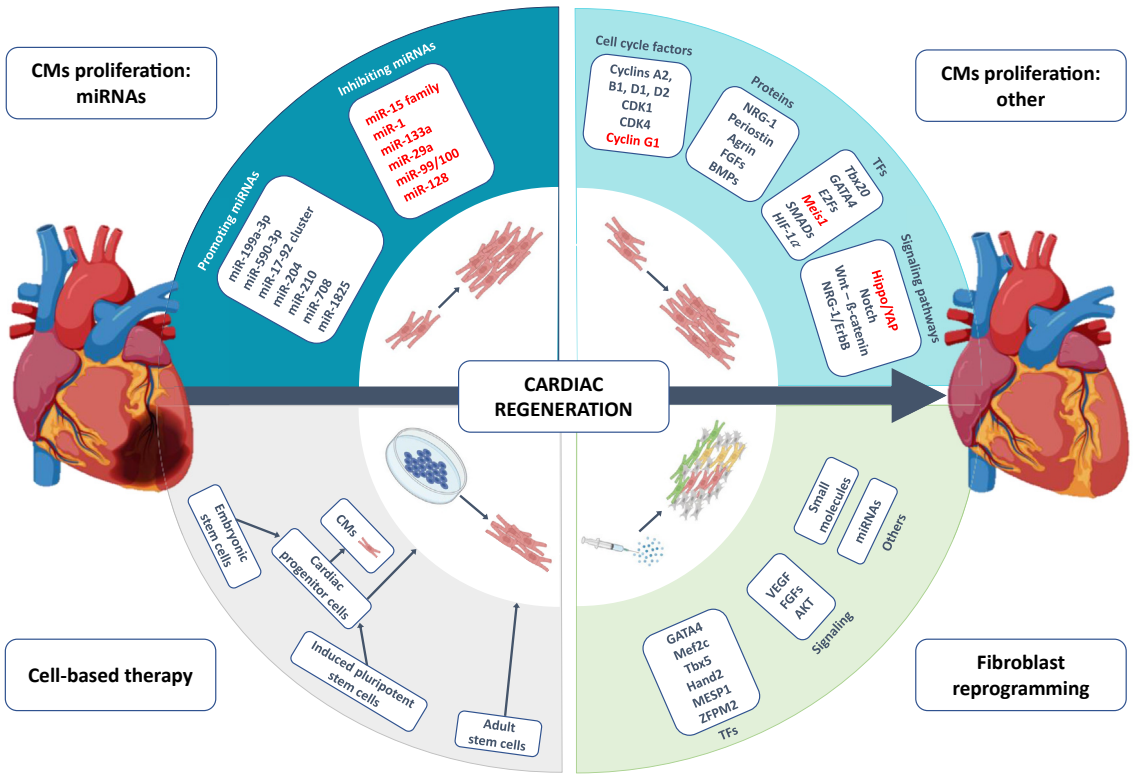
FIGURE 1 Cardiac Regeneration



ART = arterial tachycardia; CM = cardiomyocyte; FGF = fibroblast growth factor; miRNA = microRNA; VEGF = vascular endothelial growth factor.

CMs. Because gene expression is tightly regulated by miRNAs, inhibition of specific miRNAs regulating CM differentiation could reactivate CM proliferation and induce cardiac regeneration. Examples are miR-1 and miR-133, which are key regulators of CM maturation by targeting cell cycle-related genes. Similarly, the miR-15 family was found to induce cell cycle arrest of CMs and inhibition of miR-15 by antagomirs increased CM proliferation and improved cardiac function in a rodent model of MI.

The discovery of miRNAs, such as miR-411, triggering CM proliferation underlines that the exit from the cell cycle is not irreversible: exit can be manipulated by miRNA overexpression. For example, miR-199-3p and miR-590-3p are potent inducers of CM proliferation and, thus, cardiac regeneration. Given the complexity of cell cycle-related processes and the high number of uncharacterized miRNAs, new pro-proliferative miRNAs will likely be discovered in future studies. In the present study, neither the concept of proregenerative miRNA therapy, nor the main signaling pathway are completely novel. The pathway targeted by miR-411 and regulating CM proliferation is the Hippo/Yap signaling pathway, which has already been shown to be the target of several miRNAs such as miR-199a-3p, miR-590-3p, or miR-1825, all of which modulate cardiac regeneration. The fact that the Hippo/YAP signaling pathway has been shown—by independent research groups—to be targeted by multiple miRNAs regulating CM proliferation underscores the fundamental role of this pathway in CM proliferation. The present study suggests *Foxo1* to be a target of miR-411, but currently the direct or the most important target of miR-411 involved in CM proliferation has not been identified and might even reside outside the Hippo pathway.[3]

In this study, miR-411 increased both survival and proliferation of CMs, and an important unanswered question is which of these 2 mechanisms is most important in limiting infarct size. This question will become even more important when studied in models of chronic ischemic cardiac disease, with a pathophysiology based on cardiac remodeling, which occurs after the phase of acute CM death. Also, most studies in the field of cardiac regeneration focus on CMs, and less attention is paid to other cell types in

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the heart or to cell-cell communication. After MI, the cellular composition of the infarcted area changes dramatically: the number of CMs drops; there is a massive influx of inflammatory cells (mostly macrophages); fibroblasts proliferate and differentiate in myofibroblasts; and endothelial cells are activated in the process of neo-angiogenesis. The extent of the myocardial scar will not only depend on how many CMs die and how many new CMs are formed, but also on the number and activity of the other cell types in the heart. Moreover, cell-cell communication is not only important in regulating normal myocardial biology, but also this communication plays a role in regulating CM proliferation. Classic examples are the promitogenic properties of neuregulin-1 (secreted by endothelial cells) or periostin (secreted by fibroblasts) on CMs. In this regard, important questions to be answered are whether miR-411 influences the following: macrophage infiltration; fibroblasts proliferation or activation; angiogenesis; among others.

miRNAs have a real translational potential, but before miRNA-based therapies will increase CM proliferation in patients, a number of important issues will have to be solved. First, to identify a lead candidate, it is important to conduct a real head-to-head comparison of all identified miRNAs inducing CM proliferation. Second, optimization of this lead should be performed by modifying the sequence and chemical structure to improve efficacy as well as pharmacokinetic and toxicological properties. Third, an effective and targeted delivery strategy should be selected. The method selected in the current study—to deliver miR-411 by local intramyocardial injection—is excellent for a proof-of-concept study, but this method decreases translational potential. Local injection limits expression to a small spot at the injection site, limiting potential off-target effects, but at the same time requiring numerous injections, either surgically for epicardial injections or with complex interventional approaches for endocardial injections. Lessons learned from stem cell research in the past 2 decades have pointed to the many drawbacks of these approaches for the treatment of ischemic heart disease.

Systemic delivery of miRNA therapies could be a viable alternative to local injection. Efforts have been made in creating better delivery systems, with higher target specificity, longer duration of expression, and lower off-target toxicity. In patients, the delivery system will be as, if not more, important than the miRNA to minimize side effects and to enhance the efficacy of miRNA therapy. Viral-based delivery systems have been proven to be efficient to induce miRNA expression in the rodent myocardium. Nevertheless, there are concerns regarding immunogenicity, potential insertional mutagenesis, and high production costs. Additionally, expression needs to be tightly controlled in space and time to prevent uncontrolled CM proliferation and the resulting arrhythmogenicity. Nonviral methods, by which synthetic miRNA is formulated with lipids or polymers such as polyethyleneimine nanoparticles are potentially safer, but in general those methods have lower transfection efficiency. Nevertheless, progress in delivery of synthetic miRNA mimics or antagomirs can be expected in the near future.[5]

A fourth important issue in the translation of miRNA-based therapies is the need for well-controlled large-animal studies. Data of the effectiveness of miRNAs in large-animal models of MI are scarce, with the best example being miR-199a. Studies in large animals are not only important because cardiac physiology is closer to human, but also to test more realistic delivery strategies, interspecies differences, and immediate markers of effectivity, which will help in designing clinical trials.

Ultimately, the therapeutic potential of miRNA-based therapies resides in their position between small molecules and protein therapeutics. miRNA-based therapies potentially allow us to target pathways that are difficult to target with small molecules and at the same time allow for more flexibility in design, production, and delivery than recombinant proteins do.

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