Editorial

Synthetic MicroRNAs Stimulate Cardiac Repair

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MicroRNAs (miRNA) are short noncoding double-stranded RNAs that were found in several life forms, such as viruses, plants, animals, and humans. Their main function is to regulate gene translation by post-transcriptional binding of the RNA and prevent ribosomal translation (RNA silencing).[1,2] miRNAs are important for the regulation of many biological processes in our body, including organ development, maintaining stable, and steady-state function of tissues during adulthood and after injury or disease.[1,2] Although several miRNAs were found to be beneficial for cardiac regeneration, miRNAs are currently not in clinical use.[3–5] In 2012, Dr. Mauro Giacca Laboratory showed that human miRNAs can induce neonatal cardiomyocyte proliferation.[6] They identified 40 miRNAs that significantly increased both DNA synthesis and cytokinesis in neonatal mouse and rat cardiomyocytes. They then selected two of these miRNAs (hsa-miR-590 and hsa-miR-199a) and showed their ability to promote adult cardiomyocytes cell cycle re-entry in vivo. In addition, they showed that in a mouse myocardial infarction (MI) model, delivery of adeno-associated vectors (AAV) encoding hsa-miR-590 or hsa-miR-199a induced cardiac regeneration with almost complete recovery of cardiac functional parameters. Although viral-derived vectors such as AAV could be efficient tools to increase miRNAs expression, they have certain limitations that can hamper their use in inducing cardiomyocytes proliferation. The long-term expression using AAV vectors may lead to uncontrolled proliferation of the transfected cells increased cardiomyocytes size and cardiac hypertrophy. A recent report from the same laboratory, by Lesizza et al[7] in *Circulation Research*, circumvented this problem by using synthetic miRNAs (single-stranded RNAs) in the heart after MI. In their work, they focus on the two key candidate miRNAs mimic (hsa-miR-590-3p and hsa-miR-199a-3p) that show ability to induce cardiomyocytes proliferation and cardiac regeneration when were delivered in AAV9 vector (Figure).[6] In this study, they identify lipid vehicle (RNAiMAX) as the optimal delivery vehicle for delivery of synthetic miRNAs mimic in vitro and in vivo. Pharmacokinetic analysis of the synthetic miRNAs mimic shows activity for ≈12 days with most of the activity disappearing by day 20. In a murine MI model, single synthetic miRNAs mimic administration (hsa-miR-590-3p or hsa-miR-199a-3p) in lipid vehicle resulted in improved cardiac function (increase significantly % Ejection Fraction and % Fractional Shortening) and a significant decrease in scar size and an increase in cardiac wall thickness 8 weeks post-MI. This improvement in cardiac function post-MI was also accompanied by significant increase in survival compared with control miRs (cel-miR-67) post-MI. In addition, Lesizza et al[7] were able to confirm that hsa-miR-590-3p or hsa-miR-199a-3p increase adult cardiomyocytes proliferation and reduce apoptosis 12 or 2 days post-MI, respectively.

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Lesizza et al[7] show that synthetic miRNAs mimic can be efficiently used in vivo for inducing cardiomyocytes proliferation and regeneration post-MI. The short but sufficient pharmacokinetics (12 days) for induction of cardiac regeneration indicates similar to modified mRNA (modRNA),[8,9] short-term gene manipulation immediately post-MI may lead to substantial effects on cardiac[7] and cardiovascular[8] regeneration or formation of epicardial fat.[9] The fact that hsa-miR-590-3p or hsa-miR-199a-3p induce cardiomyocytes proliferation, and cardiac regeneration post-MI indicates that the miRNAs other strands (hsa-miR-590-5p or hsa-miR-199a-5p), that were delivered in the AAV9 vectors, had small or no influence on the beneficial effects observed by the 2 key candidate miRNA mimics. Also, the ability to use only on strand and not the other is beneficial as safety issues can arise from nonspecific miRNA activity. It will be important to test whether there are any synergistic effects of combining the 2 candidate miRNAs mimics in terms of cardiac regeneration. Also, while the authors used acute MI models for both published works,[6,7] it will be interesting to test the effects of the 2 candidate miRNAs mimics on more chronic MI models once ventricular dysfunction has been established. In addition, as these synthetic miRNA mimics are commercially available and can easily be scaled up to be used in larger animal models (eg, sheep or pigs), it will be clinically relevant to test these synthetic miRNA mimics on cardiac regeneration in large animal models post-MI.

It will be interesting to test the synergistic effects of combining modified RNAs and synthetic miRNA mimics for induction of cardiac regeneration in vivo. Both platforms are single-stranded RNAs that have been shown to be successfully delivered in the same lipid vehicle (RNAiMAX) in vitro and in vivo. Although synthetic miRNA mimics inhibit mRNA translation and prevent protein formation, modRNA increase mRNA translation and protein formation. That will allow the research community to manipulate genes of interest by upregulating and downregulating them. One main caveat for using synthetic miRNA mimics, is that unmodified single-stranded mRNA may elicit an immune response via activation of toll-like receptor 7/8. Nucleotide modification of single-stranded mRNA change

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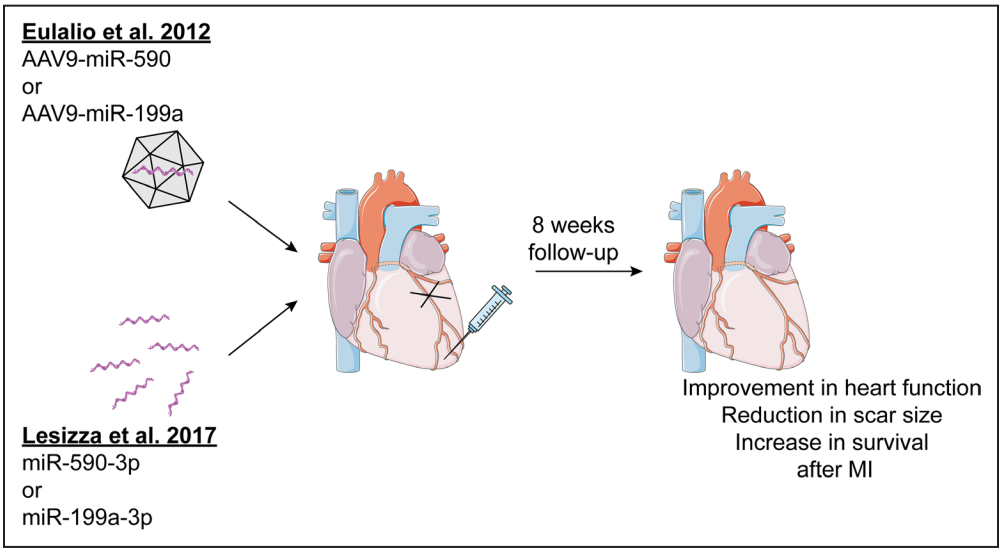

Figure. Synthetic miRNA mimics or AAV9 encoding for miR-590 or 199a, improve outcome after myocardial infarction (MI). Delivery of synthetic miRNA mimics miR-590-3p or hsa-miR-199a-3p post-MI, similar to delivery of AAV9-miR-590 or AAV9-miR-199a AAV9 reduces scar size, improves cardiac function and increases survival post-MI. AAV indicates adeno-associated vectors.

their secondary structure and allow them to escape toll-like receptor recognition (such as the case of modRNA). However, because of the short size of synthetic miRNAs mimics (22–23nt), the secondary structure cannot be manipulated.

In conclusion, this study will positively affect cardiac regenerative medicine by showing the use of synthetic miRNA mimics miR-590-3p or hsa-miR-199a-3p as potential novel therapeutic targets for cardiac regeneration after injury. More work is required to understand the molecular mechanisms involved in cardiac regeneration and to test this strategy in large animal models before translation into the clinical setting.

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Disclosures

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