In vivo Activation of a Conserved MicroRNA Program Induces Mammalian Heart Regeneration
Aguirre et al

The capacity of the adult mammalian heart to repair itself after injury is limited. In contrast, lower vertebrates such as Zebrafish can completely regenerate the organ after damage. A recent article from Aguirre et al\(^1\) in Cell Stem Cell shows that this difference is because of a microRNA program that is active in Zebrafish but silent in mammals. Crucially, reactivation of this dormant microRNA program in the murine heart induces regeneration of the myocardium.

The adult mammalian heart possesses a limited capacity to regenerate lost or damaged cardiomyocytes after cardiac insult.\(^2\) This is, in part, because of the low proliferative capacity of adult cardiomyocytes. Myocardial injury increases cardiomyocyte proliferation; however, it is clearly insufficient to completely regenerate the myocardium, and many strategies to enhance the weak regenerative response of the mammalian heart to injury are currently under investigation. The neonatal mammalian heart potentially possesses a higher regenerative capacity compared with the adult organ. In one study, cardiac regeneration was observed in 1-day-old neonatal mice after partial surgical resection.\(^4\) Using genetic fate mapping techniques, the researchers showed that the regenerated myocardium originated from preexisting cardiomyocytes proliferating in response to injury.\(^3\) This regenerative capacity was lost by day 7.\(^4\) Other studies have also pointed to an enhanced natural regenerative capacity in neonatal mice.\(^5,6\)

In contrast to mammals, lower vertebrates such as Zebrafish retain the ability to regenerate their hearts throughout life. In the case of the Zebrafish, this natural ability is particularly robust as complete cardiac regeneration has been observed even when \(\approx20\%\) of the ventricular myocardium was removed.\(^7\) The proliferation of partially dedifferentiated cardiomyocytes underlies this process.\(^8\) Specifically, cardiomyocytes that are adjacent to the site of injury break down their sarcomeres, express the embryonic cardiogenesis gene Gata4, and enter the cell cycle. Why lower vertebrates and mammals respond so differently to cardiac injury has been open to conjecture. It is possible that regenerative mechanisms active in lower vertebrates are silent in mammals. Similarly, evolutionary selection pressure may have influenced the appearance of a strong natural cardiac regenerative mechanism in lower vertebrates.

In this context, the recent article by Aguirre et al\(^1\) identified a microRNA program that was activated by cardiac injury in the Zebrafish. This microRNA program was found to be silent in the adult murine heart; importantly, activation of this program through molecular approaches was sufficient to regenerate the murine myocardium.\(^1\)

The premise of this elegant study was to determine the mechanisms underlying cardiac regeneration in the Zebrafish. The authors focused on changes in expression of microRNAs, a class of small noncoding RNAs that regulate genes at the post-transcriptional level.\(^9\) MicroRNAs were chosen by the authors for their ability to control multiple gene expression pathways simultaneously. The authors reported that significant changes in \(\approx60\) microRNAs were observed 3 days postamputation of the ventricular apex. Most of these \(\approx60\) microRNAs were downregulated. The authors decided to focus on microRNAs that displayed a high degree of conservation across vertebrates. This approach identified miR-99/100 and Let-7a/c. Using bioinformatics programs, the authors identified Fntβ and Smarca5 as potential targets for miR-99/100; targets subsequently confirmed by standard biochemical assays. One would expect there to be other targets for miR-99/100; however, these were not reported in the article. Significant elevation of Fntβ and Smarca5 protein expression was observed in the regenerating Zebrafish heart, paralleling the downregulation of miR-99/100. Immunofluorescence analysis further correlated Fntβ and Smarca5 expression with proliferating cell nuclear antigen and histone H-3 phosphorylation in cardiomyocytes, indicative of cell-cycle entry. Injecting miR mimics to prevent the downregulation of miR-99/100 inhibited Fntβ and Smarca5 expression, prevented cardiomyocyte proliferation, and inhibited regeneration of cardiac tissue. The authors did not directly manipulate Fnt or Smarca5 expression in this experiment; however, pharmacological inhibition of Fnt with a specific antagonist, tipifranib, significantly impaired cardiac
regeneration and decreased the number of proliferating cardiomyocytes. Interestingly, miR-99/100 antagoniRs significantly increased cardiomyocyte proliferation and ventricle size even in the absence of injury.

The authors then studied mammalian hearts with respect to miR-99/100 and Let-7a/c expression and functionality. Using immunofluorescence, the authors found that cardiomyocytes in embryonic (E11) mouse hearts had low levels of miR-99/100, whereas Fntβ and Smarca5 expression was high. In contrast, in the adult mouse heart miR-99/100 was expressed at a high level, whereas the expression of Fntβ and Smarca5 was barely detectable. Intriguingly, this expression pattern of Fntβ/Smarca5, high in young individuals and low in adults, was also observed in postmortem human heart tissue. In contrast to Zebrafish, miR-99/100 and Let-7a/c levels were sustained after injury to the adult murine heart. The authors examined whether the limited cardiac regeneration observed in the murine heart after injury could be explained by the failure to reduce miR-99/100 and Let-7a/c levels. They reported that silencing of miR-99/100 or Let-7a/c in isolated adult cardiomyocytes increased the expression of Gata4, a marker of cardiomyocyte dedifferentiation. The dedifferentiated cardiomyocytes acquired a proliferative phenotype as typified in the regenerating Zebrafish heart. Moreover, silencing of miR-99/100 or Let-7a/c in ex vivo cultured myocardial tissue induced sarcomere disassembly and Connexin-43 downregulation. The authors took these findings to be indicative of cardiomyocyte dedifferentiation. It should be noted that sarcomere disassembly also precedes cell death which may confound some of their interpretations. Notably, overexpression of Fntβ/Smarca5 bypassed the requirement for downregulation of miR-99/100.

Finally, they tested whether recapitulating the natural pathway in Zebrafish by reducing the expression of miR-99/100 after adult murine cardiac injury would promote regeneration of the damaged myocardium. Using a LAD (left anterior descending) ligation model for myocardial infarction, the authors found that lentiviral mediated knockdown of miR99/100 or Let-7a/c significantly improved cardiac function 15 days postinfarction. Lentiviruses do not efficiently transduce cardiomyocytes in vivo, so the functional benefits they observed may relate to effects of these microRNAs in other cell types besides cardiomyocytes. Going one step further, the authors then repeated the study using the cardio-tropic adenovirus-associated virus serotype AAV2/9. Again knockdown of miR-99/100 or Let-7a/c significantly improved cardiac function after myocardial infarction, as well as reducing the area of fibrosis. Furthermore, using immunohistochemistry, the authors were able to show that reducing the expression of miR-99/100 in the infarcted mouse heart increased the numbers of cardiomyocytes expressing Gata4, indicating cardiomyocyte dedifferentiation. Moreover, there was an increase in the number of cardiomyocytes expressing the mitotic markers anilin and aurora-B kinase, suggesting re-entry into the cell cycle.

For a long time, scientists have been attempting to understand why adult mammalian cardiomyocytes do not respond to injury by proliferating. It was originally thought that the complexity of contractile apparatus was a barrier to cardiomyocyte proliferation. However, embryonic mammalian cardiomyocytes are able to both contract and proliferate. Thus, a differentiated state is not an impediment to cardiomyocyte proliferation in the developing mammalian heart. After birth, mammalian cardiomyocytes withdraw from the cell cycle. Birth dramatically changes the cardiomyocyte environment, and several events that occur such as increased oxygen tension and mechanical stretch forces, as well as a change in the ECM (extracellular matrix) composition, have been posited to explain the withdrawal from the cell cycle. The molecular mechanisms that regulate mammalian adult cardiomyocyte proliferation are under intense investigation. It is clear from several laboratories that microRNAs are critically important regulators of cardiomyocyte proliferation. In this context, the study of Aguirre et al has provided an important understanding of the molecular basis for microRNA regulation of cardiomyocyte proliferation. It will be interesting to determine the mechanism by which their miR targets, Fntβ and Smarca5, promote adult mammalian cardiomyocyte proliferation. Smarca5 is involved in chromatin remodeling and the response to DNA damage. Notably, oxidative damage to DNA is believed to block cardiomyocyte proliferation at birth. Previously, several strategies have been used to increase cardiomyocyte proliferation, the effects have been relatively modest. This study has yielded some of the most impressive results to date and has provided evidence for a molecular basis of cell cycle blockade in the cardiomyocyte. The therapeutic implications of the findings are significant, suggesting that regeneration of the human heart may be accomplished by reactivating a dormant pathway conserved across species.

In addition to providing important insight into the mechanisms underlying cardiac regeneration in mammals and Zebrafish, this study is a further example of the attractiveness of microRNAs as therapeutic agents. These small noncoding RNAs have emerged as crucial regulators of gene expression and are key players in many human diseases. Moreover, microRNAs can improve the standard gene therapy currently used, notably by enhancing specificity. Furthermore, naturally occurring multicistronic microRNAs can be modified to express several microRNAs in a dose-dependent fashion, and this approach has been used to target hepatitis C virus and human immunodeficiency virus infection. In this context, we have previously shown that a specific combination of microRNAs (miR combo: miR-1, miR-133a, miR-208a, and miR-499-5p) known to be involved in cardiac development can directly reprogram cardiac fibroblasts into cardiomyocytes both in vitro and in vivo. This miR combo when injected into the infarcted heart induced new cardiomyocyte formation from lineage-traced fibroblasts. These microRNA-induced cardiomyocytes expressed sarcomeric structures and exhibited excitation–contraction coupling, and action potentials characteristic of mature ventricular cardiac myocytes. These cells were integrated into the myocardium. Moreover, we documented that this miR combo promoted progressive and significant improvement in cardiac function. Our results demonstrated the therapeutic potential of miR for cardiac regeneration.

Previous studies have also shown that specific microRNAs expressed in response to myocardial injury might mediate cardiac pathology. The blockade of these miRs using antagoniR
prevented or reversed the pathology. For example, administration of an antagonist to miR-21 (antagomiR) reduced fibrosis/hypertrophy and preserved cardiac function under conditions of pressure overload. AntagomiRs block miR activities by binding via sequence complementarity with microRNA. Anti-miRs can be chemically modified to contain cholesterol and phosphorothioate moieties to increase their stability. Importantly, the study of Aguirre et al. showed that using specific antagomiRs to recapitulate a developmental program in the injured heart could promote cardiac regeneration.

In summary, the recent studies of miR expression in the heart have advanced our understanding of the molecular mechanisms of cardiac development and diseases. Importantly, they elucidated the significant potential of manipulating miR expression as a therapeutic strategy for cardiac disease especially for regeneration and repair.

Disclosures

None.

References

Conserved MicroRNA Program as Key to Mammalian Cardiac Regeneration: Insights From Zebrafish
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