HEART REGENERATION

Small RNA: From development to regeneration

Ge Tao,[1,2] Jun Wang,[1,2] James F. Martin[1,2,3,4]*

A microRNA cluster that targets the Hippo pathway can reintroduce terminally differentiated cardiomyocytes into the cell cycle, promoting heart regeneration (Tian *et al.*, this issue).

MAMMALIAN HEART REGENERATION: MYTH OR MIRNA?

The heart provides the physiologic capacity to live a long and productive life. And yet, despite its critical life-providing function, the heart's reparative capacity after injury is remarkably limited. Indeed, the notion that the heart is a terminally differentiated organ with a nonrenewable supply of heart muscle cells is firmly embedded into the dogma of modern medicine. The evidence supporting the idea of a nonrenewable heart is compelling and will not be reviewed here, other than to say that cardiomyocyte renewal in the adult heart is a very rare event (*1*). However, in recent years several insights have suggested to many investigators that it is time to take a fresh look at the mammalian heart to search for developmental cues that may trigger cardiomyocyte renewal after injury. In the current issue of *Science Translational Medicine*, Tian *et al.* discover microRNAs (miRNAs) that enhance cardiomyocyte renewal after injury and that may have therapeutic efficacy when overexpressed (*2*).

As is often the case, clues from experiments in genetically tractable model organisms, such as zebrafish and fruit flies, have played an essential role in moving this difficult biological challenge forward. The concept that there is some regenerative capacity in the heart was sustained by work on nonmammalian vertebrates. For example, adult zebrafish have a robust cardiac regenerative capacity. Resection of the adult zebrafish cardiac apex induces a regenerative response that results in complete repair in 1 month (*3*). In addition, work in zebrafish has contributed insights into conserved mechanisms of heart regeneration in mammals. For example, new zebrafish cardiomyocytes have been shown to derive from preexisting cardiomyocytes that go through a process of dedifferentiation to repair the injured heart (*3*).

Data from mice, including in Tian *et al.*, indicate that preexisting cardiomyocytes that dedifferentiate are an important source of new heart muscle (*4*). Porrello *et al.* demonstrated that mammalian hearts have regenerative capacity in the immediate postnatal period. Mice can regenerate their heart for 6 days after birth, but by postnatal day 7 (P7), the heart loses its regenerative capacity (*4*). This regenerative-to-nonregenerative transition point provides a valuable spatiotemporal window in which to investigate mammalian heart regeneration. Cardiomyocyte proliferation is crucial for regeneration, and the mechanisms that switch off cardiomyocyte proliferation in the postnatal transition are only beginning to be unraveled.

HIPPO IN HEART DEVELOPMENT

After birth, as the expression of genes responsible for cell-cycle reentry, mitosis, and cytokinesis falls precipitously, the majority of neonatal cardiomyocytes exit the cell cycle. Hypertrophic growth remains to enlarge the heart and maintain proper physiological functions. One genetic pathway that extends the heart's regenerative window beyond the P7 limit when deleted is the Hippo signaling pathway (*5*).

Investigation of the Hippo pathway in *Drosophila* has provided important clues for understanding proliferation in mammalian cardiomyocytes. In *Drosophila*, Hippo pathway activation acts as a "stop proliferating" signal to the cell. In the developing mouse heart, many key Hippo pathway components are functionally conserved throughout evolution (*6*). In mammals, Mst1, Mst2 (Hippo in *Drosophila*), and their regulatory protein WW45 (Salv in *Drosophila*) form an activated complex, which then phosphorylates the large tumor suppressor homolog kinases (Lats) 1 and 2 (Warts in *Drosophila*) (*6*). The most downstream Hippo pathway components are the transcriptional coactivators Yap (Yki in *Drosophila*) and Taz, which promote transcription of pro-proliferative genes. Lats kinases phosphorylate mouse Yap at Ser^{127} and Taz at Ser^{89}, inhibiting their nuclear function by promoting their nuclear exclusion (*6*).

In a low-Hippo context, such as quickly growing embryonic cells, Yap and Taz localize to the nucleus, where they serve as coactivators of TEA-domain family members (TEAD) of DNA-binding transcription factors. Together, the Yap/Taz–TEAD complex promotes gene programs that favor proliferation and cell survival. Deletion of Hippo pathway components leads to organomegaly as the result of increased mitosis and, in some contexts, decreased apoptosis (*6*–*9*). Cardiac-specific deletion of the Hippo pathway kinases or expression of constitutively active Yap results in dramatic cardiomegaly owing to elevated cardiomyocyte proliferation during development. Conversely, Yap deletion causes myocardial hypoplasia and heart failure.

The exceedingly rare proliferation observed in mature mammalian cardiomyocytes results in only a limited reparative response within the heart after an ischemic insult, such as a myocardial infarction. Owing to its role in development, recent work has focused on investigating the Hippo pathway in the context of heart regeneration after injury. The developmental studies in mice—indicating that the Hippo pathway is a critical negative regulatory pathway that prevents cardiomyocyte proliferation—suggested that removing Hippo inhibition in the adult heart may have a beneficial effect on its reparative capacity. Indeed, removing Hippo pathway components or overexpressing a constitutive active form of Yap resulted in substantially improved adult heart function with increased cardiomyocyte proliferation and reduced scar formation after myocardial infarction in adult mice (*9*).

MIRNAS IN HEART REGENERATION: DEVELOPMENT REDUX

The heart can be induced to repair itself, from endogenous cardiomyoctes, by inhibiting the Hippo pathway, a key component of mammalian development. One means of modulating cell signaling and phenotype is miRNA—small, noncoding RNAs that inhibit gene expression posttranscriptionally through Watson-Crick base pairing to

[1]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030, USA. [2]Cardiovascular Research institute, Baylor College of Medicine, Houston, TX 77030, USA. [3]Texas Heart Institute, Houston, TX 77030, USA. [4]Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA.
*Corresponding author. E-mail: jfmartin@bcm.edu

sequences, called "seed sites," within the 3′ untranslated region (3′UTR) of target genes. Several studies have investigated miRNAs in myocardial regeneration, but without making a direct connection to an established genetic pathway that regulates cardiac regeneration.

In this vein, Tian *et al.* examined the miRNA 302-367 cluster (miR-302-367) because of its role in maintenance of tissue-specific progenitors in early development. Using both loss- and gain-of-function studies in the mouse heart, the authors showed that miR-302-367 promoted embryonic cardiomyocyte proliferation. miR-302-367 gain of function led to cardiomegaly in fetal and juvenile hearts with a more undifferentiated cardiomyocyte phenotype, similar to developing hearts with disrupted Hippo signaling (*5*). Indeed, in their screening studies Tian *et al.* linked miR-302-367 to key Hippo pathway components, including *Mst1* and *Lats2* that have been directly connected to cardiomyocyte growth control and *Mob1b* encoding a Lats interacting protein (*2*). Thus, Tian *et al.* make a firm connection between the miR-302-367 cluster, the Hippo pathway, and myocardial regeneration.

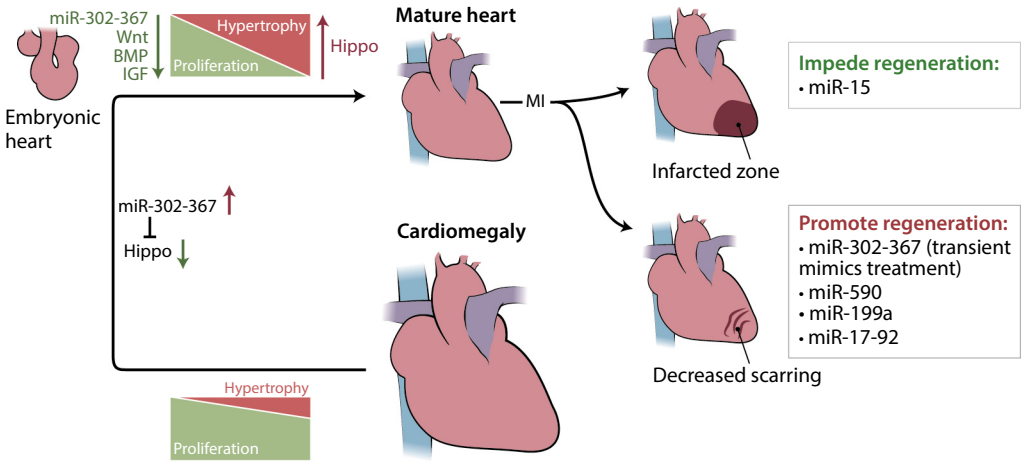

Fig. 1. miRNAs in cardiac development and regeneration. Spatiotemporal regulation of cardiomyocyte proliferation is crucial for building a functional heart of proper proportions. Embryonic cardiomyocytes rapidly proliferate to build a functional myocardium and are regulated by Hippo as well as Wnt, bone morphogenetic protein (BMP), and insulin-like growth factor (IGF) signaling during development. The maturing heart then undergoes a transition from hyperplastic to hypertrophic growth, and the majority of neonatal cardiomyocytes exit the cell cycle shortly after birth. Increased cardiac Hippo signaling is crucial for inhibiting cardiomyocyte proliferation in the normal heart. After myocardial infarction, transient activation of miR-302-367 or other miRNAs, such as miR-17-92 (*10*), improves functional recovery of damaged mouse myocardium by promoting adult cardiomyocyte proliferation, as shown by Tian *et al.* (*2*). However, constitutively active miR-302-367 leads to deficient Hippo activity—via *Mst1*, *Lats2,* and *Mob1b*—and cardiomegaly in mice.

It is important to add a cautionary note that miRNAs have multiple targets and, depending on context, may have different functional effects. miRNAs have the potential to control multiple processes during cardiac repair, including cell death, metabolism, and proliferation (*10*), and miRNA-promoted myocardial recovery does not simply rely on cell proliferation. A case in point of a miRNA that has distinct, context-dependent function is the miR-199a-214 cluster that facilitates a maladaptive metabolic shift from fatty acid utilization observed in the healthy heart toward increased glucose metabolism associated with heart failure in a pressure overload context (*10*). In contrast to what was found in the myocardial infarction model in which miR-199a overexpression had a beneficial effect, in the pressure overload model antagomirs inhibiting miR-199a and miR-214 improved cardiac function and reduced fibrosis and hypertrophy (*10*).

Knowing the effects of miR-302-367 on regeneration is important in developing a viable therapeutic for clinical translation. Infarcted mouse hearts treated systemically with a miR-302-367 mimic demonstrated decreased cardiac fibrosis and improved heart function as compared with those of control mimics. However, the authors found that persistent reexpression of miR-302-367 in the postnatal heart led to a prolonged dedifferentiated state and, ultimately, heart failure. The timing of miRNA mimic therapy must therefore be considered carefully when moving into human trials. In order to make miR-302-367 mimic therapy safe, there will have to be more studies into mimic pharmacokinetics. Moreover, once the stability of the mimic has been determined, protocols will have to be established in order to carefully monitor heart function in patients undergoing treatment.

MIR-BASED THERAPY: PROMISE AND PITFALLS

Although considerable recent progress has been made in the field of cardiac regeneration, current therapeutic strategies remain under investigation (*10*). Inhibiting miR-34a or miR-15 activity or overexpressing miR-590 may improve cardiac function after adult myocardial infarction (*10*). Yet another example is the miR-17-92 cluster that shares some seed sites with the miR-302-367 cluster implicated by Tian *et al.* Increased miR-17-92 expression induced cardiomyocyte proliferation in mice that led to improved cardiac function with a reduced scar size after myocardial infarction (*10*). Although it is clear that miRNA-based therapeutics hold translational potential in regulating myocardial proliferation and regeneration, definitive results from clinical trials are still pending.

The current study by Tian *et al.* has yielded many insights that may help to overcome this barrier in translation. Hypothesizing that the disassembly of rigid sarcomere structure within cardiomyocytes is critical for cytokinesis to occur, the authors discovered and manipulated a miRNA complex that enhances cardiomyocyte dedifferentiation with sarcomere breakdown. Thus, the strategy to promote cardiomyocytes to acquire a developmentally more primitive phenotype was successful and may be useful in future efforts. In the future, miRNA targeting of master heart development regulatory genes may permit more tailored approaches to treat specific heart disease. Tian *et al.* further emphasize that delivery of miRNA-based therapeutic constructs is an attractive option even when done systemically (Fig. 1). The miRNA-based therapy is technically feasible, given that miRNA levels can be simply ma-

nipulated by "antagomirs" or locked nucleic acid (LNA) miRNA antagonists to inhibit miRNA function—or, conversely, miRNA mimics or AAV-encoded miRNAs to overexpress miRNAs in a pathologic context.

Although more work needs to be done, regeneration-promoting miRNAs, such as miR-302-367, likely have evolutionarily conserved functions from mouse to human, making it possible to experimentally define miRNA function in depth and in many pathologic contexts using mouse models. Moreover, studying the heart-regenerative miRNAs in different in vivo contexts may enable investigators to identify new druggable gene targets.

Hurdles remain in the road to achieving long-term benefit from miRNA treatment. Understanding all relevant side effects of potential miRNA treatment is crucial. The same concern applies to the target genes that are manipulated by miRNAs. It will be essential to understand how to safely manipulate pro-regenerative signaling pathways in order to enhance regenerative potential while minimizing toxic side effects. Tian *et al.* raised this concern by showing that persistent expression of miR-302-367 in genetically engineered mice failed to restore the heart function and instead caused damage. Moreover, although Hippo pathway disruption will lead to myocardial regeneration, the precise mechanisms that regulate Hippo are yet to be defined. Specifically, at what level should Hippo be inhibited to optimize heart functional recovery? Therapies directed toward promoting cardiomyocyte proliferation may need to be transient in nature so as to avoid prolonged cardiac dysfunction (*2*).

The exciting findings from Tian *et al.* tighten the connection between developmental biology and regenerative medicine. Their data support the notion that knowledge of the developing heart provides a useful roadmap that can be used to navigate strategies to enhance human cardiac tissue regeneration. In the future, it will be important to test other developmental signaling pathways for regenerative potential in order to have a complete genetic toolbox to repair the heart and other human organs.

REFERENCES AND NOTES

1. J. H. van Berlo, J. D. Molkentin, An emerging consensus on cardiac regeneration. *Nat. Med.* **20**, 1386–1393 (2014).
2. Y. Tian, Y. Liu, T. Wang, N. Zhou, J. Kong, L. Chen, M. Snitow, M. Morley, D. Li, N. Petrenko, S. Zhou, M. Lu, E. Gao, W. J. Koch, K. M. Stewart, E. E. Morrisey, A microRNA-Hippo pathway that promotes cardiomyocyte proliferation and cardiac regeneration in mice. *Sci. Transl. Med.* **7**, 279ra38 (2015).
3. K. Kikuchi, K. D. Poss, Cardiac regenerative capacity and mechanisms. *Annu. Rev. Cell Dev. Biol.* **28**, 719–741 (2012).
4. E. R. Porrello, A. I. Mahmoud, E. Simpson, J. A. Hill, J. A. Richardson, E. N. Olson, H. A. Sadek, Transient regenerative potential of the neonatal mouse heart. *Science* **331**, 1078–1080 (2011).
5. T. Heallen, Y. Morikawa, J. Leach, G. Tao, J. T. Willerson, R. L. Johnson, J. F. Martin, Hippo signaling impedes adult heart regeneration. *Development* **140**, 4683–4690 (2013).
6. D. Pan, The Hippo signaling pathway in development and cancer. *Dev. Cell* **19**, 491–505 (2010).
7. B. Zhao, K. Tumaneng, K. L. Guan, The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. *Nat. Cell Biol.* **13**, 877–883 (2011).
8. G. Halder, R. L. Johnson, Hippo signaling: Growth control and beyond. *Development* **138**, 9–22 (2011).
9. H. Wackerhage, D. P. Del Re, R. N. Judson, M. Sudol, J. Sadoshima, The Hippo signal transduction network in skeletal and cardiac muscle. *Sci. Signal.* **7**, re4 (2014).
10. J. Wang, J. F. Martin, Macro advances in microRNAs and myocardial regeneration. *Curr. Opin. Cardiol.* **29**, 207–213 (2014).

Acknowledgments: This work was supported by grants from the National Institutes of Health (NIH) (HL 118761 to J.F.M.), the Vivian L. Smith Foundation (J.F.M.), and the American Heart Association (14SDG19840000 to J.W. and 13POST17040027 to G.T.).

10.1126/scitranslmed.aaa7538

Citation: G. Tao, J. Wang, J. F. Martin, Small RNA: From development to regeneration. *Sci. Transl. Med.* **7**, 279fs12 (2015).

Science Translational Medicine

Small RNA: From development to regeneration

Ge Tao, Jun Wang and James F. Martin

Sci Transl Med **7**, 279fs12279fs12.
DOI: 10.1126/scitranslmed.aaa7538