Heart Regeneration
A Tale of Cell Reprogramming

Aitor Aguirre, Ignacio Sancho-Martinez, Juan Carlos Izpisua Belmonte

In vivo cardiac reprogramming contributes to zebrafish heart regeneration
Zhang et al.

Is reprogramming a physiological repair mechanism occurring during cardiac regeneration?

Heart failure is one of the main health problems worldwide, and the urgent medical needs relating to it have positioned cardiovascular research as one of the most actively evolving fields in regenerative medicine. Many different strategies aiming to revert, palliate, or ameliorate the deleterious effects of heart failure have been intensively pursued, and excellent reviews on the topic exist.1-3 Briefly, approaches used to tackle cardiac repair have been focused mainly on the use of stem cells to replenish lost muscle mass (cell transplantation or mobilization of resident cardiac stem cells), reduction of cardiomyocyte hypertrophy or prevention of fibrosis, promotion of angiogenesis, and, most recently, in vivo reprogramming strategies.4-6 Although these significant advances are contributing to the development of more efficient therapies, successful and efficient heart repair strategies are by and large still lacking. Are there alternative approaches beside those mentioned above? A possible line of attack is to look at how nature deals with this issue.

Regenerative animal models are able to regenerate the heart completely.7 They do so by preventing scar formation, replenishing lost muscle tissue, and eliciting a controlled proangiogenic response. All these processes lead to a complete repair of the injured heart. Whereas it is well known that these processes occur naturally only in lower vertebrates, it has been a long established dogma that the mammalian heart is not able to regenerate. However, although the adult mammalian heart is not able to regenerate after massive myocardial damage, it can activate a healing process resembling that of regenerative organisms.8 Although the numbers of newly generated cardiomyocytes are insufficient for healing of the heart, it is clear that endogenous repair mechanisms are present in the mammalian heart, thus providing a potential target for therapeutic intervention and activation of proliferation. In support of these observations, Porrello et al9 have shown the potential for neonatal murine models to regenerate the injured heart in an analogous manner to a typical regenerative animal model, such as the zebrafish. Thus, and in parallel to mammal studies, by elucidating the basic molecular mechanisms underlying heart regeneration in other vertebrates, we might find strategies for the reactivation of seemingly dormant regenerative mechanisms in the human heart.

In this regard, in the June issue of Nature, Zhang et al10 provide a new cellular and molecular understanding of the mechanisms underlying heart regeneration in the zebrafish. Using lineage tracing and cell-specific ablation experiments, the authors show that embryonic zebrafish heart regeneration relies, at least in part, on in vivo reprogramming of atrial cardiomyocytes into their ventricular counterparts. The authors demonstrate that atrial cardiomyocytes migrate through the atrium and the atrioventricular canal toward the ventricle, where they gradually shift their genetic program to finally acquire a ventricular phenotype. This mechanism of cardiomyocyte identity reprogramming is heavily dependent on Notch signaling. The importance of Notch signaling during adult zebrafish heart regeneration has been previously highlighted.11 Zhang et al extend and expand these earlier observations demonstrating that endocardial Notch activation is necessary for the regenerative response to occur. Because Notch is known to be required for heart development, these data add to the idea that regeneration and healing of the heart may necessitate the activation of previously silent developmental programs.12

Two transgenic zebrafish models were key to address the potential role of atrial cardiomyocytes in regeneration. On the one hand, a transgenic cell lineage tracing reporter for atrial cardiomyocytes (amhc:eGFP) allowed for live cell fluorescence tracking of migrating atrial cardiomyocytes to the ventricular zone. On the other hand, animals carrying a transgenic reporter in combination with a chemical ablation system hinging on ventricular myosin heavy chain (vMHC) promoter (vmhc:mCherry-NTR), allowed for precise, time-controlled injury. Whereas the former could be seen as a more traditional system for live cell imaging, the latter represents a refined dual-purpose transgenic model. By relying on the vMHC promoter, a gene specific of ventricular cardiomyocytes driving the expression of a mCherry reporter with an attached bacterial nitroreductase, the authors were able to perform both lineage tracing and population-specific ablation studies. Administration of metranidazole, a substrate of nitroreductase, leads to the production of a toxic DNA cross-linker, resulting in quick and autonomous cell-specific death.13 Because ventricular cardiomyocytes are the only...
cells expressing this isoform of MHC (vMHC), metanidazole effects cause specific ventricular ablation. Similar systems have been used in the past to uncover the mechanisms of regeneration in the fish with a high degree of success, also in adult specimens.13–15 In the report by Zhang et al, injury during embryonic development led to a significant reduction in ventricular mass followed by enlargement of the atrium. At 48 hours postinjury, a mass of migrating cardiomyocytes was observed in the atrioventricular canal moving toward the ventricle. The appearance of these cardiomyocytes, which are positive for both atrial GFP and ventricular mCherry, was associated to ventricular function improvement, thus suggesting in vivo reprogramming of atrial to ventricular phenotype. Genetic lineage tracing with amhc:CreERT2 transgenic animals confirmed that new ventricular myocytes were of atrial origin, and that such reprogrammed populations were still present in regeneraged adult animals. These reprogrammed atrial cells present a rounded morphology, with downregulation of atrial MHC and N-cadherin, disorganized sarcomeres, and expression of proliferative markers, suggesting a dedifferentiated state similar to that described by us and others in the regenerating adult fish14,15 as well as in the neonatal mouse.9 Subsequently, dedifferentiated atrial cardiac cardiomyocytes are able to redifferentiate to ventricular cardiomyocytes by upregulating cardiac specification transcription factors, such as Gata4, Mef2, Tbx5, and Hand2. Noticeably, these genes have also been reported to be reliable drivers for the transdifferentiation of resident cardiac fibroblasts to cardiomyocytes.5,6 This last process, however, occurs in the absence of a dedifferentiation phase, and it is thus unknown whether it is a consequence of dedifferentiation in the zebrafish or a driver phenomenon in pure transdifferentiation processes. Expression of GATA4, MeF2c, and other early cardiac lineage transcription factors has also been related to endogenous cardiac progenitor cells in mammals.2,3 The lineage tracing and live cell imaging experiments provided by Zhang et al argue against the contribution of immature cardiomyocytes or cardiac progenitor cells during zebrafish heart regeneration and provide robust evidence of transdifferentiation.

Together, these results support the notion that the reprogramming of cellular fates may be a natural physiological process in organisms capable of cell regeneration in the adult heart. For example, in the axolotl, cells in the limb blastema are able to dedifferentiate to reacquire proliferative and multilineage differentiation capabilities that facilitate regeneration.16 The same process has been observed in the zebrafish tail blastema, with evidence of epigenetic reprogramming.17 Therefore, physiological dedifferentiation seems to be a requisite for regeneration. This step is connected to the need for reprogramming toward a proliferative, malleable, intermediate cell state, at least in the case of the heart.18 In clear contrast, pure transdifferentiation has been mainly attributed to the unnatural or artificial imposition of target cell identities, and its natural occurrence during endogenous tissue repair seems improbable.19,20 In mammals too, recent reports seem to support this view, although the potential of dedifferentiating to an early progenitor state and then to redifferentiate into different lineages seems far more restricted. Taken together, these observations suggest that reprogramming approaches mimicking regenerative responses by leading to a partially dedifferentiated cellular state might provide a more suitable strategy for healing the heart.

Despite the promise of these elegant investigations, several questions remain unanswered, which are certain to instigate further studies. For example, how do these observations correlate with those reported by Porrello et al9 during neonatal mouse heart regeneration? Is the process reported by Zhang et al9 a true regenerative response that could be relevant to the induction of regeneration in adult organisms, or is it only a natural consequence of developmental programs only present in the embryo?21 The fact that atrial cardiomyocytes failed to regenerate the adult zebrafish heart supports the latter, while highlighting that other mechanisms could govern regenerative responses in adult organisms.10,14 In any case, this should not detract from the value of the results presented by the authors, or from the outstanding contributions that have been recently reported in the field of heart regeneration. One of the most significant findings of the report by Zhang et al is the identification of atrial cardiomyocyte populations as a novel source of ventricular cardiomyocytes. Such findings will offer new insights into regenerative mechanisms suitable for manipulation, as well as provide an additional avenue for the future development of novel therapeutics. Ultimately, these studies may contribute to the improvement of heart repair strategies for mammals. Reactivation of developmental programs or the induction of regenerative responses might allow for the experimental activation of natural regenerative responses in humans with heart disease. Elucidation of the fundamental mechanisms underlying natural regeneration and their experimental reactivation represent not only an interesting and revolutionary area of basic research, but also provide an exciting platform to complement existing approaches for the restoration and healing of human tissues and organs.

Disclosures

None.

References


Heart Regeneration: A Tale of Cell Reprogramming
Aitor Aguirre, Ignacio Sancho-Martinez and Juan Carlos Izpisua Belmonte

Circ Res. 2013;113:1109-1111
doi: 10.1161/CIRCRESAHA.113.302519
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/113/10/1109

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/