Cardiovascular Genetics: A News Round-Up

A Repair Tool-(c)Kit for the Injured Heart

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Study Hypothesis

It is now accepted that the heart is a self-renewing organ, although its regenerative capacity is insufficient to restore healthy homeostasis after injury in human adults. Despite advances in characterization and attempts at modulating regeneration of the heart, the origin of newly formed cardiomyocytes is highly debated, with evidence independently supporting the existence of a critical stem cell compartment that supplies newly formed cardiomyocyte after injury or suggesting that division of mononucleated cardiomyocytes can replace lost cardiac cells.

In this study, Ellison et al hypothesize that c-kit-positive endogenous cardiac stem cells (eCSCs) are the main source for new functional cardiomyocytes responsible for anatomic and functional recovery in the setting of acute heart failure caused by diffuse injury.

How Was the Hypothesis Tested?

Ellison et al applied a method of severe diffused myocardial damage caused by a single high dose of isoproterenol (ISO), originally described in adult rats, and translated it to a mouse model to allow lineage tracing experiments. The resulting injury was spontaneously reverted after 4 weeks, creating a system to study endogenous regeneration. In this model, the authors characterized the origin of newly formed cardiomyocytes that replace the lost myocardium.

Several transgenic models were used in conjunction with proliferation staining (Ki67 and BrdU incorporation) to demonstrate that c-kit-positive, and not mature cardiomyocytes, are responsible for the generation of new cardiac cells. To rule out contribution of preexisting cardiomyocytes as the source of new cardiomyocytes, double transgenic mice in which β-galactosidase expression was replaced by green fluorescent protein (GFP) in cells committed to the cardiomyocyte fate after tamoxifen treatment (MerCreMer-ZEG) were used. In addition, single-cell suspensions from hearts in which enhanced yellow fluorescent protein labels cardiomyocytes (MerCreMer-RYP) on tamoxifen administration were also used. Bone marrow reconstitution studies transplanting GFP-labeled cells served as control for extracardiac contribution to regenerated cardiomyocytes. To establish c-kit progenitor contribution to heart regeneration, the authors combined a transgenic mouse carrying a loxP-flanked STOP sequence preceding enhanced yellow fluorescent protein (RYP reporter mice) and the lentiviral delivery of a Cre recombinase under the control of the c-kit promoter, enabling selective labeling of c-kit-positive eCSCs and their progeny in the setting of ISO-induced injury. Microarray analysis was used to confirm the identity of various c-kit-related populations.

To determine the tropism of c-kit-positive cells for ischemic myocardium and the mechanism behind this property, GFP-tagged clonal eCSCs (c-kit positive) were systemically delivered into ISO-injured and saline-treated rats. A c-kit-negative population of GFP cardiac cells without cardiomyocytes was used as control. Engraftment outcomes were documented by immunostaining and colocalization with GFP as well as fluorescence-activated cell analysis. To interrogate the effectors involved in myocardial cell retention, the authors targeted the SDF-1-CXCR4 axis by knocking down CXCR4 and α4β1 integrin expression in GFP-labeled c-kit cells before therapeutic delivery or by treating ISO-injured rats with a SDF-1-neutralizing antibody. Results of these experiments were obtained by immunostaining of the heart as well as other organs that could retain the injected cells.

As definitive proof of the capacity of eCSCs to regenerate the heart, Ellison et al ablated replicating cells in the heart (including eCSCs) by injection of 5-fluorouracil (5-FU), leading to overt heart failure and preventing generation of new eCSC-derived cardiomyocytes. To establish the relationship between eCSCs and heart regeneration, the authors replenished the lost cells with GFP-labeled c-kit-positive cells, including a set of experiments in which they could eliminate the injected eCSCs (containing a suicide thymidine kinase gene) by treating the animals with ganciclovir. In all cases, immunostaining, fluorescence-activated cell analysis, and cardiac function characterization were part of the analysis. Finally, cell fusion was...
ruled out by male-in-female and transgenically labeled cell transplantation assays, and robustness of the stem cell properties of c-kit eCSCs was assessed by serial transplantation and in vitro characterization.

**Principal Findings**

Ellison et al showed cell cycle reentry of previously quiescent c-kit-positive eCSCs after ISO injury in rats, as well as cardiac commitment of cells still expressing c-kit, resulting in immature proliferative cardiomyocytes. They also documented increasing numbers of small immature mononucleated cardiomyocytes integrated in the spared myocardium; however, the source of these cells could not be determined in this wild-type model.

To trace the origin of newly created cardiomyocytes in transgenic mice, the authors used double transgenics in which β-gal expression is substituted by GFP in Myh6-expressing cells (committed to cardiomyocyte lineage) on tamoxifen pulse. By inducing ISO injury into tamoxifen-treated cells, the authors found a significant increase in the percentage of β-gal cardiomyocytes compared with uninjured hearts, suggesting these to be derivatives of a noncardiomyocyte population, specifically c-kit eCSCs. A significant portion of these noncardiomyocyte-derived cardiomyocytes was proliferative, as revealed by BrdU incorporation. These results, together with the smaller immature phenotype of the newly generated cells, were confirmed in single-cell preparations from hearts expressing enhanced yellow fluorescent protein only in committed cardiomyocyte before ISO injury. The authors ruled out the direct contribution of bone marrow cells to heart regeneration in this model based on the absence of GFP-positive cardiomyocytes in injured mice whose bone marrow had been reconstituted with transgenic GFP cells.

Replenishment of the cardiomyocyte pool from resident eCSCs was next evaluated. At 4 weeks after injury in mice whose eCSCs had been labeled specifically in the myocardium (with efficiencies between 38% and 65% of the total and apical eCSCs), 10% of the cardiomyocytes were BrdU-positive, of which 40% (left ventricle) to 75% (apex) showed YFP labeling, confirming their eCSC origin. Microarray analysis of resident eCSCs, eCSC-derived cardiomyocytes, and adult cardiomyocytes revealed that regenerated cells had a cardiomyocyte-committed, although immature, profile.

With respect to the predilection of eCSCs for the damaged myocardium, systemic injection of GFP-labeled c-kit cells unraveled high levels of tropism and engraftment of transplanted eCSCs into the injured heart when compared with uninjured counterparts or non-c-kit, noncardiomyocyte populations. Injected c-kit cells differentiated mainly toward immature cardiomyocytes but were also able to contribute to other cardiac lineages, including smooth muscle, endothelium, fibroblasts, and resident CSCs.

The SDF-1/CXCR4 ligand–receptor axis was involved in the migration and engraftment of eCSCs in the damaged heart. Knockdown of CXCR4 in c-kit–transplanted cells resulted in accumulation in the spleen and lungs with no signs of cardiac preference. Similar results were observed when injured rats were treated with an anti-SDF-1–neutralizing antibody before eCSC treatment, eliminating the chemotactic properties of the injured myocardium.

Finally, to test that eCSCs are necessary and sufficient for functional regeneration, Ellison et al used a treatment with 5-FU to eliminate proliferating cells, including eCSCs. 3 days after ISO injury, limiting the endogenous regenerative potential of the heart. This resulted in a severe cardiomyopathy that evolved into heart failure, in contrast to the spontaneous recovery observed in animals treated with saline after ISO injury. Conversely, when ISO plus 5-FU–treated animals received c-kit eCSCs, their regenerative and functional capacity was restored with up to 8% of the total cardiomyocytes derived from the transplanted progenitors in a 2-month period. The observed reparative effects were reverted by selective elimination of transplanted eCSCs and their derivatives that contained a suicide gene, suggesting that eCSC-dependent regeneration is required for recovery of ISO-induced injury. In fact, the authors demonstrated that c-kit eCSCs can be clonogenically isolated and serially transplanted while maintaining their in vivo and in vitro properties.

**Implications**

Although we are beyond the times when the heart was considered a postmitotic organ, there are still gaps in our knowledge regarding the sources of new myocytes and how they vary in health and disease. Currently, 2 major lines of evidence seem to compete, including mononuclear cardiomyocytes capable of reentering the cell cycle and stem cell progenitors with a broader plasticity. In this study, Ellison et al demonstrated the important role of endogenous c-kit-positive CSCs in regeneration of the heart after diffuse cardiac injury. Although technical limitations such as inefficient lentiviral labeling of c-kit cardiac cells do not rule out contribution of other cell types, it seems that eCSCs play a key role in the rejuvenation of the myocardium in this model of diffuse cellular death. With this endogenous potential in mind, development of pharmacological strategies aimed at activating CSC proliferation poses an attractive challenge for noninvasive, self-repair platforms. In addition, the authors demonstrated the importance of SDF-1/CXCR4 signaling for the efficient homing and engraftment of CSC in the heart. Furthermore, the results suggest that the heart has a limited number of niches adequate for hosting CSCs. These 2 concepts highlight the need for concomitant therapies that optimize migration and homing of reparative biologics into the heart.

Regardless of whether the c-kit regenerative potential excludes participation of other cellular sources, this study underscores compelling properties of the eCSCs, highlighting a highly translatable reagent for cardiac therapy.

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