Are Resident c-Kit+ Cardiac Stem Cells Really All That Are Needed to Mend a Broken Heart?

Jeffery D. Molkentin, Steven R. Houser

Adult c-Kit+ Cardiac Stem Cells Are Necessary and Sufficient for Functional Cardiac Regeneration and Repair

Ellison et al


The ability of the heart to regenerate through new myocyte formation is a subject of intense investigation. Here we will discuss a provocative new publication from Nadal-Ginard and Torella and colleagues where they report that c-kit resident cardiac stem cells are necessary and sufficient to explain cardiac regeneration fully.

Nadal-Ginard and Torella and colleagues have provided yet another exciting installment in the evolving cardiac regeneration and stem cell saga. The ongoing debate centers on if and how the normal adult mammalian heart renews its myocyte pool and if and how new myocytes are generated when diseases induce myocyte death. Their recently published article1 provides supportive evidence that c-kit-marked resident cardiac stem cells (CSCs) are both necessary and sufficient for cardiac regeneration, and that the differentiation of CSCs into myocytes constitutes the primary mechanism for cellular replacement following diffuse, isoproterenol (ISO)-induced injury to the heart. Although many of the approaches and conclusions put forth are already represented in the literature, the unique aspects of this report are the injury model used as well as the simply overwhelming amount of supportive experimentation presented. However, as in any provocative ongoing scientific saga, new results engender skepticism and lead to new questions that can only be answered by additional studies from other groups.

In the recent past, 2 diametrically opposed views have emerged as to whether the heart regenerates after cardiac injury by forming new myocytes. One group2–6 has presented strong data that the heart is a highly malleable organ that is capable of near-complete regeneration, although the cell source responsible for this regenerative potential varies from the myocytes themselves, to c-kit-positive bone marrow cells, c-kit-positive resident CSCs, cardiospheres, resident fibroblasts, or tissue residence mesenchymal stem cells of various markers. The other group,6–8 with polar opposite views, has also published a number of articles providing equally strong data that the absolute quantity of cellular replacement monitored in the heart over time or with injury is below a biologically meaningful threshold. Members of this latter camp have also suggested that CSCs (c-kit-positive or otherwise) are not appreciably involved in contributing to the formation of new myocytes in the myocardium.7,8 The reasons for such discordant results remain unclear, although the new report by Nadal-Ginard and Torella and colleagues adds significant new data to this debate. In this Commentary, we try our best not to take sides in this debate. Instead, we try to evaluate the data presented in this new report carefully with no inherent prediction for either group.

Nadal-Ginard and Torella and colleagues used a relatively unique model of cardiac injury that involves a single injection of ISO in the rat at 5 mg/kg and 200 mg/kg in the mouse, which causes diffuse killing of myocytes primarily within the endocardium and apex of the left ventricle of the heart.1 Importantly, this approach seems to spare the resident CSCs, as shown in their data. Although this model is not applicable to most forms of human cardiac disease, it is suited to address the question of cardiac regeneration because the more clinically relevant model of myocardial infarction also seems to kill progenitor cells within the injury area. Using this ISO injury model as the basis for nearly all their subsequent experimentation, the authors first show that a single injection of ISO kills about 8% of the cardiac myocytes and that cardiac function is acutely depressed. Over the next month, heart structure and function spontaneously return to normal, purportedly because of endogenous cardiac repair. When bromodeoxyuridine (BrdU) (to label newly formed DNA) was injected into ISO-treated animals, ≈5% to 10% of rat myocytes within the heart became labeled 28 days later, suggesting that these labeling events resulted from new myocyte formation. The caveat with this approach, which is true for many previously published articles as well, is that BrdU first and foremost shows DNA replication and even repair in extreme circumstances, not necessarily new cell formation, although new myocytes generated from a progenitor pool would likely be BrdU+.

The other caveat worth noting is that the ISO model used here was adopted from the previous work of Ellison et al,9 which was from the Nadal-Ginard laboratory. This model shows restoration of ejection fraction and fractional shortening 6 days after ISO injection, which is likely too soon for meaningful maturation of newly minted myocytes to restore normal cardiac function. Indeed, the other reference for this injury model in the mouse is a study in which Shao et al10 used a higher single dosage of ISO (400 mg/kg), which also showed...
normal cardiac function 1 week after the injection. However, Shao et al.\(^\text{10}\) suggested that the temporary loss of cardiac performance in this model was because of a stunning effect of the drug rather than myocyte death. This issue notwithstanding, the correlation between ISO-induced myocyte death and BrdU-labeled myocytes shown by Nadal-Ginard and Torella and colleagues supports the idea that these hearts have an ability to repair themselves via new myocyte formation. In addition, the observation that BrdU+ myocytes were smaller than BrdU− myocytes is also consistent with the idea that these are new myocytes derived from a stem cell pool. Their results are similar to those published previously in a large animal model of ISO-induced cardiac injury.\(^\text{11}\)

The authors then proceeded to define the source of BrdU+ myocytes. They started by using an approach developed by Hsieh\(^\text{12}\) in which a majority of existing cardiac myocytes are genetically marked to express green fluorescent protein (GFP) using the α-MHC-MerCreMer transgene.\(^\text{13}\) Once most myocytes are labeled, it is expected that any newly generated cells derived from a mature GFP+ myocyte will be GFP+. whereas those myocytes derived from a nonmyocyte source will be GFP−. Nadal-Ginard and Torella and colleagues showed that ISO injury resulted in a significantly greater proportion of GFP+ myocytes in the heart, suggesting that myocytes were replaced from a nonmyocyte cell type, as also initially suggested by Lee and colleagues with their injury/stress models.\(^\text{12}\)

A creative aspect of this portion of the study was that BrdU labeling was also performed, which showed that BrdU+ myocytes were GFP−, further supporting the idea of replacement from a stem cell pool. Importantly, Nadal-Ginard and Torella and colleagues showed that infiltration of bone marrow-derived cells into the injured heart was not a source for this new myocyte formation (based on a bone marrow transplantation approach in conjunction with a genetic tagging system).

The remainder of the study by Nadal-Ginard and Torella and colleagues explores the hypothesis that resident CSCs are the source of BrdU+ myocytes found after ISO injury. To us, these are the most critical and novel experiments that could provide important new information. We will focus our attention on the 2 experimental approaches that we think were the most definitive and provocative in formulating the conclusion and title of the article itself. The first approach uses a lentivirus that expresses Cre recombinase under the control of the Kit gene upstream regulatory region (c-kit promoter) to the lineage trace c-kit expressing CSCs in the heart when used in conjunction with a LoxP-inactivated yellow fluorescent protein reporter allele in mice. The concept here is that while this virus will infect divergent cell types equally within the heart, it should only express Cre recombinase and label cells yellow fluorescent protein\(^\text{*}\) if they were from the c-kit lineage. This approach, if technically sound, is arguably the most direct means of proving that endogenous c-kit cells become myocytes in vivo. The Lenti-Cre was then painted on the heart and injected into the apex, where it seemed to infect slightly greater than half of the total c-kit-positive CSCs in the heart, with an even greater fraction of the c-kit cells in the cardiac apex. After 28 days, the investigators showed that in control mice <0.06% of the myocytes were yellow fluorescent protein\(^\text{*}\), whereas after ISO injury, 3.4% to 7.7% of myocytes were yellow fluorescent protein\(^\text{*}\), consistent with them being progeny of c-kit CSCs. A major concern here is that if the c-kit regulatory region contained within this vector is expressed outside of c-kit cells, it cannot be reliably used for lineage-tracing experiments. For example, if ISO induces expression of this Lenti-Cre vector in myocytes themselves, they would be inaccurately assessed as coming from the c-kit lineage. In our view, this approach is essentially the linchpin of the entire article and one of its most novel aspects; to our knowledge, no one has yet definitively shown that endogenous c-kit CSCs directly make new myocytes in vivo. To rule out that myocytes might be directly labeled by this Lenti-Cre vector after ISO treatment, the authors generated an entirely novel transgenic mouse in which 14.7 kb of c-kit regulatory region was used to drive GFP directly, so that any fields of expression could be visualized throughout the mouse. These c-kit-GFP transgenic mice were then treated with ISO to show exactly that myocytes did not express GFP. Hence, by extrapolation, the Lenti-Cre approach with its segment of the c-kit promoter will never express in myocytes and, therefore, be valid for lineage tracing. However, this comparison between the transgenic mice and Lenti-Cre approach would require that the c-kit regulatory regions used in these 2 sets of experiments be identical, which is, in fact, not the case based on what is stated in methods and the source of these constructs. The lentivirus, given strict packaging limits, could only have used the 6.7-kb promoter fragment (or smaller) from the quoted source,\(^\text{15}\) whereas the transgenic mice were made with a much larger Kit regulatory region (construct 3) consisting of 6.7 kb upstream promoter region and 8 kb (14.7 kb total) of additional first intron sequence.\(^\text{16}\) Thus, the transgenic mice are probably irrelevant for determining if the lentivirus was indeed specific for the c-kit lineage, and to us, this makes the results of the entire approach questionable. Indeed, Cairns et al\(^\text{17}\) even showed that the 6.7-kb region of c-kit promoter is not expressed properly in vivo, with the majority of transgenic lines producing ectopic expression, and none generating expression in hematopoietic sites of the mice. It should also be noted that even mutations 154 kb upstream of the Kit locus can severely affect its expression; hence the only valid means of lineage tracing for c-kit cells in any tissue might be with a knock-in approach because the regulatory region (promoter) of this locus is extremely complicated and extended.\(^\text{18}\) Given that the lentiviral lineage-tracing approach is of uncertain validity, the conclusion that endogenous c-kit-positive CSCs are the source for new myocytes within the heart seems to be based on indirect evidence and not definitively proven. However, the authors do provide numerous lines of less direct evidence that confirm results of others in making a stronger case for their conclusions, as well as an additional new approach that will be discussed below.

A final critical and unique approach presented by Nadal-Ginard and Torella and colleagues is the use of a chemotherapeutic agent to neutralize c-kit cell activation in the heart after ISO injury, thereby addressing the requirement of these cells in regeneration. The authors show that 5-fluorouracil (5-FU), when given after the single dosage of ISO, prevents c-kit CSC expansion, new myocyte formation in the heart, and functional recovery of the myocardium. Our first inclination when assessing these data was to suspect that the combination of 5-FU and ISO
had a synergistic toxic effect, rather than altering myocyte regeneration solely through c-kit cells. However, the authors provide evidence to the contrary, because injecting c-kit CSCs into the tail vein after ISO plus 5-FU was able to rescue the myocardium fully, both in terms of function and new myocyte generation. This is quite an amazing result because the rescuing stem cells were delivered 28 days after ISO injury, when cell death should have been exacerbated by the evolving heart failure and should have led to replacement fibrosis and a lack of available niches. However, the results of this new study would suggest that CSCs can repair the heart even after a mature injury, unless the ISO injury model has some additional unique features in priming the heart for stem cell activity that is not normally observed after myocardial infarction. Nadal-Ginard and Torella and colleagues even went one step further to document that CSC-mediated repair involves formation of new cardiac tissue (myocytes, blood vessels, and fibroblasts). Here, c-kit-positive CSCs were engineered to contain a thymidine kinase viral gene so that ganciclovir treatment could be used to kill these cells and their progeny. These engineered c-kit CSCs were then injected into the tail vein whereupon they again induced full repair of the ISO-injured heart, but with ganciclovir treatment, this rescue was subverted. The authors even removed the reconstituted CSCs from ISO-plus-5-FU–rescued hearts, clonally and functionally expanded them, and showed that they retained full differentiating ability and could be transplanted back into new ISO-plus-5-FU–treated rats to again mediate rescue. Overall, these results suggest that transplanted c-kit-positive CSCs are valid therapeutic tools to be further investigated. These results support the use of autologous cardiac-derived stem cells in ongoing clinical trials to treat patients with cardiac damage from myocardial infarction.4,14–16 These findings also suggest that c-kit-positive CSCs could be used to treat chemotherapy-induced cardiomyopathy in cancer patients.

In summary, Nadal-Ginard and Torella and colleagues have certainly provided additional provocative data to be weighed with all previous data as the field contemplates if or how to best use stem cells to treat patients with select forms of heart disease. Overall, the study was well controlled, and all but 1 of the experimental approaches seem to be rigorously performed and documented. Additional experimentation is clearly needed to resolve the numerous questions that we have raised here. It will be important to readdress the lineage-tracing studies using an independent system to further investigate if endogenous c-kit cells are truly used by the heart as part of physiological cardiac repair, as well as to provide additional convincing evidence that these cells can be used as therapeutics for patients with debilitating cardiac diseases and that their potential benefit is because of bona fide new myocyte formation.

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