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Neonatal Heart Regeneration: Mounting Support and Need for Technical Standards

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Regenerating the adult heart is by many standards the holy grail of modern cardiovascular medicine. As a result, this field has been fraught with innumerable conflicts and controversies. Everything from the rate of cardiomyocyte turnover and regulators of cardiomyocyte proliferation in the adult heart, to the existence of cardiac progenitors and their therapeutic role, has been heavily contested.

In 2011, we published a report outlining the regenerative potential of the neonatal mouse heart in the first few days of life. We found that the newborn mouse heart is capable of regeneration following apical resection of 15% of the left ventricle with only minimal scarring seen in a small percentage of samples. Moreover, we showed that this process is mediated primarily by proliferation of preexisting cardiomyocytes.[1] These studies were inspired by the well-known ability of early neonatal myocytes to divide, even in culture. Our findings have been widely reproduced in mice and rats, and for the first time provided tools and insights for studying regulators of mammalian heart regeneration,[2–15] whereby loss of regenerative potential of the neonatal heart or prolongation of the postnatal regenerative window can be studied in the context of cardiomyocyte cell cycle regulation.

The current report by Konfino[16] and colleagues elegantly compared 2 different forms of neonatal heart injury (namely, apical resection and left anterior descending coronary artery (LAD) ligation), which we first reported in 2011 and 2012, respectively. The results confirmed our previous findings that apical resection is associated with a robust regenerative response and induction of cardiomyocyte proliferation above the basal levels seen in the neonatal heart. However, the current report observed significantly larger scar formation following LAD ligation compared to our findings, associated with lack of induction of cardiomyocyte proliferation.

In the 17th century, Robert Boyle argued that reporting scientific methodology is critical for ensuring reproducibility of experimental results.[17] The technical difficulty of performing neonatal heart injury is likely an important reason why these studies have not been attempted prior to our initial report. Technical considerations such as neonatal anesthesia, survival after thoracotomy in the absence of mechanical ventilation, exsanguination after apical resection, and maternal cannibalization are all significant obstacles that can hinder successful neonatal cardiac injury. As a result, we set out to disseminate this surgical technique by training over 30 laboratories, sharing our protocols with numerous others, and publishing a dedicated protocols report.[18] Importantly, many labs with surgical expertise independently established this model following our reports. These efforts undoubtedly helped with standardization and reproducibility of the surgical techniques.

Nevertheless, several important technical considerations remain unclear. For example, based on a recent report by the Lee group, it is clear that there is a limit to the degree of neonatal heart regeneration where larger injuries are associated with a higher incidence of incomplete regeneration. Similarly, in the current report by the Leor group, they observed complete regeneration following apical resection, but incomplete regeneration following myocardial infarction. In our 2012 report of neonatal regeneration following LAD ligation, we observed a small scar at the site of the LAD ligature, which we attributed to the persistence of the ligature. The Leor group made a similar observation, albeit they may have observed a larger anterior wall scar in close proximity to the ligature. Interestingly, almost immediately after online publication of our initial neonatal myocardial infarction report, another group reported complete regeneration with lack of anterior wall scarring following LAD ligation.[8] This complete lack of scarring may be due to the group's ability to directly visualize the LAD and therefore minimize the amount of myocardium included in the ligature. Although it is difficult to discern the precise technical discrepancies between the 3

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groups, an inherent limitation of the LAD ligation model, both in neonates and adults, is the difficulty in standardization of injury size. Given that a proximal LAD occlusion can result in infarction of as much as 40% of the left ventricle (LV), it is highly plausible that larger ischemic injuries, similar to larger resection injury, are incompatible with complete regeneration.

An interesting finding of the current report by the Leor group is the lack of increase in myocyte proliferation following myocardial infarction as compared to resection. This is in contrast to previous myocardial infarction studies by our group and others,[2,8] and cannot be simply explained by a discrepancy in injury size. For example, in the recent study by the Lee group,[19] both small and large resection injuries resulted in increased myocyte proliferation, although larger injury was more likely to be associated with incomplete regeneration.[19] Therefore, apart from the type of injury, larger myocardial injury per se does not seem to inhibit myocyte proliferation. Other technical variations could potentially explain some of the discrepancies; For example, the Leor group used isoflurane anesthesia as well as 100% oxygen supplementation during their surgical procedure, neither of which was used in our protocol. Both inhalation anesthetics[20] and hyperoxemia[12] have been shown to induce significant DNA damage, which is a major inhibitor of neonatal cardiomyocyte proliferation.[12] Regardless of the underlying mechanism, an important question here is whether the lack of an appreciable increase in myocyte proliferation (above a high background level of myocyte cell cycle activity in the neonatal heart) is reflective of a lack of myocardial regeneration, or merely lack of an enhanced regenerative response? The fact that the scar formation following LAD ligation in the current report is focal, rather than inclusive of the entire LAD territory, certainly supports the later scenario.

In the current report, the Leor group also performed a cardiac organ culture postinjury, a technique that we have not previously used in our studies. Interestingly, they witnessed both myocyte sprouting as well as some expansion of an unidentified c-kit-positive cell population. The question of involvement of c-kit cells in neonatal heart regeneration is interesting, especially in light of the intense debate surrounding the role of c-kit cells in adult heart regeneration. Based on results by our group, genetic fate mapping of cardiomyocytes does not support a significant contribution of nonmyocytes in postresection or myocardial infarction injuries. However, it is possible that there is minor contribution by c-kit cells in the small subset of myocytes that were not labeled by our genetic-fate-mapping approach. Moreover, the type of injury may be a critical determinant of the regenerative response. For example, the Kotlikoff group observed incomplete regeneration following cryoinjury in the neonatal heart.[10] Similarly, a recent report by the Lien group observed complete regeneration following mild cryoinjury but not severe cryoinjury.[10] Our group has also made similar observations (unpublished data). Interestingly, the Lien group did not observe an increase in cardiomyocyte proliferation either with mild or severe cryoinjury, although their method for assessment of myocyte proliferation (nuclear co-localization of Mef2c and pH3) has not been previously tested. Nevertheless, these reports highlight important discrepancies in the neonatal regenerative response, and could help uncover important regulators of mammalian heart regeneration.

As the number of groups studying neonatal heart regeneration grows, it may be prudent to examine not only methodologies, but also our basic definitions of myocardial regeneration. For example, what constitutes myocardial regeneration? Is it the formation of new myocytes resulting in long-term restoration of myocardial function and architecture? Is it the absence of full-thickness scar following transmural injury? Or is it restoration of cardiac architecture and function in the absence of any extracellular matrix deposition, whether interstitial, endocardial, or epicardial? While scar formation in the adult heart is the default healing mechanism in the absence of any appreciable regenerative response, extracellular matrix deposition in the neonatal heart is a normal component of the regenerative response,[1,4] and its persistence may not reflect lack of neomyogenesis or even incomplete regeneration. These seemingly simple questions can be a source of contention and controversy and are best resolved through communication and careful analysis. Undoubtedly, there is still much to learn about neonatal heart regeneration, and how it can help uncover the regenerative potential of the adult heart.

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Disclosures

None.

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