Cardiovascular Regenerative Medicine
The Developing Heart Meets Adult Heart Repair

Despite recent advances, heart disease kills more people than all other major diseases combined. Most commonly, coronary occlusion attributable to plaque rupture results in episodes of ischemia and reperfusion that destroy significant number of cardiac myocytes, causing impairment in cardiac function and heart failure. In the absence of transplantation, the long-term survival of heart failure patients is worse than that associated with several types of cancer. This is because existing therapies can only slow the inexorable downward progression of patients with heart failure but do not tackle the fundamental problem, ie, progressive loss of myocytes.

A recent explosion of studies aimed at repairing and regenerating heart tissue with cell therapy, however, promises to radically change the landscape of cardiovascular disease mortality and morbidity. The excitement is palpable everywhere. However, these are still early days and the field is rife with controversy and debate about how to proceed. A panoply of daunting questions face investigators who seek to study cell therapy. How to safely and ethically use embryonic stem cells to reconstruct damaged areas of the heart? Which specific types of stem cells are likely to be most effective? If adult stem cells are safe and efficacious, why use embryonic stem cells? Should we avoid embryonic cells altogether and focus on the use of stem cells present in the adult bone marrow or the adult heart? Alternatively, or in addition, can induced pluripotent stem cells replicate the properties of embryonic stem cells and possibly supplant them? Can heart cells divide, and, if so, can we develop strategies to stimulate the growth and differentiation of the cardiac cells left in the injured heart to promote recovery of tissue mass and function? Clearly, these are important questions that require thoughtful research, analysis, and debate. In this brief update, we highlight a spate of recent studies that point toward new directions and new frontiers in cardiovascular cell therapy.

Human Cardiac Cells Meet Their Fate Slowly
The first step in devising a rational regenerative strategy is to understand how the heart develops during embryogenesis; how embryonic cells form heart cells; how lineage commitments are determined; and what factors regulate the development of primordial cells into fully functional cells that form cardiac myocytes, endothelial cells, and smooth muscle cells. In the mouse embryo, multipotent cardiac progenitor cells have been shown to give rise to all of the major types of cells in the heart. The situation in human embryos is less clear. A step toward understanding the generation of cardiovascular cells during human embryogenesis has been taken by a recent study by Kenneth Chien and his colleagues of the Massachusetts General Hospital. These investigators demonstrate the existence of human “primordial progenitor” cells that multiply and diversify into multipotent cardiovascular cell lineages for several weeks; in effect, these cells give rise to the panoply of cells that form the heart. These cells express the transcription factor islet1 (ISL1) and persist during human embryogenesis for several weeks. In contrast, progenitors expressing the murine ISL1 homolog multiply and diversify for only a few days. In human hearts at 11 and 18 weeks of gestation, these cells were found in the right atrium and the outflow tract as well as in the left atrial wall and appendage, but not in the left ventricle. During later stages of development, these cells were gradually lost. No ISL1+ cells remain in the adult heart indicating that they appear transiently during development and give rise to cells committed to a cardiovascular lineage.

By attaching fluorescent tags to these cells, Chien and his colleagues were able to obtain a pure population of ISL1+ cells and to assess their differentiation potential into all 3 major cardiovascular lineages. Also, they were able to expand these cells in vitro and to maintain their pluripotency, raising the possibility that they could be studied by themselves or used in transplantation studies. These findings may open up new avenues for understanding why sometimes cardiac development goes awry during embryogenesis giving rise to congenital malformations. Because cardiac malformations account for more that 50% of all birth defects, development of procedures for the isolation and the maintenance of progenitor cells in vivo is welcome news for those studying fundamental mechanisms of cardiac development and cardiac birth defects.

Do ISL1+ cells have any potential therapeutic value? In theory, there is the possibility that ISL1+ cells isolated from embryos could be used for transplantation and regeneration of the injured myocardium, but this scenario seems far-fetched at present. Several formidable obstacles make therapeutic application of ISL1+ cells very problematic. “An important problem,” says Piero Anversa of the Harvard Medical School and Brigham and Women’s Hospital, “is that there is no cell surface marker to isolate the cells for use in adult.” This is critical because specific cell populations are identified and isolated by flow cytometry, which separates cells based on their cell surface antigens. Without knowing their surface characteristics, it is difficult to isolate these cells, to expand them in vitro, and to use them for transplantation. Furthermore, as is the case for other embryonic stem cells, transplantation of heterologous ISL1+ cells is bedeviled by rejection and potential for tumor formation. Also, the ability of ISL1+ cells to regenerate cardiac tissue in vivo remains to be
demonstrated. Clearly, much work remains to be done to develop procedures for isolating and studying these cells and to determine whether they have any utility for regenerative purposes. Nevertheless, identification of such cells is a first step and ongoing research is addressing questions associated with the use of these cells. Says Chien, “the field can now explore the role of these endogenous human heart progenitors and take clues from developmental pathways and principles and apply them to regenerative therapeutic goals.” Whether this promise will be fulfilled remains to be seen.

**Once Irreplaceable Cells, Now Replaced Slowly**

Using embryonic stem cells to generate cardiac tissue is still a distant possibility and one riddled by multiple ethical and methodological issues. Whether embryonic stem cells offer enough advantages over adult stem cells to justify the cost and effort involved in harnessing them as a practical therapeutic approach for heart disease is still a matter of opinion and ongoing debate, particularly after the advent of induced pluripotent stem (iPS) cells, which are touted as having properties similar to embryonic stem cells but are derived from adult cells. Another approach may be to stimulate the heart’s own capacity to grow new cells. However, the adult heart has long been thought to be incapable of generating new cells. This belief stems from many factors. First and foremost are the difficulty in demonstrating postnatal generation of cardiac myocytes in humans and experimental animals and the poor ability of the heart to regenerate new myocytes after injury. In addition, adult cardiac cells maintain only a sparse abundance of positive cell cycle regulators but are rich in negative cell cycle regulatory genes. Researchers have cited the infrequency of oncogenesis in the heart as evidence of a nondividing cardiomyocyte. Primary cardiac tumors are rare, suggesting that the adult heart has limited growth potential. There are teleological arguments as well. Cell division is believed to be bad for the heart because it could lead to the accumulation of errors during DNA replication or because it might temporarily disrupt the electric syncytium of the heart, leading to the generation of low resistance pathways generated by newly formed cells. Moreover, the highly organized myofibrillar structure of the heart seems to constitute an insurmountable cell cycle check point, and it is difficult to imagine how it can be dismantled during cytokinesis.3 On the other hand, for cardiomyocytes to lack turnover, they would have to be remarkably resilient, if not immortal, with the capacity to beat more than 3 billion times in a person who lives 100 years or more. That may be too much to ask of a cell. Hence, some researchers have suggested that the “postmitotic,” nondenuding model of the heart is untenable.

Anversa points to research that he and his colleagues have been publishing since the early 1990s suggesting that adult cardiomyocytes can reenter the cell cycle and duplicate.4,5 According to Anversa, “homeostasis of the adult heart requires cardiomyocyte replacement”; that is, myocytes lost because of wear and tear must be replaced by new cells to maintain uncompromised cardiac performance over a lifetime. Stephanie Dimmeler, a researcher at the Goethe University in Frankfurt, Germany, cites mounting data from multiple groups who describe, “a continuous replacement of myocardial cells during the lifetime of mammals.”

Among the studies that Dimmeler cites is a particularly elegant work published earlier this year by Jonas Frisén and colleagues from the Karolinska Institute in Stockholm, Sweden,6 which measured DNA integration of carbon-14 generated in the atmosphere by surface nuclear bomb testing during the Cold War. They found that the 14C concentrations in all individuals born around or after the nuclear bomb tests corresponded to atmospheric concentrations several years after the subjects’ birth, indicating significant postnatal DNA synthesis. By specifically purifying DNA from cardiac myocytes, they were able to show that the 14C concentration in cardiomyocyte DNA corresponded to the concentration several years after birth, indicating that there was substantial postnatal DNA synthesis specifically in cardiac myocytes. After ruling out DNA synthesis resulting from polyplodyization, increase in cardiac myocyte number, binucleation, and DNA repair, they were able to estimate that cardiac myocytes are renewed at a rate of about 1% per year at the age of 25 and 0.45% at the age of 75. At this rate, by the age of 50 years, 55% of the cardiomyocytes would be expected to have been replaced, whereas the remaining 45% are retained from birth. The actual magnitude of myocyte turnover, however, may differ from these estimates; what is important in Frisén’s study is not whether renewal of cardiac cells occurs at a rate of 1%, or less or more, but the very demonstration that turnover of myocytes in the adult heart occurs at all: a concept that for a long time has been rejected or vigorously questioned by large segments of the scientific community.

Additional studies are required to understand the source of the new myocytes. Cardiomyocyte entering mitosis would stop beating, posing a significant paradox particularly for the already diseased heart. As Steven R. Houser of the Cardiovascular Research Center at Temple University points out, “Dismantling them during mitosis would induce weakening in the heart muscle.” An alternative to myocyte mitosis would be formation of cardiomyocyte from progenitor/stem cells. Says Houser, “It may make more sense for the heart to use primarily stem cells instead of inducing division in differentiated cells to repair myocardium.” Indeed, the lead author of the 14C study, Frisén, says that he and his colleagues could not determine whether the new cardiomyocytes derive from cardiomyocyte duplication or from stem or progenitor cells, “because both would result in similar 14C integration in DNA.”

If adult cardiac myocytes divide, how does this happen? The process remains mostly unknown. A recent study by Bernhardt Kühn and coworkers at the Children’s Hospital in Boston suggests that trophic factors can induce cardiac myocytes to proliferate. Previous studies have shown that stimulation with fibroblast growth factor-1 and periostin can induce cardiomyocytes to reenter the cell cycle. Kühn and colleagues report extensive evidence showing that stimulation with neuregulin (NRG)1, which binds to the receptor kinases ErbB2 and -4 of the epidermal growth factor family, enhances cardiomyocyte cell cycle entry via a phosphatidylinositol 3-kinase–dependent mechanism.7 By genetically deleting the ErbB4 gene, the authors show that Erb4 is required for postnatal cardiomyocyte proliferation and
that transgenic overexpression of ErbB4 increases myocyte proliferation in vivo.

“Bernard Kühn’s study suggests that noncell treatments that stimulate generation of new heart cells are feasible,” says Richard T. Lee of the Partners Research Facility at the Harvard Medical School. In comparison with controls, the NRG1 injections were reported to reduce postinfarct scarring by 46%. It is also noteworthy that Kühn and colleagues conclude that the injections stimulated proliferation of differentiated cardiomyocytes, an important conclusion, because it suggests that postnatal cardiomyocytes can reenter the cell cycle, confirming the observations made by Anversa many years ago.4,5

According to Anversa, however, “it is not clear whether the proliferating myocytes in the study were from progenitors or from differentiated cardiomyocytes.” Dimmel er also errs on the side of caution. “Although the study by Kühn and colleagues convincingly shows that cardiomyocytes can be stimulated to proliferate, and thus the study makes an important point, the absolute number of proliferating cells is still very low.” She adds that the significance of cardiomyocyte proliferation in regeneration remains to be determined and wonders whether “the therapeutic benefit seen by injecting NRG1 is mediated by cardiomyocyte proliferation or by the proangiogenic and antiapoptotic effects of NRG1 described by Liu et al.” The study she cites demonstrates that NRG1 by itself could improve cardiac function.8

In response to these comments, Kühn says that the evidence from his study demonstrates that the “bulk of the functional and structural improvement can be attributed to differentiated cardiomyocytes.” On the other hand, he does not dismiss the possibility that NRG1 could have stimulated proliferation of stem cells. Demonstrating proliferation of differentiated cardiomyocytes, he says, is different from excluding another process, and determining the role of stem cells in NRG1 “would be another study.”

Could NRG1 become a practical tool for coaxing adult myocytes to divide and regenerate dead tissue? A note of caution is in order because more research is required to determine whether NRG1 could induce cancer as a result of its stimulation of the kinase receptor ErbB4. This is particularly important because a related kinase receptor, ErbB2, has been shown to be associated with breast cancer. Accordingly, Philippe Menasché of the Hôpital Européen Georges Pompidou in Paris, France, cautions that “boosting this proliferative potential by compounds like NRG1 is attractive but one can easily foresee major safety issues related to the means of controlling this proliferation rate.” Kühn says that after 12 weeks, when the mice were killed, there was no evidence of overgrowth with the use of NRG1. Nevertheless, more studies and a longer follow-up will clearly be necessary.

Cardiovascular Regenerative Medicine
Going Forward

The field of cardiovascular regenerative medicine is in its infancy and in complete flux. “Nobody knows at the time being what will be the best therapy for our patients,” says Dimmel er. “We may need different cells for different patients and different cells for drug discovery or tissue engineering.” Which cell(s) will ultimately prove to be useful in patients is a matter of opinion. Some researchers such as Menasché express confidence that embryonic cells hold the key to myocardial regeneration. “What looks reasonable is to state that only these early cardiac progenitors, regardless of their phenotype, which still needs to be more precisely characterized, will likely be more efficacious than adult stem cells for effecting a true ‘regeneration’ of the myocardium.”

Although Chien is investigating the role of fetal cells in myocardial regeneration, he places significant value on transplantation and delivery of adult cells. “The science itself, and the clinical studies that were spawned from these studies, have been informative and valuable to the field, and are clearly meritorious.” Chien continues that researchers know “relatively little about the therapeutic potential of human ES and iPS- derived heart progenitors, which play a key role in generating cardiac muscle in the first place.”

Eduardo Marbán of the Cedars-Sinai Heart Institute in Los Angeles recommends that scientists “continue to pursue primordial cells for their long-term potential, while realizing that adult stem cells are here and now.” Marbán also says that primordial cells face obstacles that require further consideration, “including the risk of tumor, immune rejection, worsening of arrhythmias, and regulatory hurdles to genetic manipulation.” Chien acknowledges that there is more to learn, saying that “these are early days, but nevertheless exciting.” Whether the use of adult cells versus embryonic cells is a controversy or a question of investigative priorities, according to Lee, such differences help “people devise completely new approaches to sort out the truth, and those new perspectives allow us to reach consensus based on multiple lines of evidence.”

References


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doi: 10.1161/CIRCRESAHA.109.211243

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