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Regeneration Gaps

Observations on Stem Cells and Cardiac Repair

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Substantial evidence indicates that cell transplantation can improve function of the infarcted heart. A surprisingly wide range of non-myogenic cell types improves ventricular function, suggesting that benefit may result in part from mechanisms that are distinct from true myocardial regeneration. While clinical trials explore cells derived from skeletal muscle and bone marrow, basic researchers are investigating sources of new cardiomyocytes, such as resident myocardial progenitors and embryonic stem cells. In this commentary, we briefly review the evolution of cell-based cardiac repair, discuss the current state of clinical research, and offer some thoughts on how newcomers can critically evaluate this emerging field. (J Am Coll Cardiol 2006;47:1777–85) © 2006 by the American College of Cardiology Foundation

Stem cells are the building blocks through which tissues are developed and maintained. We and many other groups predict that stem cells will prove tremendously useful in clinical medicine. Possible uses include systems for high-throughput drug screens, as in vitro models of disease and, eventually, in treating diseases associated with cell deficiency. As one of the least regenerative organs in the body, the heart stands to benefit greatly from addition of new parenchymal cells. Cardiovascular researchers have risen to this challenge and, as a result, cardiac repair is arguably the most advanced program in the emerging field of regenerative medicine. Progress in this field has been rapid, from humble beginnings with committed skeletal (1–3) or cardiac muscle cells (4–6), moving to multipotent adult stem cells (7–9) and, most recently, to embryonic stem cells (10–13).

In this brief commentary, we review some important recent developments in stem cell-based tissue repair. (Readers wishing additional basic and clinical information are directed to several recent in-depth reviews [14–16] on the field.) Like other areas involving stem cell-based regeneration, the field of cardiac repair has its share of controversies. These will also be touched upon, with an aim of separating experimental observation, on which there is much agreement, from interpretation, which varies widely at the moment.

EVERY CELL TYPE SEEMS TO IMPROVE CARDIAC FUNCTION

A few calculations are helpful in assessing the scope of regenerating a human myocardial infarct. Myocardium contains approximately 20 million cardiomyocytes per gram of tissue (17). The average left ventricle is approximately 200 g and therefore contains approximately 4 billion cardiomyocytes. To cause heart failure, an infarct needs to kill approximately 25% of the ventricle (for comparison, infarcting 40% of the ventricle results in acute cardiogenic shock) (18). Therefore, the myocyte deficit in infarction-induced heart failure is on the order of one billion cardiomyocytes. True cardiac regeneration would therefore require restoring approximately one billion cardiomyocytes and ensuring their synchronous contraction via electromechanical junctions with host myocardium.

Although today's technology clearly cannot achieve this goal of true regeneration, we are fortunate that substantial physiological benefit (repair) currently can be derived from transplanting cells into the infarcted heart. What is most surprising, however, is that almost every cell type tested seems equipotent: benefit is derived from cardiomyocytes (6,19,20), skeletal myoblasts (21–23), smooth muscle cells (24), fibroblasts (25), endothelial progenitors (26), mesenchymal stem cells (27), hematopoietic stem cells (7,28), other marrow populations (29), resident myocardial progenitors (30), and embryonic stem cells (13). Consequently, the mechanism underlying the benefit has been difficult to pin down. Although most studies sought to restore systolic function to an infarcted region, it seems clear that most of the benefit results from something other than graft cells beating in synchrony with host myocardium. One of these unanticipated benefits is improvement of the infarct's passive mechanical properties and subsequent amelioration of ventricular remodeling (23). Improved passive mechanics may result in part from a mechanical buttressing of the infarcted wall by the transplanted cells. A second benefit is the so-called paracrine effect, which refers to the production of local signaling molecules that may improve perfusion to chronically ischemic tissue (31) or promote survival of tenuous cardiomyocytes (26). Indeed, Gnecchi et al. (32) recently reported that most of the benefit of mesenchymal stem cell transplantation in the heart could be reproduced by injecting the cell-free supernatant recovered from mesenchymal stem cell cultures.

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Abbreviations and Acronyms

EGFP = enhanced green fluorescent protein
ESC = embryonic stem cell
LacZ = beta-galactosidase
Sca = stem cell antigen
SDF = stromal-derived factor

Improvement of the heart's function by so many cell types, most of which are non-cardiogenic, is at once frustrating and encouraging. Frustration arises because, despite much hard work, no group has developed reproducible techniques that generate substantial amounts of new, beating myocardium in the infarcted heart. However, it is encouraging that cellular therapies, even with current limitations, can significantly improve function of the infarcted heart. These therapies open the door to testing existing cardiac repair strategies in the clinic while basic scientists work on deciphering mechanisms that will lead to bona-fide cardiac regeneration.

MULTIPLE CELL TYPES HOME TO THE INJURED HEART

It has been known for many decades that myocardial infarction is an inflammatory disease, which results first in the homing of neutrophils and later monocytes from the circulation (33). We have appreciated only recently, however, that marrow-derived progenitor cells circulate and home to injured tissues similarly to leukocytes, where they contribute to formation of new tissues (34,35). A "natural" human experiment occurs when female hearts are transplanted into male patients. This procedure permits one to use Y chromosome in situ hybridization to track the male cells in the female allografts, coupled with immunostaining to define the identity these cells have acquired (similar processes likely occur outside this specialized setting but are more difficult to track). Multiple groups have shown that most components of myocardium can be derived from extracardiac progenitors, but the frequency of repopulation varies widely by cell type (36–41). In our studies (37), endothelial cells were the most commonly derived from progenitors, averaging 24%. These were followed by perineural Schwann cells at 11% and coronary smooth muscle cells at 3%. Cardiomyocytes, unfortunately, were only derived rarely from circulating progenitors, averaging 0.04% (36). The likely source of such cells is the bone marrow, as Deb et al. (40) has shown similar myocardial repopulation in patients with male-to-female bone marrow transplants. The lesson we draw from such experiments is that circulating progenitor cells are promising for revascularization of ischemic tissues, but they are much less promising for remuscularization of infarcts.

In addition to tracking endogenous cells, several exogenously administered cell populations have been shown to home to the injured heart after intravenous injection, including endothelial progenitors isolated from blood or marrow (26,42), mesenchymal stem cells from the marrow (35,43), and stem cell antigen (Sca)-1–positive cells from the myocardium (44). Indeed, one of the big surprises from work with adult stem cells is how many have the ability to home to injured tissues. This discovery has led to a search for factors that mediate mobilization and homing. The cytokine stromal-derived factor (SDF)-1 and its cognate receptor CXCR4 have emerged as important players in this regard. Activation of hypoxia-induced transcription factors induces ischemic tissues to produce SDF-1 (45), and SDF-1 appears to mediate homing of endothelial progenitors and other cells to the acute infarct (46). Manipulating this pathway appears promising for control of cell homing to the heart. A clinical trial of intravenously administered mesenchymal stem cells in acute myocardial infarction recently has been initiated by scientists from Johns Hopkins University and Osiris Therapeutics (47).

ADULT STEM CELL TRANSDIFFERENTIATION: THE RISE (AND PARTIAL FALL)

Transdifferentiation can be defined simply as the acquisition of an unexpected phenotype in a cellular lineage. Hence, when a hematopoietic stem cell gives rise to an erythrocyte or granulocyte, this is normal, expected differentiation. If the same hematopoietic stem cell gives rise to a cardiomyocyte, this is unexpected and therefore qualifies as transdifferentiation. Even this simple definition has two critical elements, however: cell lineage and cell phenotype. The imprecise use of these terms often leads to confusion. Cell lineage strictly refers to a genetic line of descent, telling us the ancestry of a cell in question. Lineage does not specify what specialized features a cell may have. Conversely, phenotype refers to specific cellular features at the time of study, such as gene expression patterns or physiological function, and it does not imply where the cell came from. It is critical that lineage and phenotype markers be shown to intersect in the same cell. Errors associated with these parameters, or their colocalization, likely explain much of the current controversy in stem cell research.

The late 1990s and first few years of this decade were marked by dramatic reports of transdifferentiation in multiple organ systems. Examples included marrow → skeletal muscle (48), marrow/blood → endothelium (49,50), neural stem cells → blood (51), skeletal muscle → blood (52), marrow → lung (53), marrow → brain (54,55), marrow → liver (56), and marrow → heart (7). Indeed, it seemed for a while that the rules of development did not apply to adult stem cells and that simply placing these cells into new environments was sufficient to induce them to acquire the phenotype of their surrounding tissue.

After a few years, however, several of these conclusions were shown to result not from transdifferentiation but from novel processes not known to occur at the time of the initial

report. For example, formation of blood from skeletal muscle turned out to result from "ectopic" hematopoietic stem cells that unexpectedly resided in skeletal muscle, rather than conversion of muscle-specific stem cells into blood (57). Formation of liver from marrow resulted from the fusion of blood cells with hepatocytes, and subsequent reprogramming of the leukocyte nucleus to a hepatic phenotype (58,59). Other observations were not reproducible when subsequently attempted or are suspected to result from experimental artifact. For example, formation of blood cells from neural stem cells could not be reproduced by another laboratory despite exhaustive effort (60). Additionally, the postulated formation of pulmonary epithelium from marrow has recently been suggested to be an artifact related to autofluorescence or close approximation of marrow-derived leukocytes and lung epithelium (61,62).

DO HEMATOPOIETIC STEM CELLS TRANSDIFFERENTIATE INTO CARDIOMYOCYTES?

Of particular relevance to the cardiovascular community was the hypothesis that bone marrow cells can give rise to new cardiomyocytes. A provocative study from Orlic et al. (7) suggested that directly injecting hematopoietic stem cells resulted in extensive myocardial regeneration. These authors took bone marrow from mice expressing enhanced green fluorescent protein (EGFP), depleted the differentiated cells, sorted the remainder for expression of the stem cell marker c-kit, and then injected them into acutely ischemic myocardium. They reported that nine days after injection, regenerating myocardium derived from the donor marrow occupied the majority of the infarct region. Further, mice receiving stem cell injection showed reduced ventricular dilation and increased fractional shortening by echocardiography. This article generated tremendous excitement in both the basic and clinical research communities.

To understand the mechanism through which marrow cells transdifferentiated into cardiomyocytes, our group, working in collaboration with Loren Field's group, devised a genetic screen using cardiac-restricted and ubiquitously expressed promoters (63). We isolated hematopoietic stem cells from transgenic mice wherein the cardiac-specific alpha-myosin heavy-chain promoter drove expression of nuclear-targeted beta-galactosidase (LacZ). In these transgenic mice, cardiomyocytes express LacZ in the nucleus, which can be identified by a sensitive histochemical stain, but all other cells in the body (including the marrow) are LacZ-negative. Hematopoietic stem cells were isolated according to the protocol of Orlic and Anversa and injected into acutely ischemic myocardium of wild-type (non-transgenic) mice. Transdifferentiation into cardiomyocytes would be accompanied by activation of the cardiac-specific transgene, thereby using a single marker to track both the donor lineage and the cardiac phenotype. Despite the use of an assay capable of detecting a single LacZ-positive nucleus in a wild-type heart, we were unable to detect a single transdifferentiation event in 42 infarcted hearts. Variations in the myocardial status (cautery injury, isoproterenol-induced injury, normal myocardium) and different strategies for stem cell isolation also failed to yield a single transdifferentiation event in another 66 animals. To rule out artifacts associated with our specific transgene, we transplanted hematopoietic stem cells from an additional cardiac-specific transgenic line (alpha-myosin heavy chain/EGFP) and mice ubiquitously expressing EGFP from the chicken beta-actin promoter. None of these 37 additional animals showed evidence for cardiac transdifferentiation. Finally, we compared sarcomeric actin and myosin expression patterns in mice receiving stem cell transplants to those receiving sham injections. There was no difference in the distribution of actin-positive or myosin-positive cells, indicating that significant regeneration did not occur. It should be emphasized that the grafted cells were readily identified in the injured hearts. They were small, round, myosin-negative and morphologically consistent with leukocytes.

Two other groups reported similar findings around the same time. Balsam et al. (28) found that hematopoietic stem cells did not transdifferentiate into cardiomyocytes, and they also showed that these cells instead acquired a leukocyte (predominantly granulocyte) phenotype within the infarct. Nygren et al. (64) reported that hematopoietic stem cell formed almost exclusively leukocytes within the infarct, with no activation of cardiac genes in the transplanted cells. They also showed that mobilization of marrow stem cells with cytokines did not result in formation of new cardiomyocytes, although endogenously derived circulating cells were noted to fuse with host cardiomyocytes. The basis for the discrepant findings among laboratories has not yet been discovered. An exchange of experimental samples among the different groups seems like a reasonable step toward resolving the differences.

In any case, a very large body of evidence leads us to conclude that there is no significant cardiac differentiation after direct injection of hematopoietic stem cells. A few circulating cells have been documented to fuse with host cardiomyocytes (64,65), giving rise to hybrid cells, but these are too few to influence contractile function. One implication of this work is that clinical trials of bone marrow for cardiac repair are unlikely to generate significant numbers of new cardiomyocytes. It remains quite possible, however, that marrow cells could influence remodeling of the ventricle by improving the connective tissue framework or promote angiogenesis to chronically ischemic regions.

RESIDENT MYOCARDIAL PROGENITORS

In recent years, several groups have reported the isolation of cardiac stem cells, also termed cardiac progenitor cells, from rat, mouse, dog, and human myocardium. In most studies, the isolation is based on the absence of cardiomyocyte, smooth muscle or endothelial markers and the presence of primitive cell markers. Examples include cells expressing the

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receptor for stem cell factor (c-kit) (66), cells expressing Sca-1 but not expressing c-kit (44), or cells expressing the transport protein Abcg2 (so-called "side population"), which are low in c-kit (67) but have been reported to express Sca-1 (68). Another study has simply used the ability of cells isolated from murine and human myocardium to form self-adherent clusters termed "cardiospheres" (in analogy to neurospheres formed by neural stem cells) in vitro (69).

Beltrami et al. (66) first isolated c-kit+ cells from adult myocardium of the rat. A histological analysis revealed that these cells were distributed in small clusters in the interstices between cardiomyocytes throughout the ventricular and atrial myocardium with a higher density in the atria and the ventricular apex. The cardiac c-kit+ cells were self-renewing, clonogenic, and multipotent, giving rise to three different cardiogenic cell phenotypes, that is, cardiomyocytes, endothelial cells, and smooth muscle cells and after direct injection regenerated the infarcted rat hearts. These cells have recently been reported to traverse the vascular barrier and participate in regeneration after intracoronary injection (30).

Just a month after the discovery of c-kit+ cardiac stem cells, Schneider's group reported the existence of adult mouse heart-derived cardiac progenitor cells expressing Sca-1 (44). These cells initially expressed no cardiac-specific genes, but a small percentage activated cardiac genes (but did not beat) in response to DNA demethylation with 5′-azacytidine. In vivo, the Sca-1+ cells homed to injured myocardium after ischemia/reperfusion. Using Cre recombinase techniques, the apparent cardiac differentiation was shown to be due to fusion in approximately 50% of cases. Shortly afterward, Matsuura et al. (70) reported isolation of Sca-1+ cells from adult murine hearts. These cells differentiated into beating cardiomyocytes when treated with oxytocin (yielding 1% beaters), whereas in their hands 5′-azacytidine failed to induce cardiac differentiation.

Another cardiac-derived subpopulation with progenitor potential has been studied by the groups of Martin et al. (67) and Pfister et al. (68). This rare population was isolated from mouse hearts based on their ability to exclude Hoechst 33342 dye, so-called side population cells, and the authors show that the transport protein Abcg2 confers the side population cell phenotype. These cells, which are present throughout cardiac development and in the adult heart, also express Sca-1+ (but are c-kit^{low}) and appear capable of differentiation into cardiomyocytes after coculture with rat cardiomyocytes.

The most recent addition to the burgeoning field of resident cardiac progenitors is cells expressing the homeobox gene islet-1 (isl-1). During development, isl-1+ cells contribute to formation of the outflow tract, the atria and the right ventricle, which develop from the "secondary heart field" (71). Laugwitz et al. (72) showed that a population of isl1+ cells persists in neonatal mouse hearts, which express the cardiac transcription factors Nkx2.5 and GATA4, but not Sca-1, CD31, or c-kit. The isolated progenitor cells demonstrate both self-renewal and maintain the ability to differentiate into functional cardiomyocytes in vitro and in vivo. However, isl-1+ cells have only been isolated from neonatal animals, and their existence or potential in older adults, where most infarcts occur, is currently unknown.

Taken collectively, these data argue that the postnatal heart has one or more populations of resident progenitor cells, which under some circumstances, can be induced to form new cardiomyocytes. The role such cells play in normal cardiac homeostasis or response to injury is not clear at present. Most scientists find it surprising that four nonoverlapping populations of progenitor cells reside in a tissue that repairs itself so poorly after infarction. However, other tissues that are known to have stem cells, such as the gut or brain, also respond poorly to infarction, and therefore failure to regenerate after injury should not be equated with the absence of stem cells. It is possible that resident progenitors are involved in a much-slower myocyte turnover, replacing occasional cell dropout but incapable of regenerating large injuries like an infarct. Without doubt, a multipotent cardiac stem cell would be a welcome candidate for cardiac repair. To be of clinical use, one would need either to activate the proliferation of endogenous progenitors and promote their migration to the site of injury, or isolate these progenitors, expand them sufficiently without sacrificing potential, and reintroduce them into the injured heart. Currently, not even the researchers from the best laboratories know how to do this with human cells, but reports exist that describe clonogenic expansion while preserving the functionality of cardiac stem cells from mouse, rat, and dog. For this area to move forward, it will be important for each candidate population to be confirmed by independent groups and to learn what their endogenous roles are.

EMBRYONIC STEM CELLS

Multiple groups have shown that cardiomyocytes can be reliably obtained from embryonic stem cells (ESCs), derived from the inner cell mass of preimplantation mouse and human embryos (reviewed in reference 73). Embryonic stem cell-derived cardiomyocytes express cardiac molecular markers, including Nkx2.5, GATA4, sarcomeric myosin heavy chain, and cardiac troponin I. Ultrastructural studies showing myofibrillar assembly and formation of intercalated disks indicate potential for a high degree of developmental maturity (74). Electromechanical coupling and electrophysiologic specialization also have been observed (75,76). The versatility of ESCs is their greatest asset, but it also makes the isolation of the cells of interest more challenging. Current protocols yield cardiomyocytes in sufficient quantities for most basic research needs. However, a limitation of the use of ESC for therapeutic purposes is the inefficiency with which cardiomyocytes are generated (typically 1% of a differentiating culture). Strategies based on developmental paradigms have used directed differentiation to increase cardiogenesis (77). These methods have had some success; however, not surprisingly, no one has been able to fully

recapitulate the complex mixture of cardiogenic factors and environmental cues that efficiently induce cardiomyogenesis during embryonic development.

Other groups have suggested that transplantation of undifferentiated ESCs into the heart provides the appropriate signals to induce cardiomyocyte differentiation that results in an improvement of contractile function (78,79). As appealing as this approach might be, subsequent studies, including our own, have shown that this method leads to the formation of teratomas (a tumor type composed of cells derived from all three embryonic germ layers) (80,81). Teratomas are arguably the greatest risk associated with ESC-based therapy. In fact, recent studies have shown that the formation of teratomas can counteract the benefit provided by the intended cellular therapy (82,83). Therefore, predifferentiation and purity are prerequisites to the application of cell-based therapies using ESC-derived cells. In an effort to increase purity, Klug et al. (84) selected cardiomyocytes using a cardiac-restricted promoter to drive the expression of a neomycin resistance gene, which allowed for the isolation of a population containing 99.6% ESC-derived cardiomyocytes. Other groups have enriched for cardiomyocytes using restricted promoters in combination with fluorescence-based cell sorting. The promise of the genetic selection strategy has been further validated by scalability studies (85).

Increasing the number of cells through mitogenic stimulation is another approach that would allow for the purification of higher numbers of ESC-derived cardiomyocytes. This strategy has been difficult because mouse-derived ESC cardiomyocytes have low mitotic rates (86). A surprising characteristic of human ESC-derived cardiomyocytes is that, unlike their mouse counterparts, they have a high proliferative capacity (87–89). The advantages of this capacity are multifold. First, it provides a unique human model system to analyze the biochemical mechanisms that control cardiomyocyte proliferation. Initial analyses have already shown that the proliferation of these cells can be regulated by the IGF-1/PI3 kinase/Akt signaling pathway (89). In vivo studies have also shown that grafts of transplanted human ESC-derived cardiomyocytes grow in size seven-fold over a four-week period (11). The elucidation of the cellular mechanisms that control this process can then be exploited to expand the cardiomyocyte population before transplantation and/or to increase proliferation post-transplantation in situ.

CLINICAL TRIALS OF CARDIAC REPAIR: PRIMUM NON NOCERE

After nearly 10 years in preclinical animal models, cell-based cardiac repair trials have begun in humans. (For a detailed review, please see Laflamme and Murry [14] and Murry et al. [90].) The first clinical trials were performed with autologous skeletal myoblasts (91), which, by virtue of needing several weeks to expand in culture, have all been performed in patients with chronic ischemic heart disease. Trials of autologous bone marrow cells began a few years later, involving both acute infarction and chronic ischemic disease (92–95). Most of these have been safety and feasibility trials, neither designed nor powered to provide data on efficacy, and therefore caution in interpretation is clearly indicated at this early stage. Nevertheless, there are tantalizing trends toward improved function in most of the cell-treated hearts that are supported by the findings of early randomized, controlled trials (96,97).

The editors of the *Journal of the American College of Cardiology* charged us to address the following questions about current cardiac repair trials: 1) Should stem cell trials be in the clinic? 2) If so, for acute infarction or chronic ischemic disease? 3) What are the optimal cells? 4) Which outcomes should we be looking for?

These are lucid and relevant questions but, unfortunately, we do not have enough information to answer all of them. It is our opinion that clinical trials of cell-based cardiac repair are warranted, based on a large volume of data showing preclinical safety and efficacy. Animal models, although critical, cannot reproduce all aspects of human heart disease. Conversely, once safety and efficacy have been established in the animal, it is reasonable to move carefully to the clinic while the underlying mechanism is being established.

The optimal timing of cell-based therapy after myocardial infarction is currently unknown. A recent infarct still in the healing phase is almost surely a more favorable substrate for cell-based repair than is an old scar within a dilated ventricle. On the other hand, many patients with infarcts do well with standard treatment and may not need such an advanced intervention as cell-based therapy. An improved ability to predict which patients will progress to heart failure would help in this regard. We think the current approach of testing both acute and chronic ischemic disease (as well as dilated cardiomyopathy) is reasonable, given our current information.

Current evidence does not permit one to rationally choose the best cell for cardiac repair. Autologous cells with limited plasticity, such as skeletal muscle or bone marrow, are likely to be the safest populations for initial trials. This is particularly true if one is studying patients with recent infarcts, for whom standard treatment is reasonably effective. On the other hand, more potent stem cells, such as embryonic stem cells, offer greater opportunities to truly regenerate myocardium. However, stem cell potency is a double-edged sword, and as highly potent cells are explored clinically, risks for complications such as arrhythmias and the formation of tumors may increase. For highly potent cells, it may be prudent to begin in patients targeted for heart transplantation, for instance, implanting cells at the time of left ventricular assist device placement. Patients on left ventricular assist devices should be least affected by arrhythmias or cardiac tumors, and cardiectomy at the time

of transplantation should prove a definitive therapy should a teratoma appear.

We believe that cardiac repair trials need to focus on ventricular function and anatomy as primary end points. Although these measurements are less powerful than morbidity and mortality, the field is not sufficiently advanced to support such outcome-based end points. If we are able to reliably demonstrate that cardiac repair works to improve ejection fraction and prevent/reverse ventricular dilation, we then can begin to test whether morbidity and mortality are reduced. Because the mechanism through which cell therapy acts is still being characterized, clinical trials that establish mechanistic correlates will be most helpful. For example, studies using magnetic resonance imaging in patients suggest that cell therapy might alter the rate of infarct repair or influence the amount of scar contraction (93). Positron emission tomography studies have demonstrated increased glucose uptake and enhanced myocardial blood flow in cell-engrafted regions (94,95,98), which provide important information regarding effects on tissue metabolism and perfusion. Another very useful mechanistic end point for clinical trials is the ability to track cells after they are implanted, for instance, through use of paramagnetic particles visible by magnetic resonance imaging (99), positron-emitting isotopes (100), or molecular tracers (101). Finally, we believe that, whenever possible, tissue-based analyses should be included in clinical trial design, either by evaluation of explanted hearts at the time of transplantation (102) or by autopsy of patients who die following cell therapy (103).

THINKING STRAIGHT ABOUT STEM CELL RESEARCH

It is important to evaluate all studies of cardiac regeneration with a critical mind, and studies claiming great advances merit particularly careful scrutiny. One need not have detailed training in stem cell biology to formulate reasonable opinions. When evaluating a new study, the first thing the reader should do is identify how the authors tracked the cells' lineage (who begat whom) and the cells' phenotype (what were the cells at the beginning, what were they at the end). As stressed previously, stem cell studies are only as good as their ability to track these two parameters. After that, it is sufficient to use common sense and remember the fundamentals of experimental design. For example, we must never underestimate the importance of control experiments. Most of us have read articles in which readers are whisked down a path that involves only stem cell treatments, leaving us to infer what might have happened in control animals. Controls do not always do what we predict, which is exactly what makes them so important. Another critical facet of regenerative therapy, too often omitted, is blinding of the observers. When one experimental outcome leads to publication, speaking invitations and research funding, and the alternate outcome leads only to more toil, it is imperative to protect ourselves even from subconscious biases. We submit that all stem cell therapy studies need to have their end points read by blinded observers, with the code broken at the study's end.

The early phases of research in cardiac repair used histological outcomes as their principal guide, which provided good insights into the fates of transplanted cells but told little about effects on contractile function. During the last five years, however, there has been a pronounced shift toward physiological outcomes as principal end points. This shift is reasonable—patients care more about their heart's function than they do its histology. However, the shift toward physiology has made mechanisms less evident, and multiple reports have erroneously reasoned that, because ventricular function was improved, the heart was regenerated. Following this line of logic, one might also conclude that beta-adrenergic blockers and angiotensin-converting enzyme inhibitors regenerate the heart. Both drugs reduce remodeling and improve ventricular function after an infarct, but few would assert that this is achieved through regeneration. As we read the existing literature, it is important to bear in mind that many paths lead to improved function, and not all proceed through regeneration.

CLOSING THOUGHTS

Stem cells offer the chance to rebuild damaged tissues like the infarcted heart from their component parts. What was a radical notion 10 years ago is now a mainstream experimental concept, and early clinical trials are underway throughout the world. The scientific and lay communities are extremely enthusiastic about the promise this field offers. For researchers in the field, however, enthusiasm is not enough. We must be rigorous, skeptical, and willing to have our own work subjected to public scrutiny and criticism. Our interventions have to be understood mechanistically (to permit rational improvement), tested in the best animal models (including large animals whenever possible), and reproduced by independent groups before they move to the clinic. The high expectations for stem cell research are reminiscent of the gene therapy field a decade ago, where an overzealous push to the clinic resulted in an adverse clinical outcome and a marked setback to the field (104). Stem cell therapy must not repeat gene therapy's trajectory. If we do it right, however, stem cells may give us the tools to reactivate processes of embryological development in diseased tissues. If so, the potential benefit to human health will be great.

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