Advances in Cell-Based Therapy for Structural Heart Disease

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Congestive heart failure and coronary artery disease are the leading causes of morbidity and mortality in the United States despite substantial therapeutic advances in the last half century. Only very recently have studies arisen that support the possibility of regenerating tissue of damaged human organs including the heart. In this regard, there is growing pre-clinical and clinical evidence demonstrating the safety and efficacy of cell-based myocardial regeneration using a variety of cell lines. Although the data on the exact mechanism of action and the fate of the administered cells is controversial, there is consistent evidence for improved cardiac function and myocardial regeneration using different cell types. This extraordinarily exciting scientific advance has forced cardiovascular scientists to re-evaluate the long-held paradigm of cardiac myocyte terminal differentiation and life-long longevity of the cardiac myocytes that comprise the heart. Whereas, these new ideas originated with attempts to perform cellular transplantation using exogenous stem or precursor cells, mechanistic insights have rapidly evolved to the realization that adult organs harbor stem cells with significant plasticity, capable of repopulating their respective organ. Indeed these cells may be harnessed as a therapeutic agent or may represent the target of regenerative therapeutic strategies.

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**Embryonic Stem Cells**

The field of ESC-based approaches to cardiac regeneration are described in the article by Christophorou and Gerhardt. Embryonic stem cells are conceived as a highly promising therapeutic approach because they are totipotent. In this regard, they are characterized by their capacity to proliferate in an undifferentiated state while maintaining their capacity to differentiate into cell lines from all 3 embryonic germ layers. Human ESCs when cultivated in suspension form contracting areas, embryoid bodies, which are positive for cardiomyocyte markers such as myosin heavy chain, α-actinin, desmin, and troponin I. Embryonic stem cells are capable of differentiating into cardiomyocytes with the capacity to propagate action potentials, as well as endothelial and smooth muscle cells. Administration of ESCs in an animal model of myocardial infarction results in engraftment, improved left ventricular (LV) function and reduced LV remodeling.

Embryonic stem cells have obvious important limitations as a therapeutic agent given their propensity toward teratoma formation, immunogenecity, and the ethical issues raised by their use. Indeed, the greatest value of ESCs currently is it being a model system for development, but indeed they hold substantial promise as a potential therapeutic agent.

**Skeletal Myoblasts**

In the quest to develop adult cell-based therapies, both skeletal myoblasts and bone marrow–derived cells have been studied in detail at mechanistic, translational, and clinical levels. Based on the concept that they could possess plasticity sufficient to give rise to cardiac muscle, skeletal myoblasts obtained from skeletal muscle biopsies were among the earliest cell types used for cardiac regeneration. Among their postulated advantages are the ability to derive autologous cells, thereby eliminating the need for immunosuppression. In addition, skeletal myoblasts have high proliferative capacity at a later stage of differentiation and are resistant to ischemia. These cells are committed to a myogenic lineage and as such are less teratogenic than ESCs.

As reviewed by Menasche, preclinical and clinical studies of post myocardial infarction (post-MI) administration of skeletal myoblasts demonstrate engraftment and functional improvement. However, engrafted myoblasts have not proven to differentiate into cardiac myocytes, leading to the idea that the beneficial effects of their administration derive from paracrine effects, autonomous contraction, infarct size reduction, or alteration in mechanical properties of the scar. Myoblasts secrete a variety of angiogenic and antiapoptotic factors similar to paracrine factors released from the bone marrow.

A variety of clinical studies have assessed safety and efficacy of skeletal myoblasts using both intracoronary and intramyocardial modes of delivery in patients with acute myocardial infarction and chronic ischemic cardiomyopathy. Inability of skeletal myoblasts to transdifferentiate to cardiomyocytes and to form electrical junctions has raised potential concerns regarding formation of substrate for ventricular reentry tachycardia.

**Bone Marrow–Derived Mononuclear Cells**

Another early foray into the field of cellular therapeutics involved the use of the bone marrow, motivated by the idea that bone marrow contains, in addition to hematopoetic stem cells, other stem cells with the plasticity to form cardiac myocytes. Two notable examples are bone marrow–derived mesenchymal stem cells and cells that express the c-kit receptor. Using c-kit–positive bone marrow–derived cells to improve cardiac function in mice after MI, Orlic et al stimulated extraordinary enthusiasm for the use of the bone marrow as a therapeutic strategy in humans. In rodent models, BMC contribute to regeneration of ischemic cardiomyocytes and endothelial cells after injection into infarcted myocardium. Subsequent studies that stimulated bone marrow mononuclear cells (BMCs) mobilization with granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) demonstrated improved ventricular function and regeneration of cardiomyocyte, endothelial, and smooth muscle cells. Work in this area is reviewed by Liao et al.
Clinical Trials of Bone Marrow–Derived Cells

The preclinical evidence for the regenerative capacity of BMCs prompted the conduct of clinical trials using bone marrow–derived mononuclear cells in both acute MI\(^3\) and chronic ischemic cardiomyopathy.\(^3\) The BOOST trial was an early trial where 60 patients were randomized to receive either autologous mononuclear cell therapy or conventional therapy. The BMC group had significant improvement of LV function compared with the control group; after 6 months, left ventricular ejection fraction (LVEF) increased 6.7 percentage points in the bone-marrow-cell group vs 0.7 in the control group.\(^3\) However, in a follow-up report, at 18 months, there was no longer a difference in LV ejection fraction between the 2 groups\(^3\) due to a catch-up increase in the placebo group, suggesting that BMCs accelerated recovery in ejection fraction (EF). Intramyocardial injection of BMC in patients with severe ischemic cardiomyopathy has also improved both LV function and myocardial perfusion.\(^3\) The recent results of reinfusion of enriched progenitor cells and infarct remodeling in acute myocardial infarction (REPAIR-AMI) revealed that intracoronary infusion of BMC 3 to 7 days after MI resulted in improved LV function at 4 months and reduction in combined clinical end point of death, recurrence of myocardial infarction, and any revascularization procedure at 1 year.\(^3\)

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are nonhematopoietic multipotent stem cells initially identified in bone marrow that have the potential to differentiate into a variety of mesodermal cell types.\(^4\) Importantly, MSCs or MSC-like cells are also found in umbilical cord blood, adipose tissue, and the heart. Bone marrow–derived MSCs lack HLA class II and B7-costimulatory molecule expression, which permit MSCs to exist even in inflammatory environments without activating host T-cells.\(^5\) MSCs have been used in rodent models of myocardial infarction, and they resulted in improved remodeling, and reduction of infarct size by transdifferentiating to cardiomyocytes and endothelial phenotypes.\(^4\) We demonstrated in a porcine model of MI that intramyocardial injection of allogeneic MSCs administered 3 days after MI resulted in reduced infarct size, improved LV function, and myocardial regeneration, without causing an inflammatory reaction due to rejection.\(^6\) Although MSC retention declined over time and there was no evidence of differentiation of injected MSCs into cardiomyocytes,\(^7\) there was clear evidence of the reappearance of myocardial tissue and improved regional LV function, suggesting that MSCs stimulate endogenous repair mechanisms. Several studies have suggested that improving the survivability of these cells by transfecting them with the gene encoding Akt,\(^8\) or administration of insulin-like growth factor-1,\(^9\) and MSC modification using a hypoxia-regulated heme oxygenase-1 vector\(^10\) translates into better response to MSC therapy.

Endothelial Progenitor Cells

As reviewed by Young et al,\(^11\) bone marrow–derived or circulating endothelial progenitor cells (EPCs) are functional precursors of endothelial cells, expressing AC133,\(^12\) and other endothelial cell surface markers.\(^13\) In a rat MI model, intravenously administered human EPCs showed selective migratory properties for ischemic cardiomyocytes and resulted in neovascularization of the ischemic tissue and reduction of apoptosis in the treated animal.\(^14\) Local injection of these cells into the infarcted myocardium also resulted in proliferating myocytes and vascular structures.\(^15\) The mechanism of action of these cells is mediated by neovascularization and formation of larger-size capillaries, which potentially protect the hypertrophied cardiomyocytes in the peri-infarct zone from apoptosis.\(^16\) Intracoronary EPC infusion resulted in improved LV function, myocardial perfusion, and reduction of infarct size when transplanted after recanalization of chronic total occlusion in a randomized clinical trial.\(^17\)
There is a single report of intracoronary infusion of autologous bone marrow–derived mesenchymal cells given to patients after MI resulting in improved LV function and myocardial perfusion, although a clear description of the cellular preparation is lacking in this report. Autologous and allogeneic MSCs have been used in several clinical trials including Crohn disease, osteogenesis imperfecta, and graft-vs-host disease. A multicenter, phase I, placebo-controlled, double-blind trial of allogeneic MSCs has been completed and will provide information regarding the safety and efficacy of intravenously administered cells in patients with acute MI.

Cardiac Stem Cells

In the quest to advance cellular therapeutics for heart disease, a series of laboratories pursued the identification of stem or precursor cells from the heart itself. Indeed, as described by Barile et al, candidate cells have been identified on the basis of either antigen panning, ability to exude Hoechst dye, or culturing approaches. These cells express c-kit, sca-1, Islet1, and MDR1. The origin of the cardiac stem cells is thought to be the cardioblasts during embryogenesis, although the analysis of the posttransplant organs of sex-mismatched heart transplants indicates that there could also be an extracardiac source of circulating stem cells that can replenish the pool of cardiac stem cells. The c-kit+ subset of cardiac stem cells is clonogenic in vitro and is able to undergo differentiation into cardiomyocytes, endothelial, and smooth muscle cell. These cells can also undergo in vivo differentiation into cardiomyocytes when injected into the heart tissue. The sca-1+ subset of cardiac stem cells has been shown to have both in vitro and in vivo capacity to differentiate into cardiomyocytes. Morretti et al recently reported that multipotent isl1+ cardiovascular progenitors can give rise to endothelial, cardiac, and smooth muscle cells.

The cardiac stem cells express markers of early and late stages of cardiomyocyte differentiation, suggesting that these cells are committed to the cardiomyocyte lineage. There, cells increase in number after MI and differentiate to cardiomyocytes. Cardiac stem cells require a nurturing environment to maintain hemostasis. Stem cell niches are described as structural and functional units that control the proliferation and differentiation of the stem cells. Anversa et al defined the cardiac stem cell niche as a microenvironment that nurtures cardiac stem cells and maintains the stem cell viability and tissue hemostasis. The cardiac stem cells and early lineage–committed cells are nested together and are connected structurally and functionally to myocytes and fibroblasts by junctional and adhesion proteins. This anatomic and functional arrangement is similar to the niche structure found in the bone marrow and brain.

Several studies have demonstrated that the administration of cardiac stem cells after myocardial infarction can result in myocardial and vascular regeneration. Studies have also shown that these cells can be stimulated via paracrine effect to reduce postinfarct remodeling. Cardiac stem cells can be grown from endomyocardial biopsy samples, raising the possibility of using these cells for autologous therapy.

Mechanism of Action

Almost all cell-based therapies have provided evidence for improved LV function and ameliorated remodeling, without necessarily forming new cardiac myocytes. As a result, a variety of mechanisms of action have been considered.

Differentiation Vs Fusion

One of the major controversies in the field of cardiac regeneration is whether injected stem cells differentiate into cardiomyocytes or not. Several animal studies using genetic and fluorescent labeling supported differentiation of the administered cell, whereas other studies challenge this mechanism despite the presence of LV function improvement. Other proposed mechanisms include fusion of the administered cells with the host tissue or endothelial regeneration.

Paracrine Effect

Several studies suggest that stem cell therapy improve LV function and reduce LV remodeling via secretion of antiapoptotic and angiogenic factors. For example, bone marrow stromal cells secrete factors that prevent cell death.
of cultured cardiomyocytes and endothelial cells under ischemic conditions. The observation that cytokines and hormones (eg, estrogen) mobilize bone marrow–derived stem cells and stimulate angiogenesis further supports a paracrine action hypothesis.

Activation of the Endogenous Repair Mechanism

The recent identification of the endogenous cardiac stem cells and the possibility that the heart has stem cell niches suggest intrinsic repair mechanisms in the heart. Because stem cell mobilization from the circulation contributes to replenishing cardiac stem cells, cell-based therapy could operate by potentiating endogenous repair mechanisms as reviewed by Liao et al.

Ethical Issues

As reviewed by Sugarman, the new field of cell-based therapy for human disease raises important ethical issues. Notable is the debate surrounding the ethics of performing clinical trials. Some investigators have argued that existing data are sufficient to justify the conduct of large human clinical trials, whereas others continue to question the safety of the use of stem cell therapy in clinical studies. Historically, there has always been a discrepancy between the preclinical and clinical results of a treatment strategy due to the complex biology and safety profile of treatments in clinical trials. Only the conduct of carefully designed randomized clinical trials can answer the question of safety and efficacy of stem cell therapy in cardiac regenerative therapy in human subjects. Consent issues, additional mechanisms to oversight the stem cell research, patient selection in early stage of clinical trials, and both financial and nonfinancial conflicts of interest of professional groups are among the key ethical issues raised by Sugarman.

Future Directions

Although there is growing evidence from preclinical and clinical studies to suggest that different classes of stem cells can improve LV function and remodeling, the predominant molecular mechanism for their effect is not well defined, and the optimal cell type has not been characterized. Identifying a common molecular pathway used by different cell-based therapies will help develop novel strategies targeting myocardial and endothelial regeneration. Emerging imaging techniques, as reviewed by Hoshino et al., will help investigators target the most suitable site for cell administration and will provide strategies to track cells in vivo, thereby contributing to mechanistic insights regarding this emerging field.

Current clinical trial results signal a maturation of the field away from surrogate measures of LV structure and function and are beginning to explore clinical benefits. These studies are beginning to provide valuable information about cell therapy’s safety profile. For example, emphasis is being placed on risks of lethal arrhythmias and tumorogenesis, both theoretic yet dreaded potential complications.

Despite the controversies raised in the field regarding scientific underpinnings and the ethical debates surrounding the clinical development, this area of clinical work and cardiobiology represents one of the most exciting new developments in all of medicine. The results of scientific and translational studies have both ushered in the exciting concept of permanently healing the injured heart and additionally have stimulated a reappraisal of some critical and long-held scientific paradigms. The emerging data suggest the possibility that cell-based therapies may enter clinical use in the not too distant future. The articles contained within this series outline key concepts and review the state-of-the-art in this rapidly evolving field.

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