Cardiac Regenerative Medicine

— Cellular Therapy and Tissue Engineering —

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Interest in regenerative medicine has grown worldwide, not only in academic circles, but also in the mass media. Cardiac disease is a leading cause of death, and many more randomized controlled trials investigating the use of regenerative therapy have been reported for the heart than for other organs. This review discusses the candidates for donor cells to be used in cell transplantation and the mechanisms for improving injured heart function. (Circ J 2009; Suppl A: A-61 – A-67)

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Society is increasingly affected by degenerative diseases that need to be treated by modern medicine. In the advanced stages of such diseases, modalities for cure are often not available. Heart disease is the primary cause of death throughout the world, despite dramatic improvements that have been made in the treatment of cardiovascular diseases over the past decades. At present, heart transplantation and ventricular assist devices are the last line of therapy for patients with disparate types of heart failure. Heart transplantation is the definitive treatment, with survival rates of 86.1% at 1 year and 78.3% at 3 years. However, several problems are associated with transplantation as a therapeutic modality, including a shortage of donors (in Japan, fewer than 10 transplantations are performed each year), long waiting period, infection, and carcinogenesis because of immunosuppressive drugs, as well as concerns about organ commercialism. Ventricular assist devices represent an alternative to heart transplantation, with the capability to function as a bridge-to-transplantation, destination therapy or bridge-to-recovery in certain cases of dilated cardiomyopathy. Although device-based therapy also has disadvantages, such as durability, infection, hemorrhage, and neurological events, several types of implantable ventricular assist devices have been released and have demonstrated excellent survival and quality of life. In Japan, clinical trials using the EVAHEART, Jarvik 2000 and DuraHeart have been completed for premarket approval.

Since the 1960s, numerous experiments using amphibians and newts have clearly demonstrated the regenerative potential of the heart. However, the concept that homeostasis may be maintained in the adult heart with cell renewal capacity via the stem cell system has been challenged by a series of experiments claiming the heart to be a non-regenerative organ. The regenerative capability of the heart is not sufficient to compensate for myocardial cell loss following infarction. Recently, there have been many reports of pluripotent stem cells/progenitors obtained from diverse fetal and adult tissues, ranging from skeletal muscle and bone marrow to amniotic fluid, placenta, umbilical cord, and fetus. Many investigators have demonstrated that murine bone-marrow-derived stem cells/progenitors carry over their transdifferentiation ability to generate an array of progeny types tested in the context of cardiac regeneration. However, they are unable to differentiate into cardiomyocytes and form electrophysiological connections with host cardiomyocytes. The results do not uniformly demonstrate effectiveness in improving cardiac function. However, the safety issues have been addressed through these clinical trials, which involved more than 1,000 patients who underwent regenerative therapy. The issues that remain to be solved include delivery methods, conditioning of the host, timing of implantation, and dose of cells, as well as cell population. This review explores the recent scientific and clinical advances that are likely to have a strong impact on regenerative medicine in the field of cardiology. In particular, we discuss the stem cell system in the heart and the mechanisms of cell therapy for heart diseases.

Skeletal Myoblasts

Autologous skeletal myoblasts were among the first cell types tested in the context of cardiac regeneration. Myoblast survival and differentiation into mature myofibers in scarred myocardium demonstrated their resistance to ischemia and ability to regenerate after injury. However, they were unable to differentiate into cardiomyocytes and form electrophysiological connections with host cardiomyocytes. The possibility that lethal ventricular arrhythmia may be induced following myoblast transplantation could not be eliminated through any modification of the cells, necessitating implantation of a defibrillator simultaneously during the transplant procedure. Several human trials are still ongoing, although the lack of efficacy has resulted in their premature termination.
Bone-Marrow-Derived Cells

Murine experiments have suggested that bone-marrow-derived hematopoietic stem cells could differentiate into cardiomyocytes and that their grafting into the heart following infarction could induce replacement of lost cardiac cells. However, subsequent studies with genetically labeled cells have indicated that bone-marrow-derived stem cells do not transdifferentiate into cardiomyocytes. A number of large-scale, randomized controlled trials have been performed in which autologous bone-marrow-derived stem cells/progenitors were grafted with or without following in vitro expansion into intracoronary artery or ventricular muscle. The majority of those studies used bone-marrow mononuclear cells and showed, at best, modest improvements in cardiac function. Furthermore, several studies did not demonstrate a significant difference in cell therapy for heart diseases. Thus far, the conclusive consensus from randomized controlled trials seems to be that although very few, if any, bone-marrow-derived cells integrate into the heart, an appreciable improvement in myocardial function is likely to occur following cell transplant; the mechanism by which cardiac function is improved remains to be disclosed. It is important to emphasize that no significant adverse events related to this procedure using bone-marrow-derived cell transplantation were observed during the trials. In animal experiments, ectopic differentiation, such as bone formation and excessive extracellular matrix (ECM) generation, microembolism of grafted cells into microvasculature, worsening of atherosclerosis in the coronary artery, and arrhythmia induction, had raised concerns prior to the clinical phase about possible effects in humans.

Endogenous Cardiac Stem Cells

For a long time, the adult heart was believed to be a post-mitotic organ comprising only terminal differentiated cells, which must survive a lifetime without being renewed. Recently, the identification of resident stem cells/progenitors within the heart, which was carried out independently by several institutes, has opened new avenues for cardiac repair using these cell populations.

C-kit

Cell surface markers are used to identify stem cell populations in the hematopoietic system and to isolate stem cells from the heart. Among the surface markers, the c-kit+ population in the adult rat heart was first isolated as a putative stem cell candidate. c-kit, a proto-oncogene, encodes a receptor for tyrosine kinase located on the W locus, and is activated by the stem-cell factor, c-kit ligand. Binding leads to target mobilization, anti-apoptosis, and cell proliferation. This cell population was reported to be clonogenic, self-renewing, and multipotent (i.e., able to differentiate into 3 main types of cells: cardiomyocytes, smooth muscle, and endothelial cells). In addition, bone marrow reconstitution with c-kit+ cells in c-kit mutant mice and vice versa disclosed that c-kit+ cells played a critical role in prohibiting expansion following myocardial infarction. The findings suggested that bone-marrow-derived c-kit+ cells may migrate in response to an insult to the heart, and that these progenitors may then generate their progeny, thus losing the self-renewal capability. The original reservoir of the stem-cell system may thus be the bone marrow, and the cardiac environment may represent a temporary location rather than an independent setting for maintaining the system.

Sca-1

Another representative hematopoietic stem-cell marker, Sca-1, also known as lymphocyte activation protein-6A/E (Ly6A/E) is a glycosyl phosphatidylinositol-anchored cell surface protein. A Sca-1+ population, which could be differentiated into cardiomyocytes after 4 weeks of exposure to the chemical demethylation agent, 5-azacytidine, was isolated from the heart, although the self-renewing and clonogenic potential remain undetermined. Their authenticity as cardiac stem cell (CSCs), however, has been debated because 5-azacytidine demethylates an entire genome in a nonspecific and uncontrolled fashion. Transplantation of Sca-1+ cells following reperfusion injuries has shown that their progeny integrate into the native heart as divergent types of cells constituting the heart (i.e., cardiac muscle, and smooth muscle cells) along the ischemic border zone, resulting in a 3.5% increase in the number of ventricular cells.

Side Population (SP) Cells

In addition to the methodology using antibodies to purify stem cells/progenitors, SP cells have been enriched on the basis of their dye exclusion properties, which express an ATP-binding cassette transporter (Bcrp1/ABCG2). SP cells from neonatal heart can differentiate into cardiomyocytes after exposure to oxytocin or trichoestatin A, but their clonogenic potential and self-renewal capacity are not yet determined. Heart-derived SP cells infused following insult to the heart show differentiation to cardiomyocytes, endothelial cells, and smooth muscle cells.

Cardiosphere-Derived Cells

Endogenous CSC/progenitors in the mouse fetal and adult heart have prompted investigations into similar populations in the human heart. An initial study demonstrated the existence of a heterogeneous population of cells in a suspension culture from human atrial and ventricular biopsy samples that possessed long-term self-renewal and in vitro and in vivo differentiation capabilities. In the same way that a neural stem cell population forms a sphere in a non-adherent culture dish, termed a "neurosphere", the population of these CSCs also forms self-adherent clusters, termed "cardiospheres". Another group investigated this cell population from human cardiac biopsies by transplanting them into the border zone of infarcted myocardium, which resulted in an increased number of cardiomyocytes in the infarcted area. The surface profile of the cardiosphere-derived cells is similar to that of bone-marrow-derived mesenchymal stem cells, and they exist predominantly in the right heart. The heart might thus exchange cells with bone marrow to maintain homeostasis of this cell population.

Isl1

The knock-out of the LIM homeodomain transcription factor is1 demonstrated complete absence of the outflow tract, right ventricle, and much of the atria. During cardiogenesis, is1+ cells arise from the cardiac crescent and mark the second heart field. Postnatal is1+ cardiac progenitors are coexpressed with known early cardiac transcription factors, Nkx2.5 and GATA4. Postnatal is1+ progenitors can differentiate into cardiomyocytes by coculture with neonatal cardiomyocytes. However, is1+ cells are detectable in human neonates, but are absent in adult hearts.
Epicardium-Derived Stem Cells

A new subset of vascular progenitors is derived from the proepicardial organs (PEO), which are derived in turn from the developing liver. Epicardial cells from the PEO migrate through the pericardial cavity to the heart, envelop it to form the primitive epicardium, and form components of the coronary arterial tree, the microvasculature, and interstitial cells via an epithelial–mesenchymal transition (EMT). The differentiation of CSCs/progenitors may depend on another important population of cells in the heart, termed “cardiac fibroblasts”, which arise from the PEO and epicardium, generate ECM, and provide the heart with elasticity and mechanical strength. Human adult epicardial cells could undergo EMT to produce the smooth muscle cells of the coronary vessels and the perivascular and intermyocardial fibroblasts under certain culture conditions. The epicardium is essential for maintaining myocardial architecture, as the interstitial fibroblasts migrate from the epicardium into the subepicardial space to support the cardiomyocytes and comprise a significant portion of the myocardial wall. In addition, the epicardium and myocardium engage in reciprocal paracrine and cell-to-cell interactions that are required for growth and development. A recent study has shown that epicardium-derived c-kit+ cells in mice and humans could differentiate into endothelial cells, smooth muscle cells, and even cardiomyocytes. Epicardium might supply cardiac progenitors to ischemic regions, demonstrating that cardiac function in infarct mice in which the pericardium was left open had greater preservation of left ventricular function, in contrast to that in infarct mice in which the pericardium was left open. Transcription factor Wt1 is restricted to the PEO and epicardium, Wt1+ cells arise from Nkx2.5+/ Isl1+, and could differentiate into cardiomyocytes. Another study examining T-box transcription factor, tbx18, expression showed that tbx18+ cells migrating from the PEO onto the surface of the heart constituted the epicardium and contribute to the cardiomyocyte supply in the ventricle. The actin-binding protein, thymosin β4, stimulates quiescent epicardium-derived progenitor cells into EMT. Administration of thymosin β-4 was approved by the FDA in 2005, and is currently subject to phase IA clinical trials for treating acute myocardial infarction.

Human Embryonic Stem Cells (ESCs)

Following differentiation induction into cardiomyocytes, most human ESC derivatives acquire only a fetal phenotype and do not fully mature into functional cardiomyocytes unless the cells are placed in an environment resembling normal tissue. A transplantation study described successful long-term survival of human ESC-derived cardiomyocytes and formation of gap junctions with host cardiomyocytes. Neither in-situ guided differentiation nor immunological tolerance induction was possible in murine ESC transplantation. The average efficacy of cell transplant did not surpass 5% improvement in fractional shortening, almost the same as that of somatic stem cells/progenitors. Moreover, none of the studies to date has reported long-term functional improvements. These data suggest that merely generating and injecting more cardiomyocytes to increase functional improvement is unlikely to be successful.

Fetal Cells

The first attempts at fetal cell transplantation were made in the 1920s. In 1987, studies involving transplantation of human fetal mesencephalic tissue, which is rich in dopaminergic neurons, to the striatum in patients with Parkinson’s disease were started. In the most successful cases, patients have been able to withdraw from l-dopa treatment after transplantation and resume an independent life; on the other hand, severe dyskinesia as a major adverse event following fetal cell transplantation has been a concern. At the annual meeting of the International Society for Minimally Invasive Cardiothoracic Surgery in 2005, Benetti, who is a pioneer of off-pump coronary artery bypass, presented the results of clinical experiments showing excellent improvement of cardiac function following fetal cell grafting to advanced heart failure. Japan lacks clear laws to guide the investigation of fetal cells, tissues, and organs. Although it is premature to consider fetal cell transplantation as a workable clinical therapy, results from both human trials and animal experiments suggest it has the potential to rescue and restore damaged organs. We need to establish definitive criteria to investigate fetal materials.

Cell Sheet

Tissue engineering has been developed as a basic technology for regenerative medicine. The fundamental components used in tissue engineering approaches are isolated cells, growth factors, and ECM. As alternatives for ECM, 3-dimensional biodegradable scaffolds have been used for the reconstruction of various tissues and organs. Recently, a multilayered cell sheet without the use of any biodegradable ECM overcame several hurdles associated with the use of biodegradable scaffolds. After cardiomyocyte sheets comprising a few layers were implanted subcutaneously in rats, their contractions were recognizable for up to 1 year. The cell sheet has the potential to repair damaged hearts with improvement in systolic function and reverse remodeling.

Cytokines

Many cytokines are involved in the pathophysiological processes of the heart. In heart failure, several cytokines might play important roles in mobilizing exogenous stem cells and stimulating endogenous repair mechanisms. Granulocyte colony-stimulating factor (G-CSF) is involved in the proliferation, maturation, and survival of granulocytes, and in the mobilization of bone marrow cells, including hematopoietic stem cells. Its receptor, G-CSFR, encoded by the CSF3R gene, is distributed not only in neutrophils, monocytes, and platelets, but also in cardiomyocytes. The actions of G-CSF are presumed to be primarily the recruitment of bone-marrow-derived stem cells reducing the amount of granulation tissue in the infarcted myocardium, and anti-apoptotic effects. Several clinical trials of G-CSF in the treatment of myocardial infarction have been
transplantation, adequate adhesion molecules may need to proliferate, grow, survive or differentiate. Prior to cell transplantation and bFGF administration solved some of the controversial aspect of experimental studies and human trials.

Engraftment and survival of grafted cells has not been sufficient, cytokine therapy should be examined as an alternative to ischemia, and is governed by a complex cascade of trophic cytokines, ECM components, cell surface receptors, and signaling pathways. Preclinical experiments and clinical trials have been performed to evaluate the use of angiogenic cytokines, such as basic fibroblast growth factor (bFGF or FGF-2) and vascular endothelial growth factor. There have been two large randomized, double-blind, placebo-controlled trials that have intra-arterially administered FGF-2 protein. In the first trial, the targets were patients with coronary artery disease in whom coronary intervention was not possible. Based on the results of that trial it was concluded that intracoronary injection of FGF-2 protein was not efficacious in the treatment of myocardial infarction. The TRAFFIC trial, in which FGF-2 protein was also administered intra-arterially, focused on patients with moderate to severe intermittent claudication. The treadmill test and data on quality of life failed to demonstrate significant improvement in the treatment group.

From the results of large randomized clinical trials, successful treatment by a single administration of a single cytokine for myocardial infarction appears not to be a novel treatment modality. The clinical success or failure of cardiac regenerative medicine depends not only on the cytokine administered, but also on the delivery method, timing, and preconditioning of the host. Currently, preclinical research is under way to identify a cytokine cocktail or combination of cytokines and drugs with complementary or synergistic effects for cardiac repair. Sustained release of bFGF using biodegradable materials would be an effective approach to overcoming the limitations of cytokine protein therapy. Recently, a combination of swine endogenous CSC transplantation and bFGF administration solved some of the problems associated with the individual treatments used alone, such as poor cell survival and insufficient intervention in the regenerative process, respectively. Hepatocyte-growth-factor gene transfer with myoblast grafting improved cardiac function in a hamster model of dilated cardiomyopathy. Although the results of major clinical trials have been less than ideal, cytokine therapy should be examined as an important component of cardiac regeneration therapy.

Mechanism

Transdifferentiation and Direct Differentiation

Transdifferentiation, also termed plasticity, or direct differentiation of grafted stem cells/progenitors is a controversial aspect of experimental studies and human trials. Engraftment and survival of grafted cells has not been sufficient to improve organ function. Although the process of cellular engraftment is not very well understood, it must involve the binding of implanted cells to host matrix elements. Without physical tethers to matrix elements, cells will not proliferate, grow, survive or differentiate. Prior to cell transplantation, adequate adhesion molecules may need to be expressed in both donor and recipient.

Paracrine Effects

In the cell transplantation strategy into the heart, the number of donor cells is very low relative to the total cardiac cells. Moreover, because of stressful conditions at the site of cell injection and the harsh milieu of the failing heart, substantial cell losses are common immediately after cell grafting. Despite these significant cell losses, reverse remodeling has been unequivocally observed. A small number of cells could thus have a profound impact on the local environment, if they secrete key stimulatory factors for repairing organs.

Angiogenesis

In animal models of myocardial ischemia, administration of angiogenic growth factors, either as recombinant protein or by gene transfer, could improve perfusion by inducing angiogenesis. Even angiogenesis in the absence of myogenesis might improve cardiac function via reverse remodeling. Increased perfusion may salvage hibernating cardiomyocytes and also aid in the restoration of injured ECM, which in turn may facilitate donor cell incorporation and donor-to-host cell communications at the site of cell transplantation.

Transplantation of bone-marrow-derived mesenchymal stem cells rescued murine infarcted heart through angiogenesis and myogenesis. Cell transplantation has been used in non-ischemic models with similar induction of angiogenesis and improvement in cardiac function.

ECM

The ECM is a frame of tissues or organs and a dynamic signaling network that assembles and maintains a variety of cells. The ECM becomes dysfunctional in cardiac remodeling after myocardial infarction, in hypertensive hearts, and in cardiomyopathy. The fibrillar collagen network is the primary matrix component that maintains myocardial shape, alignment, and the transduction of cell shortening into effective ventricular ejection. An increase in interstitial collagen content causes the myocardium to become stiffer. In contrast, a reduction in collagen content, characterized by disruption of fibrillar collagen, leads to a dilated left ventricle. Moreover, collagen degradation results in contractile dysfunction without concomitant alterations in myocyte contractility, confirming a critical role for the ECM in regulating cardiac structure and function. The integrity and homeostasis of the ECM are mainly regulated by the balance between degradative enzymes, the matrix metalloproteinases (MMPs), and their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs). Smooth muscle cell transplantation in hamsters enhanced TIMPs expression and reduced MMPs expression, resulting in restoration of the ECM architecture and attenuation of chamber dilation.

Cell Fusion

Cellular or nuclear fusion is considered an alternative to cell reprogramming by transdifferentiation. Direct fusion of donor cells to host cells might contribute in the cell transplantation setting, but the specific role remains to be determined. Cell-to-cell communication is essential for living organisms. To date, diverse mechanisms for the intercellular transfer of information have been documented, including chemical synapses, gap junctions, and plasmodesmata. Recently, ultrafine intercellular structures of cultured cells, which are referred to as “tunneling nanotubes”, have been observed. Following that report, it was demonstrated that...
the same structures were formed between murine endothelial progenitor cells and cardiac myocytes. Moreover, when cells with nonfunctional mitochondria were cultivated with human MSCs that had intact mitochondria, the deficient cells received the intact functional mitochondria. Mitochondria are essential organelles in cells and are involved in many diseases. Transfer of fresh mitochondria might contribute to the mechanism of cell transplantation. However, such transfers have not been determined in vivo, even in animal experiments.

Summary

Regenerative medicine is a rapidly developing field offering great potential for the cure of disparate disorders. Many issues remain to be addressed, however, before these therapies will have advanced sufficiently to offer fully realized medical modalities. In concluding this review, we would like to cite the words of Dr Rosenzweig, “We should guard against both premature declarations of victory and premature abandonment of a promising therapeutic strategy. The ultimate success of this strategy is likely to depend on continued and effective coordination of rigorous basic and clinical investigations.”

References


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