Cell Therapy for Cardiovascular Regeneration

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A great number of cardiovascular disease patients all over the world are suffering in the poor outcomes. Under this situation, cardiac regeneration therapy to reorganize the postnatal heart that is defined as a terminal differentiated-organ is a very important theme and mission for human beings. However, the temporary success of several clinical trials using usual cell types with uncertain cell numbers has provided the transient effect of cell therapy to these patients. We therefore should redevelop the evidence of cell-based cardiovascular regeneration therapy, focusing on targets (disease, patient’s status, cardiac function), materials (cells, cytokines, genes), and methodology (transplantation route, implantation technology, tissue engineering). Meanwhile, establishment of the induced pluripotent stem (iPS) cells is an extremely innovative technology which should be proposed as embryonic stem (ES) cellularization of postnatal somatic cells, and this application has also showed the milestones of the direct conversion to reconstruct cardiomyocyte from the various somatic cells, which does not need the acquisition of the re-pluripotency. This review discusses the new advance in cardiovascular regeneration therapy from cardiac regeneration to cardiac re-organization, which is involved in recent progress of on-going clinical trials, basic research in cardiovascular regeneration, and the possibility of tissue engineering technology.

Keywords: cell therapy, stem/progenitor cell, cardiovascular disease, regeneration, cytokine

INTRODUCTION

Cardiovascular disease is one of the major causes of death. Although it requires an extended period of treatment, resulting in high medical costs, a complete recovery from cardiovascular injury is still difficult, and the patient’s QOL markedly decreases. Various technologies using devices (e.g., catheter intervention, pacemaker, including an implantable cardio-defibrillator with cardiac resynchronization system, and ventricular assist device) have been developed for cardiovascular disease, but they do not always reduce the mortality rate.1,2) In such a situation, “cell therapy,” which aims to restore the impaired function of organs by “cardiovascular regeneration” as its mechanism, has been generated from the 1990s. This innovative therapy was begun using bone marrow cells and has been performed for more than 10 years, and heterogeneous differences in clinical effects and patient’s outcomes, which are caused by cell sources and transplantation methods, have been clarified. Now, cardiovascular regeneration therapy is exactly on a turning point to break through the current aspect. Here, we show current cell therapies that have been clinically used for cardiovascular diseases and discuss their future advancement.

MECHANISTIC DIVERSITY OF VARIOUS CELL THERAPIES

Cardiovascular regeneration consists of the cardiomyocytes and skeletal muscles regeneration with angiogenesis and vasculogenesis. During the early prenatal period, vascular generation, maturation, and development are managed by “vasculogenesis,” which refers to the differentiation...
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The self-vascular regeneration process by cell transplantation (cells for vascular construction such as bone marrow-derived cells and EPCs) or an administration of some cytokines. However, previous studies on cell therapy using bone marrow mononuclear cell (BMNC) have rarely shown solid and mature vascular formation in ischemic tissue and rather clarified a new vascular network limited to collateral vessel growth by angiogenesis. Therefore, the protective effects of several cytokines released by transplanted cells have been suggested as a mechanism of the restoration of impaired function in organs.

Another important factor is the regeneration of cardiomyocytes and skeletal muscle cells in damaged cardiovascular systems. Self-repair of skeletal muscle by myoblasts and satellite cells is known to be actively performed in the damaged muscle. Therefore, in cell or cytokine therapy for peripheral artery disease (PAD) represented by atherosclerosis obliterans (ASO) and Burger’s disease, an improvement in blood flow by vascular network reconstruction promotes the regeneration of skeletal muscle cells. In addition, this occurs because skeletal muscle cells have an ability to produce several cytokines such as HGF and VEGF by autocrine or paracrine systems.
and the skeletal muscle has partial self-regeneration ability by forming abundant collateral vessels and promoting myocyte differentiation in post ischemic disorder.\(^{12}\)

On the other hand, since the mature cardiomyocyte cannot proliferate and divide by mitosis in vitro, it has been considered for a long time that the heart is the terminal differentiated organ. Indeed, myocardial necrotic tissue due to myocardial ischemia has been associated with the promotion of fibrosis and non-regeneration of cardiomyocytes. However, recent studies have suggested that cardiomyocytes lost by apoptosis are renewed at a rate of approximately 1% per year from birth.\(^{13}\) This theory may suggest an updating of the established theory of which the heart is the terminal differentiated organ. As cell sources provided in the cardiomyocyte turnover theory, bone marrow-derived stem cells\(^{14–16}\) and epicardial cardiac progenitors\(^{17}\) have been suggested. However, the incidence of new host-derived cardiomyocytes according to analysis of chimerism of the transplanted heart\(^{18}\) indicates a very low rate of cardiomyocyte turnover by bone marrow cells. Meanwhile, endogenous cardiac stem/progenitor cells (CSC/CPC) that are present in cardiac niches\(^{19}\) are also candidates of cell sources contributing to cardiomyocyte turnover. These CSC/CPC are activated under ischemic disorder, undergo induction of differentiation in the ischemic myocardium, and become a small part of the regenerated cardiomyocyte. However, this physiological cardiomyocyte turnover is fully insufficient for the self-repair of ischemic myocardium. In addition, since the differentiation mechanism of CSC/CPC in vivo is also unclear, it is still difficult to say that the heart is an organ with functional self-regeneration ability.

Therefore, to restore cardiac dysfunction caused by severe cardiovascular disease, cell therapies for functional and histological myocardial regeneration are indispensable (Fig. 1). However, the differentiation potential of bone marrow-derived stem cells is insufficient, and most regenerated cardiomyocytes in the host myocardium result due to cell fusion between these transplanted cells and host cells. Thus, bone marrow-derived stem cells have not exhibited adequate effects for regenerating functional cardiomyocytes. Instead of these cells, CSC/CPC isolated from host heart is now being focused on as one of candidates of cell sources of cardiac regeneration therapy.\(^{20–23}\) Among somatic stem cells, CSC/CPC have a high potential to differentiate into cardiomyocytes. These cells have been confirmed to differentiate into cardiomyocytes in the ischemic heart through not only cell fusion with host cardiomyocytes but also trans-differentiation and contribute to the supplementation and functional recovery of lost myocardial tissue, mainly in the border zone between fibrotic tissue and living cardiomyocytes (Fig. 1). Their clinical application has already been initiated. However, since cell transplantation into ischemic tissue is indispensable in cardiac regeneration by cell therapy, the loss of transplanted cells from host myocardium is still not unchained in cell therapy using CSC/CPCs. In recent years, to overcome this disadvantage, hybrid therapy, combined with the controlled release of cytokines that improve the low survival rate of transplanted cells has been used, and the application of an embryonic stem (ES) cell or induced pluripotent stem (iPS) cell with the highest ability to differentiate into cardiomyocytes will put us in a place to hope for cardiovascular regeneration therapy.

**CURRENT CELL THERAPIES FOR CARDIOVASCULAR REGENERATION**

**Peripheral artery disease (PAD)**

Indications for cell therapy for PAD are severe ASO (Fontaine stage III or IV) or Berger’s disease that is difficult to treat when only using revascularization techniques, such as artery bypass. The goal of cell therapy using bone marrow or peripheral blood mononuclear cells is the salvage of the ischemic limb. The TACT trial initiated in 2000 was the first clinical trial in the world using autologous human BMNC for severely ischemic limbs.\(^{24}\) Autologous BMNCs were isolated from bone marrow collected under general anesthesia, and about 2.7–2.8 \(\times 10^9\) cells were injected into 40 sites of the skeletal muscle in ischemic limbs. This trial was conducted as a randomized controlled trial, which was performed using overall survival, amputation-free survival, ankle-brachial index (ABI), \(\text{TcO}_2\), and the pain-free walking time as primary endpoints. As a result, in patients with severe ASO and Berger’s disease, particularly in the latter, the amputation-free ratio and pain-free walking time markedly improved. Based on these results, this therapy has been performed in patients with severe ASO and Berger’s disease in more than 30 institutions, in Japan. Then, the clinical application in which mononuclear cells in peripheral blood are used, which is less invasive, has also been evaluated because of the marked invasiveness of bone marrow aspiration during general anesthesia. However, since these cells contain a lower percentage of EPCs (about 0.5%) than BMNC, adequately improved
effects on limb ischemia cannot be obtained. Therefore, in another clinical trial in Japan, increased CD34⁺-EPCs by the administration of granulocyte-stimulating factor are purified by magnet cell sorting (MACS) and are transplanted into ischemic limbs.

**Acute myocardial infarction (AMI)**

Cell therapy for AMI is generally performed as follows. A bone marrow aspirate is usually collected within 3–5 days after the onset of AMI, and BMNCs are separated by the Percoll gradient method or blood apheresis. Then, $2–3 \times 10^9$ cells of BMNCs are administered into an infarct-related coronary artery that has succeeded revascularization. The BOOST trials were cardiac regeneration trials that were safely completed without adverse events. However, concerning clinical effects in these trials, the left ventricular function that was evaluated, based on the left ventricular ejection fraction (LVEF), did not significantly differ between the cell transplantation and placebo groups at 6 months and 5 years after cell therapy.²⁵ On the other hand, in the REPAIR-AMI trial, the effects of preserving LV systolic function were observed in patients with lower LV function classified in a baseline LVEF < 50%.²⁶ Therefore, the task force of the European Society of Cardiology suggested that revascularization using BMNCs is effective in AMI patients: (1) those who have revascularization delayed more than 12 hours after the onset of AMI, (2) in which the outcome is expected to be poor (decreased left ventricular ejection function) even after standard revascularization.²⁷

**Old myocardial infarction (OMI)**

In the TOPCARE-CHD trial, bone marrow and peripheral blood-derived mononuclear cells were transplanted in patients with an old myocardial infarction (OMI) instead of in those with AMI, in whom clinical effects are influenced by the result of revascularization²⁹ (Table 1). The protocol used in this trial was similar to the one used in the REPAIR-AMI trial, and the subjects consisted of 75 OMI patients after 3 months or more from the onset of AMI. In this trial, there were no limitations for the LVEF of eligible patients before cell therapy. As a result, 2.9% of LVEF from baseline to 6 months after cell therapy improved in the BMNC group but not in the peripheral blood mononuclear cell or placebo group. However, clinical efficacy was thought to be insufficient in patients with reduced LV function (LVEF < 41%), and this therapy was not considered to be useful as standard therapy for OMI patients. Stamm, et al.,²⁹ who performed direct intramyocardial injection of AC133 antigen-positive bone marrow cells concomitant with coronary artery bypass grafting (CABG), reported that a 6.3% improvement in LVEF was observed in the cell therapy group compared with CABG alone. Now, a Phase III trial is ongoing to evaluate the validating intramyocardial bone marrow stem cell therapy in combination with CABG (PERFECT trial).³⁰

As a transplant cell, other than bone marrow-derived cells, skeletal myoblasts (SMBs) have also been used as carriers of several cytokines such as IGF-1 and HGF with cardio protective effects. In the MAGIC trial, SMBs were directly injected into the ischemic myocardium in OMI patients with LV dysfunction (LVEF < 35%) concomitant with CABG.³¹ In the phase I study of this trial, since there was a concern that SMB transplantation could increase the risk of ventricular arrhythmia, an implantable cardio-defibrillator was implanted in all patients. As a result of a placebo-controlled, double-blind study, the incidence of ventricular arrhythmia did not significantly differ between the cell therapy and placebo groups, but no significant difference was observed in improvement of LVEF, 6 months after treatment.

Based on the results of these clinical trials on cell therapy using bone marrow cells or SMBs to OMI patients, another randomized phase I trial using cardiosphere-derived cardiac stem cells was initiated (CARDiosphere-Derived aUtologous stem CEIls to reverse ventriculardysfunction: CADUCEUS).³² Marban, et al., collecting myocardial tissue (278 mg) by endomyocardial biopsy from the right ventricle in MI patients with LV dysfunction (LVEF, 25%–45%) at 3 weeks after revascularization for AMI, performed the ex vivo culture of cardiosphere-derived stem cells from these tissues for 65 days (mean). The CADUCEUS trial used a protocol in which the intracoronary infusion of cardiosphere-derived stem cells into the infarcted myocardial area is performed using a balloon infusion catheter. Of 467 registered patients, 25 eligible patients were allocated into the low ($12.5 \times 10^6$ cells) or high ($25 \times 10^6$ cells) dose cell therapy group and a placebo group, and major adverse cardiac events (MACE), LVEF, and infarct volume, evaluated by Magnetic Resonance Imaging (MRI), were analyzed 6 months after the cell infusion. As a result, MACE occurred in 27% of those in the cell therapy group, but the incidence of MACE did not significantly differ from that of the placebo group (13%) in statistic analysis. Concerning efficacy, both in cell therapy groups and the placebo group, the LV dysfunction showed no significant improvement after
6 months, but the infarct volume of the cell therapy group significantly decreased from baseline to 6 month later. These results suggested that cell therapy using cardiosphere-derived cardiac stem cells is also effective in some patients with LV dysfunction after AMI.

Ischemic cardiomyopathy

There was also a clinical trial using bone marrow-derived mesenchymal stem cells (BMSCs), which can differentiate into multiple mesodermal cell types, in patients with ischemic cardiomyopathy (POSEIDON study).33) (Table 1). In this trial, 30 patients with ischemic cardiomyopathy were randomized to either autologous or allogeneic transendocardial injection of BMSCs using the Biocardia Helical Infusion Catheter, and the usefulness of BMSCs and the safety of allogeneic transplantation were evaluated. As a result, the myocardial infarction size was decreased, but no improvement was observed in the left ventricular systolic function evaluated by LVEF. However, there were no adverse events including immune responses, which were associated with allogeneic transplantation. Therefore, this is a very important trial that showed the safety of allogeneic transplantation associated with the “immune privilege” of BMSCs.

The clinical application of autologous cardiac stem cell (CSC) was also initiated to ischemic cardiomyopathy in 2009. Bolli, et al. performed a Phase I trial in which c-kit-positive CSCs were isolated from myocardial tissue of the right atrial appendage of OMI patients with LV dysfunction (LVEF ≤40%), and infused into the infarct-related coronary artery 4 month after CABG (Cardiac Stem Cells in Patients with Ischaemic Cardiomyopathy: SCIPIO trial).34) The SCIPIO trial was a partial randomized trial, and CABG was followed by placebo infusion in 7 patients as controls or CSCs infusion in 16 patients. In the control group, the LV dysfunction showed no improvement, but in the c-kit-positive CSCs group, the 8.2% of LVEF restored from baseline to 4 months after CABG.

However, although cardiac regeneration therapies using various types of cells have been attempted from the 1990s, a common issue of cell therapy, which is the low engraftment and survival rate of transplanted cells in the ischemic environment, is still unsolved. In this severe environment of the cell transplantation area, more than 80% of cells directly transplanted into the host myocardium are lost.35–37) To overcome this issue, the promotion of cell adhesion38) and transduction of proteins or genes with anti-apoptosis effects39) have been attempted for the survival of transplanted cell. Among these methods, hybrid therapy using tissue engineering is expected to be clinically applied. In an experimental study using an infarcted porcine heart model, control-release of bFGF using gelatin hydrogel combined with human CSC transplantation resulted in a 2-fold or greater increase in cell engraftment in the host myocardium until 4 weeks after cell transplantation. To evaluate the efficacy of cell transplantation combined with control-release of bFGF, a randomized controlled study was performed between bFGF alone and the transplantation of human CSCs with bFGF (hybrid therapy group). In the hybrid therapy group, the LV dysfunction showed significant improvement in LVEF (8.4%), which was more than twice the improvement in bFGF groups. Using this innovative technology, the

<table>
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<tr>
<th>Trial</th>
<th>Cell</th>
<th>LV Function</th>
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<td>97</td>
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<td>I–IIa</td>
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<td>Intracardial injection + CABG + DDS</td>
<td>09'.9</td>
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LV: left ventricular; CABG: coronary artery bypass grafting; DDS: drug delivery system
ALCADIA trial has been initiated since 2009.\textsuperscript{41,42} This is a phase I trial in which hybrid therapy consisting of autologous CSC transplantation and control-release of bFGF is performed in ischemic cardiomyopathy patients with reduced LV function (LVEF <35%) in whom CABG is needed. Autologous CSCs were isolated from myocardial tissue (about 18 mg), which was obtained by right ventricular endomyocardial biopsy, and cultured ex vivo for about 33–39 days, and $0.5 \times 10^6$ cells/kg body weight were transplanted with a bFGF-incorporating gelatin hydrogel sheet concomitant with CABG. Cell therapy was completed in 6 patients in 2 years, and the recurrence of cardiac failure as MACE was observed in only 1 patient. The postoperative cardiac function favorably improved compared with the results of clinical trials using conventional CABG without cell therapy.\textsuperscript{43}

**NEW GENERATION CELLS TO CARDIOVASCULAR REGENERATION**

The ethical hurdle for human ES cells is high, and there is still no prospect for the realization of ES cell application to cardiac regeneration therapy. Therefore, instead of ES cells, the iPS cells reported by Yamanaka, et al. have offered greater hope in cardiac regeneration therapy.\textsuperscript{44,45} The application of iPS cells for cardiac regeneration is exactly expected to make the cardiac regeneration come true, including not only the iPS cell-derived cardiomyocytes, but also the direct conversion of cardiomyocytes from cardiac fibroblasts based on advances in cell reprogramming technology.\textsuperscript{60} For clinical application, reprogrammed cells still have various problems such as their production period, proliferative ability, and teratoma formation due to differentiation-resistant cells. However, these cells will be a novel transplant cell allowing autologous transplantation obtained by overcoming the problems of ES cells, and then, further studies should be necessary.

**SUMMARY**

We reviewed the development of cardiac regeneration therapy and described its future prospects. During the past 10-year period since the appearance of cardiovascular regeneration therapy, progress in cell therapy have been made from first-generation therapy to new generations such as cardiac stem cell, tissue engineering, and iPS cells. The methodologies of cardiovascular regeneration may not be the identical among individuals, and there should be an optimal cardiac regeneration therapy for each patient. In the future, secure milestones should be provided, not only for the development of new treatment methods but also for the establishment of cardiac regeneration methods appropriate for individual patients. We expect progress in cardiac regeneration therapy in the next 10 years.

**DISCLOSURE STATEMENT**

None declared.

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**REFERENCES**


