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Review

Stem Cell Therapy in Heart Disease: Limitations and Future Possibilities

Toshikazu Sano[a]*§, Shuta Ishigami[a], Tatsuo Ito[b], and Shunji Sano[a]

[a]*Department of Surgery, Division of Pediatric Cardiothoracic Surgery, University of California San Francisco, CA 94158, USA,*
[b]*Department of Hygiene, Kawasaki Medical University, Okayama 700-8505, Japan*

Heart diseases are one of the major causes of morbidity and mortality worldwide. Despite major advances in drug and interventional therapies, surgical procedures, and organ transplantation, further research into new therapeutic options is still necessary. Stem cell therapy has emerged as one option for the treatment of a variety of heart diseases. Although a large number of clinical trials have shown stem cell therapy to be a promising therapeutic approach, the results obtained from these clinical studies are inconsistent, and stem cell-based improvements of heart performance and cardiac remodeling were found to be quite limited. Since the precise mechanisms underlying the therapeutic actions of stem cells are still under debate, researchers have developed a variety of strategies to improve and boost the potency of stem cells in repair. In this review, we summarize both the current therapeutic strategies using stem cells and future directions for enhancing stem cell potency.

Key words: heart disease, stem cell, myocardial regeneration

Heart failure is one of the most common causes of death in heart disease patients. Despite significant improvements in pharmacological and interventional treatment for heart failure, current treatment is palliative and cannot restore the cardiac function or maintain normal heart function over the long term [1,2]. In the case of end-stage heart failure, heart transplantation is currently the only available treatment. However, for many patients, transplantation is not a viable option given the strict criteria for eligibility and the long waiting period due to the limited numbers of heart donors in Japan [3].

In recent years, interest has inclined more towards myocardial regeneration with the application of stem cells [4,5]. Clinical trials have used various types of autologous transplanted cells, such as adipose tissue-derived mesenchymal stem cells [6], bone marrow-derived mesenchymal stem cells [7,8], and cardiosphere-derived cells (CDCs) [9], and achieved an increase in viable myocardial tissue and a decrease in infarct size. However, many questions remain unresolved, and for now, no cell therapy has been unequivocally shown to be effective for the treatment of heart diseases. Therefore, new strategies have been developed in order to improve the potency of current stem cell therapies.

In this review, we provide an overview of the different cell types that have been used in regenerative therapies to treat heart diseases. We also discuss promising strategies to improve the outcome of stem cell therapies.

Stem Cell-Based Therapies for the Treatment of Heart Disease

Several types of cells have been used in cell therapy and are beneficial for improving cardiac function, mainly in cases of ischemic heart disease. Stem cell-based therapy has the ability to activate endogenous regenerative processes, which include the recruitment

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*Corresponding author. Phone: 1-415-476-3535; Fax: 1-415-476-5365
E-mail: swallowtails2@yahoo.co.jp (T. Sano)
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of resident stem and progenitor cells and the promotion of cardiomyocyte proliferation [10]. Pluripotent stem cells seem to be an appropriate candidate due to their proliferative properties and their ability to differentiate into various cell type, including cardiomyocytes [11].

Skeletal myoblasts. In the field of cardiac regeneration, skeletal myoblasts were the first cell type to be tested both in pre-clinical and clinical trials in patients with ischemic heart disease. The first effective transplant of skeletal myoblasts into the damaged human heart was performed in 1994 [12]. However, in most of the clinical trials using skeletal myoblasts, a high incidence of ventricular arrhythmias was detected in patients treated with the cells [13, 14].

Bone marrow-derived stem cells. Bone marrow-derived stem cells are one class of multipotent stem cells that have attractive properties. Bone marrow contains several subpopulations of cells with the ability to transdifferentiate into both myocardial and vascular cells [15]. Although patients randomized to receive skeletal myoblasts in the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial had a higher incidence of ventricular arrhythmias, patients who received transplantation of bone marrow mononuclear cells did not show an increased rate of arrhythmias [16]. The limited survival of implanted stem cells in trials using bone marrow mononuclear cells and the fact that the favorable effects of adult stem cells are mediated by mechanisms other than direct cardiomyocyte differentiation might account for this favorable safety profile.

Mesenchymal stem cells (MSCs). MSCs, which reside in the stroma of bone marrow, are found in bone marrow, muscle, adipose tissue, and skin [17]. MSCs can transdifferentiate into cardiomyocytes under certain condition, such as in co-cultures with cardiomyocytes [18]. They also can be induced to differentiate into endothelial cells by culturing with vascular endothelial growth factors (VEGF) [19]. Notably, MSCs are considered to be immune-privileged and exhibit immunosuppressive properties, permitting them to be applied in an allogeneic setting [20].

Cardiac progenitor cells (CPCs)/Cardiosphere-derived cells (CDCs). CPCs or CDCs are believed to reside in the myocardium [21]. CDC populations may offer major advantages over extracardiac cell sources because the cardiac progenitors differentiate along both the cardiomyocytic and vascular lineages, which survive well in the myocardial environment. CDCs constitute a candidate pool of such cardiac stem cells that comprise a heterogeneous population of cardiac cells, including a potentially clonogenic stem cell subpopulation. Several basic studies have reported the validity of CPC or CDC injection for inducing angiogenesis and stimulating endogenous CPCs to promote regeneration [22, 23]. However, the main limitation in using such injections is the decreasing number of CPCs or CDCs with age [24] and their subsequent reduced potency to regenerate the myocardium [25]. Results from the CADUCEUS trial after a one-year follow-up described a subtle enhancement in the viability of cardiac muscle without benefit to cardiac function [9]. Although a meta-analysis of several cardiac clinical trials showed that cell therapy provided no benefits in terms of improving left ventricular function after myocardial infarction (MI) [26], cell therapy trials using autologous CDCs for congenital heart disease showed improvements in ventricular function by reverse remodeling, improved heart failure status [27, 28], and reduced late complications [29]. These experimental studies have suggested that the decline of cardiomyocyte replication might be associated with the absolute loss of intrinsic progenitor cells or reduced potential of preexisting mature myocyte proliferation during heart development.

Embryonic stem cells (ESCs). ESCs are derived from the inner cell mass of the blastocyst and have the ability to discriminate into cell types of all three germ layers (endoderm, mesoderm, and ectoderm) [30]. Cardiomyocytes are known to originate from the mesoderm layer, and many different *in vitro* protocols have been established to induce cardiomyogenic differentiation of ESCs despite the challenges of generating large quantities of pure and mature cardiomyocytes [31]. In 2018, the phase I clinical ESCORT trial was performed; this was the first trial to apply human ESC-derived cardiac progenitors embedded onto a fibrin matrix for the treatment of patients suffering from severe heart failure [32]. Nevertheless, clinical trials of these cells have been challenging due to ethical issues, genetic variability, the risk of immune rejection, and potential tumorigenicity [33].

Induced pluripotent stem cells (iPSCs). iPSCs were developed in 2006 from mouse fibroblasts by the retroviral introduction of four defined transcriptional factors known as Yamanaka factors, namely, c-Myc, octamer-binding transcription factor 3/4 (Oct3/4), sex

determining region Y-box 2 (Sox-2), and kruppel-like factor 4 (Klf4) [34]. These iPSCs have the full potential for cardiac regeneration, and iPSC-derived cardiomyocytes have several practical characteristics of cardiac cells, such as contractility, spontaneous beating, and ion channel expression using cytokines such as activin A and BMP4 [35]. The addition of chemical compounds that inhibit Wnt signaling was also shown to enhance the cardiac myocyte differentiation efficiency markedly [36]. However, functional analyses of iPSC-derived cardiomyocytes revealed that these cells are immature and more related to embryonic rather than adult cardiomyocytes [37]. Also, various studies described genomic instabilities in iPSC lines that could be actual risk factors for adverse effects, including malignant outgrowth [38].

Potential Mechanisms of Stem Cell Therapy in Heart Failure

Recent studies have revealed that stem cell transplantation stimulates the endogenous cardiac repair process via paracrine signaling, direct cell-to-cell interaction, and/or transfer of microRNAs from exosomes that influence the transcriptional activity of host cells [39]. Classically, it is believed that an ideal stem cell should differentiate into cardiomyocytes that integrate both mechanically and electrically with innate myocytes. However, data obtained from numerous studies led to the conclusion that the paracrine effect is the fundamental mechanism that mediates the beneficial effects of stem cell therapy [40].

Today, it is generally accepted that stem cells release factors such as cytokines, chemokines, and growth factors that affect the cellular environment. For example, stem cells reconstruct the myocardial vascular system by secreting VEGF, basic fibroblast growth factor (bFGF), and insulin growth factor-1 (IGF-1) as proangiogenic factors. IGF-1 also has the potency to inhibit the death of cardiomyocytes by apoptosis. Cells communicate with each other via released microparticles enriched in pre-microRNAs and exosomes in their cellular environment. Exosomes are membrane vesicles (40-100 nm in diameter) formed by endocytosis. Barile *et al.* showed that exosomes isolated from mouse CPCs protected H9C2 from oxidative stress by inhibiting caspase 3/7 activation *in vitro* while reducing cardiomyocyte apoptosis in a mouse model of myocardial ischemia *in vivo* [41]. Timmers *et al.* showed that injection of media conditioned by MSCs that contained the active component of exosomes reduced infarct size and improved cardiac function in a pig ischemic model [42]. A recent report claims that MSC-extracellular vesicles are sufficient to improve angiogenesis and exert a therapeutic effect on MI; the pro-angiogenesis effect of these vesicles might be associated with an miR-210-Efna3-dependent mechanism [43]. Increasing evidence suggests that exosomes may act as a vector of genetic information, and mRNAs carried by exosomes can be translated into proteins in the target cell.

Improvement Strategies to Boost the Potency of Stem Cells

A growing number of phase I and II clinical trials have proven the feasibility and safety of stem cell therapy [44]. Several meta-analyses and systemic reviews reported a slight but significant improvement in heart function of between 2-5% [44,45]; however, a meta-analysis of several cardiac clinical trials showed that cell therapy provided no benefit in terms of left ventricular function after MI [26]. This limited outcome of stem cell-based clinical trials necessitates the development of novel strategies to boost the efficiency of stem cells for cardiac repair. Two major concepts must be considered in generating the next generation of stem cell therapy: 1) pharmacological preconditioning and 2) genetic stem cell modification approaches. Using these techniques, numerous stem cell properties can be addressed, including engraftment, survival, paracrine activity, angiogenetic potential, and differentiation capacity.

Pharmacological preconditioning. Stem cell-based therapy is hindered by limited transplantation efficiency, and researchers have aimed to solve this problem with appropriate cell modification. Pharmacological preconditioning represents a convenient and cost-effective technique to stimulate the regenerative activity of stem cells. Alteration of stem cell culture conditions, cytokines and drug pretreatments are among the possible strategies. The use of hypoxia-conditioned media for the production of CPC or CDC sheets resulted in cells that secreted mRNAs and angiogenetic factors, which in turn led to better performance in cardiac function and a reduction of fibrosis [46,47]. Pretreatment of MSCs with atorvastatin improved the cell survival through induction of MSC autophagy by activating the AMPK/mTOR and MEK/

ERK pathways [48]. A recent report indicated that pretreatment of CDCs with resveratrol increased capillary density and decreased cardiomyocyte apoptosis in the peri-ischemic myocardium by the upregulation of VEGF and stromal derived factor (SDF)-1α [49]. As these cells exert their therapeutic effects on damaged tissue mainly by paracrine signaling, drug pretreatment was applied to promote their secretion activity [50].

Genetic stem cell modification. Genetic modification is highly desirable as another powerful technique to boost stem cell efficiency. Compared with non-genetic based pre-conditioning, genetic tools for specific perturbation of endogenous gene expression are usually applied to induce prolonged effects of stem cell activity. In general, three main strategies of genetic modification can be applied for stem cell therapy: 1) protein overexpression by DNA delivery, 2) gene silencing by RNAi [51], and 3) gene editing using TALENs or CRISPR/Cas9.

Overexpression of therapeutic factors, including VEGF, SDF-1, FGF, and IGF-1, can be induced using DNA-based modifications. IGF-1 overexpression has been utilized to positively influence the paracrine signature of human cardiac stem cells. Cardiac function was slightly improved with IGF-1-modified stem cells four weeks after MI, and apoptotic events in the infarcted myocardium were less frequent in stem cells engineered to overexpress IGF-1 [52]. Mice treated with SDF-1α-modified cardiac stem cells demonstrated a significant improvement of ejection fraction and reduction of scar area compared to unmodified control cells. Interestingly, these cells were shown to induce a two-fold increase in proliferating cardiomyocytes [53].

Synthetic transcriptional activators derived from the CRISPR/Cas9 system are emerging as powerful new tools for activating the endogenous expression of target genes [54]. These synthetic constructs, generated by tethering transcriptional activation domains to a nuclease-dead Cas9(dCas9), can be directed to the promoters or enhancers of endogenous target genes by single-guide RNAs (sgRNAs) to activate transcription. CDC populations may offer major advantages over extracardiac cell source, because these cells exhibit a balanced profile of paracrine factor production and provide the greatest functional benefit in MI [55]; however, the reduced differentiation potential of CDCs to develop into a functional population of cardiomyocytes has always been a significant setback. If we could boost the cardiomyogenic efficiency of CDCs to develop into a large population of cardiomyocytes by the intrinsic activation of cardio-specific differentiation factors using a CRISPR/dCas9 gene transcriptional enhancement system, this approach could potentially overcome the pitfalls of the previous cardiac regeneration efforts by achieving a large population of efficient cardiomyocytes.

Conclusion

The limited endogenous degree of cardiac regeneration is insufficient to compensate for the massive loss of cardiomyocytes occurring after acute and chronic heart failure. The transplantation of stem cells has emerged as a new approach to restore damaged myocardial tissue; however, the extent of the benefits of stem cell therapy is still controversial. Therefore, a deeper understanding of endogenous reparative mechanisms, as well as their interactions with stem cells, is needed.

Many groups are currently investigating various avenues of stem cell optimization, such as the use of genetic manipulations, or of cell preconditioning using drugs or environmental stressors, to increase angiogenesis, enhance survival pathways, and boost the promoters to differentiate into effective cardiomyocytes. The potential for optimizing stem cell therapy using preconditioning, genetic modification, or other ex vivo manipulated stem cells will significantly propel this type of therapy forward and provide invaluable information on stem cell biology.

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