Future Prospects for Regenerated Heart Using Induced Pluripotent Stem Cells

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Abstract. Induced pluripotent stem cell (iPSC) generation is an epoch-making technology. The potential applications for iPSCs are wide-ranging from in vitro disease models to drug discovery. For regenerative medicine in particular, the technology provides great hope for patients with incurable diseases or potentially fatal disorders such as heart failure (HF). However, the true realization of that promise for HF remains uncertain and moving toward the clinical application of iPSCs needs to be stepwise and careful. The establishment of “safe” iPSCs must be a major premise, while genome integration-free and oncogene-free reprogramming is also necessary. Teratoma formation also remains a risk with undifferentiated iPSCs, but it must not happen in patients’ bodies. Thus, regardless of the target organ, the differentiated cells from iPSCs must be purified to exclude any possibility of tumorigenicity. The transplantation strategies used for iPSC-derived cells are very important for the recovery of lost cardiac function. Longer engraftment of transplanted iPSCs-derived cardiomyocytes is essential particularly because their survival could be hampered by ischemia, inflammation, apoptosis, immunological rejection, and other cardiac phenomena. Providing these multistep solutions will open the new frontier of regenerative therapies with iPSCs for patients with severe HF.

Keywords: induced pluripotent stem cell, cardiomyocyte, regenerative medicine, cell transplantation, cell survival

1. Introduction

The heart works as an engine or a pump to circulate blood throughout the body for an entire lifetime. Heart failure (HF) thus increases mortality risk and decreases quality of life. Optimized medical therapies have drastically elongated life expectancy in HF patients, although the only radical therapy and possible cure for HF is cardiac transplantation. However, donor shortage for many types of organ transplantation including the heart remains a serious problem worldwide (1), and fewer than 4000 cardiac transplantations take place each year. Consequently, there has been considerable recent focus on regenerative cell transplantation therapy as a last resort for HF patients. Several cell sources are potentially available for such therapy including bone marrow stem cells, myoblasts, and cardiac progenitor cells, while BMSCs and CPCs were shown to be safe in transplanted patients (2–4). However, while all of these cell sources are functionally successful in small animal experiments, none has produced a marked improvement of cardiac function in large clinical studies (5). Thus, transplantation of purified cardiomyocytes (CMs) is eagerly awaited to achieve feasible and solid strategies for regenerative cardiac medicine.

Human embryonic stem cells (hESCs) were first reported in 1998 as a reliable cell source for generating functional CMs (6). They are the first truly pluripotent stem cells to show potential for differentiating into all cell types in vitro. CMs derived from hESCs have not yet reach the clinical trial stage, because of the difficulty in producing large-scale quantities of hESC-derived CMs in vitro and the incumbent ethical issues surrounding the use of human embryo-derived cells for clinical applications (7). Nevertheless, hESCs-derived CMs ready
for transplantation into a patient’s heart remains an excellent candidate for cell therapies. Immunosuppressive therapies will still be necessary for the transplantation, because of the allograft nature of hESCs-derived CMs for patients.

2. Induced pluripotent stem cells (iPSCs)

In 2006, murine iPSCs were reported for the first time by Professor Yamanaka’s group (8), with human iPSCs reported the following year (9). In contrast to hESCs, iPSCs present no ethical problems. Furthermore the most valuable advantage of iPSCs is the actuality of autotransplantation. Organ transplantations, including cardiac transplantation, demand immunosuppressive therapies for a patient’s whole life that might cause life-threatening problems such as malignancy and infection. Technologies involving iPSCs could potentially eliminate the need for such immunosuppression because differentiated cells for transplantation would ideally be differentiated into specific cell types as needed from the patient’s own tissue. This scenario presents the perfect therapy to restore lost organ function, although there are still several steps that need to be optimized to realize successful clinical application (Fig. 1).

The first step toward clinical application is the establishment of “safe” iPSCs. Conventional iPSC generation requires the four Yamanaka factors, OCT4, SOX2, KLF4, and c-MYC. Of these, c-MYC strongly promotes generation of iPSCs, but it is proto-oncogenic. Thus L-MYC, which is not tumorigenic and is also a strong promoter of iPSC generation, could be more suitable for clinical-grade iPSCs (10). In addition, a skin biopsy is required to acquire the necessary skin fibroblasts to generate iPSCs. While not very invasive, skin biopsies are still problematic cosmetically, and some patients may also need repeated biopsies to establish “good” iPSCs, with the best differentiation potential. Seki et al. (11) reported T cells and Sendai virus vector as useful tools for establishing iPSCs for clinical applications. This approach enabled the rapid generation of iPSCs (less than one month), minimal patient invasion, and most importantly, a reduced risk of tumorigenicity because Sendai virus is a very potent infective agent for T cells and never integrates into the host genome. This latter feature provides a strong advantage for the efficient generation of safe iPSCs.

3. Cardiac differentiation

Currently the differentiation potential of human iPSCs is seemingly similar to that of hESCs. The differentiation of human pluripotent stem cells (hPSCs) into CMs is considered according to the developmental steps re-

Fig. 1. Multiple subjects of iPSCs therapy for heart failure.
quired. Several protocols are available for cardiac differentiation from hPSCs: the embryoid body culture system, the monolayer culture system, and the inductive co-culture system (12). Cardiac differentiation is regulated using a balance of cytokines such as BMPs, TGFs, and WNTs. At first, hPSCs should be developed into endomesodermal lineage cells that express Brachyury (T), and several factors could promote the differentiation in this step; in particular, BMP4, Activin A, and WNT3A are potent factors for mesodermal differentiation (13, 14). After the differentiation into early cardiac progenitor cells, Mesp-1–positive cells, small chemical Wnt inhibitors, such as IWR, IWP, and XAV, are also useful for the next stage of development into cardiac lineage cells (15). The BMP inhibitor Noggin was also reported to support cardiac differentiation (16). On another front, fewer molecules activate the CM cell cycle. In this respect, granulocyte colony-stimulating factor is a valuable cytokine activator of cell division in fetal heart and hPSCs (17), and a combination of these cytokines and new factors will be applied to the efficient protocol of cardiac differentiation. Recently it was reported that chromatin modification also has a great impact on cardiac differentiation (18), and it might be necessary to have a deep knowledge of epigenetic mechanisms to achieve better CM differentiation protocols.

Finally, it is notable that the differentiation potential of hiPSCs to generate CMs also differs among individual cell lines, even those reprogrammed from the same individual. A selection method will therefore be required to establish the most efficient cell line to generate clinical-grade iPSCs-derived regenerative CMs.

4. Purification

The most valuable characteristic of iPSCs is pluripotency. However, this important feature can also introduce a strong tumorigenicity risk, and transplanted iPSCs-derived CMs inducing teratoma in the heart would be a tragedy for the patient. Thus, establishment of an excellent purification system for iPSCs-CMs is absolutely necessary for clinical application. Fluorescent activated cell sorting (FACS) is the most prominent method reported for selecting specific cell types, although it requires antibodies, a long processing time, and can process only small amounts of cells at one time. Because a human heart comprises more than $1 \times 10^8$ CMs, and if 10% of total CMs are lost due to myocardial infarction, $5 \times 10^7$ CMs might be needed for cell therapies. Thus, a novel large-scale purification system must be established to generate the CMs required for effective clinical use. Tohyama et al. (19) reported that CMs have different metabolic characteristics from hPSCs, which depend on glycolysis to generate energy and produce biomass. On the other hand CMs have more mature mitochondria and use the tricarboxylic acid cycle (TCA) cycle, and thus efficiently generate more ATP. Moreover, CMs could efficiently use lactate as an energy source to drive the TCA cycle instead of glucose. Glucose-depleted and lactate-enriched medium could therefore successfully eliminate the undifferentiated residual stem cell when generating CMs and such a metabolic-based selection system for generating large numbers of CMs is now established (19).

5. Transplantation strategies for regenerative cardiomyocytes

The final step to be optimized to realize clinical applications of iPSCs-derived CMs is the cell transplantation strategy. Survival of transplanted CMs has thus far remained extremely low regardless of the transplantation method, and better strategies for cell engraftment are necessary to improve cardiac function after cell transplantation therapy. Ischemia is the most critical factor for cell survival, while inflammation, apoptosis, and immunological rejection are also important issues to be solved.

Three major strategies have thus far been explored for cell transplantation: direct cell injection, cell sheets, and cell scaffolds.

5.1. Direct cell injection

Needle injection of CMs causes aggregation and necrosis of transplanted cells, and in our hands, very low percentages of CMs survive in cell transplantation therapies (20). To improve survival of transplanted CMs, the CM cluster method was then developed (21), resulting in transplanted CM clusters successfully engrafted in the heart of immune-compromised mice, with a cell attachment ratio > 80% and hPSCs-derived CMs showing a fetal phenotype. However, the engrafted cells grew in the host heart, thus cell cluster transplantation of human iPSCs-derived CMs is the better strategy than conventional direct cell injection.

5.2. Pericardial endoscopy

HF patients are likely to need repetitive cell transplantation therapy, and open chest surgery is very invasive for patients, especially the elderly. In particular, adhesions of the chest wall and heart will hinder reopening of the chest. It is also difficult to transplant a large amount of cells via a vascular catheter because of the risk of microvascular embolism. Pericardial endoscopy was invented to solve this fundamental problem because it can be inserted from the epigastric fossa into the peri-
cardial space to provide a clear view from a charge-coupled device camera on the endoscopy tip and thus enable observation of the transplanted cells by ultrasonography (22). In this way, an operator can check the depth of the injection needle, making such transplantations much safer and feasible for regenerative therapies.

5.3. Cell sheets

In cell-sheet techniques, cells are cultured in a temperature-responsive dish or a polymerized fibrin-coated dish and then piled up to form multilayered sheets (5). However, Masumoto et al. (23) reported that the functional recovery with cardiac cell sheets was mainly due to cytokine effects, and not cardiac contraction. In addition, CMs in the cell sheets were gone one month after engraftment, and sheets comprising more than three layers are susceptible of necrosis. However, the ommental blood supply to cardiac cell sheets could remarkably increase cell survival and improved cardiac function (24). Optimizing the multilayering and cell survival are thus key factors for recovering lost cardiac function with cardiac cell sheet therapy.

5.4. Scaffold and biologically synthetic organ

Cell scaffolding is the most classical method for regenerative medicine, wherein the scaffold helps the transplanted cells to engraft a host heart. Recently, biodegradable scaffolds have been developed that are more suitable for clinical application, involving bio materials such as fibrin, alginate, and collagen (25). Scaffolds can also be used as a matrix to release cytokines, which could possibly assist neovascularization and cell survival. Decellularized tissue is a particularly promising use of scaffolds, and if such tissue can be filled with sufficient CM numbers, it would generate the best synthetic heart. Indeed, decellularized rat heart filled with neonatal rat cardiac and endothelial cells that could contract synchronously has been reported (26). Decellularized mouse heart has also been reseeded with hiPSCs-derived cardiac progenitors, and contracted (27). The decellularized heart of large animals may support the engraftment of hiPSCs-derived CMs in the same way; but the key point of this technology is the preparation of large amounts of CMs and constitution of proper vascular networks.

5.5. Arrhythmogenicity after cell transplantation therapies

Arrhythmia is the most critical event of HF. The arrhythmogenic substrate usually exists in a failed heart; therefore careful observation would be required after cell transplantation in patients with severe HF. It is also well known that myoblasts induced arrhythmia in the Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial for severe HF patients (4). Even though myoblasts were transplanted as cell sheets, they still provoked arrhythmia because myoblasts do not express connexin43 or N-cadherin, and have their own automaticity (5). On the other hand, CMs can connect with a host heart, inducing less risk of arrhythmia with transplanted CMs. Moreover PSCs-derived CMs improved the arrhythmogenic status in vitro and in vivo (28, 29). This would be another advantage of transplantation of CMs, not only for treatment of HF. Arrhythmia could therefore be the next target application for cell transplantation therapy with iPSCs-derived CMs (30).

6. Conclusion

Patients with severe HF worldwide look forward to the realization of regenerative therapy with hiPSCs-CMs, although multiple steps need to be resolved before successful clinical application of these cells. However, scientific evidence is building regarding the safety and effectiveness of iPSCs-CMs and cell therapy, with iPSCs-CMs hoped to be available to every patient with HF in the near future.

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