Perspectives on Stem Cell Therapy for Cardiac Regeneration

– Advances and Challenges –

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Ischemic heart disease (IHD) accelerates cardiomyocyte loss, but the developing stem cell research could be useful for regenerating a variety of tissue cells, including cardiomyocytes. Diverse sources of stem cells for IHD have been reported, including embryonic stem cells, induced pluripotent stem cells, skeletal myoblasts, bone marrow-derived stem cells, mesenchymal stem cells, and cardiac stem cells. However, stem cells have unique advantages and disadvantages for cardiac tissue regeneration, which are important considerations in determining the specific cells for improving cell survival and long-term engraftment after transplantation. Additionally, the dosage and administration method of stem cells need to be standardized to increase stability and efficacy for clinical applications. Accordingly, this review presents a summary of the stem cell therapies that have been studied for cardiac regeneration thus far, and discusses the direction of future cardiac regeneration research for stem cells. (*Circ J* 2012; **76**: 1307–1312)

Key Words: Cardiac regeneration; Heart disease; Stem cell therapy

Ischemic heart diseases (IHD), including myocardial infarction (MI), are highly associated with mortality and morbidity in an aging society.¹ MI increases the necrosis and cellular apoptosis of cardiomyocytes, placing an excessive load on the heart, which eventually causes heart failure. Progressive loss of cardiomyocytes occurs not only in the ischemic state but also during the heart failure that occurs as a side effect of chronic hypertension.² In contrast, suppressing cardiomyocyte apoptosis in an experimental environment results in improved cardiac function.³

Drugs, interventions, and cardiac transplantation as contemporary common treatment methods have shown their limitations for improving the treatment outcomes of patients with heart disease. Thus, some research has been carried out on various methods of overcoming the limits of these current standard treatment methods. In particular, stem cell therapy began to receive attention in the early 2000s, and active relevant studies have been conducted with the hope of overcoming heart disease.⁴ Cardiomyocytes were known not to proliferate and regenerate in the adult heart, but it is now known that cardiomyocytes can undergo proliferation and division, and that the healing mechanism in mammals (including humans) activates tissues and cell self-regeneration after they are damaged.⁵

However, the ability of human cardiomyocytes to regenerate a damaged heart is insufficient for recovery from a pathological condition.

Accumulating evidence from a wide variety of animal experiments has demonstrated that damaged heart function can be recovered by inducing organ regeneration through the administration of stem cells that have been artificially amplified on a large scale in vitro and have the ability to self-proliferate and differentiate into cardiomyocytes in the damaged area.⁴ However, experimental cardiac regeneration using such stem cells has shown that the effect of the cells on functional heart improvement is rather weak, causing dispute as to whether stem cells differentiate into functional cardiomyocytes.⁴ Therefore, more research must be conducted on stem cell therapy for cardiac regeneration.

This review includes a classification of the stem cell sources that can be used to improve cardiac function and a re-examination of their effect on cardiac regeneration. Furthermore, the direction of future research on stem cell therapy for heart disease is discussed.

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Mechanisms of Cardiac Regeneration

Because the heart is a postmitotic organ, cardiomyocytes are in a state of cell cycle arrest. However, some experiments using the newt and zebrafish have confirmed that the damaged heart can be regenerated by resident cardiac cells that had self-regenerating ability. Cardiomyocytes of vertebrates, such as the newt and zebrafish, show low proliferation rates under normal conditions, as in mammals. But, when the heart is damaged, their cardiomyocytes undergo cell division by regulating DNA synthesis and the cell cycle.^{6,7}

In contrast, cardiomyocytes' proliferation capability is lost immediately after birth in mammals. Soonpaa et al reported that labeled nuclei are observed in 0.0005% of cardiomyocytes at baseline and in 0.0083% of myocytes in the border zone after injury.⁸ In humans, only about 1% and 0.4% of cardiomyocytes are renewed each year at the ages of 20 and 75 years, respectively.⁹ In contrast, about 4% of cardiomyocyte in the border zone are positive for Ki-67 in the hearts of patients who have suffered a MI.¹⁰ Additionally, mammalian cardiomyocytes undergo remarkable cell division around the border zone of the damaged heart¹¹ and overexpression of specific genes promotes division of cardiomyocytes that have been in a state of cell cycle arrest. This observation has amplified the expectation of regenerating damaged cardiomyocytes.^{12,13} These differing findings are a reflection of the lack of standardized analysis methods, although analyses of proliferation-related protein (Ki-67) expression and DNA synthesis are typically used to assess proliferation. Additionally, the number of analyzed cardiomyocytes has also differed.

Despite these controversies, stem cell research for cardiac regeneration has been actively carried out since the 2000s. Stem cells not only have self-renewal capabilities but also multipotent differentiation abilities and can differentiate into diverse cell types, including cardiomyocytes. In particular, pluripotent stem cells (PSC), such as embryonic stem cells (ESCs) and induced PSC (iPSCs), as well as bone marrow (BM)-derived stem cells (BMSCs), mesenchymal stem cells (MSCs), and resident cardiac stem cells (CSCs), are receiving high recognition as important sources of cell therapeutic agents for cardiac regeneration.¹⁴ Thus, we will now discuss the features of each of these types of stem cell.

Stem Cell Sources for Cardiac Regeneration

ESCs

ESCs are stem cells derived from the inner cell mass of the blastocyst and have totipotent ability. ESCs are the most advantageous source, as they have the ability to differentiate into various cell types, including cardiomyocytes.^{15,16}

ESCs can differentiate into cardiomyocytes under specific culture conditions, such as in cocultures with mouse visceral-endoderm-like cells, and various factors essential for cardiac differentiation promote differentiation of ESCs into cardiomyocytes through a paracrine effect.¹⁷

Xue et al showed that when human ESC-derived cardiac cells are transplanted into an experimental animal model, the ESC-derived cardiac cells are incorporated into the recipient's heart tissue and interact with the recipient heart tissue both electrically and functionally.¹⁸

Additionally, ESC-derived cardiomyocytes play an important role in regenerating the ischemic area of the heart after treatment for MI, and that also exerts a positive effect on the left ventricle (LV), as judged from a hemodynamic analysis of contraction, regional wall motion, and diastolic dimensions of

the expanded LV.¹⁹ These results suggest that ESCs can differentiate into mature cardiomyocytes, and that ESC-derived cardiomyocytes might functionally couple and interact with host heart tissue.²⁰

Despite such studies showing a positive effect of ESCs on cardiac regeneration, only a small proportion of differentiated cardiomyocytes typically have innate contractile ability.¹⁶ Additionally, the mechanisms by which ESCs differentiate into cardiomyocytes have not been completely demonstrated, although efforts are being made to elucidate the mechanism(s).²¹ Furthermore, ESCs are disadvantageous from the ethical viewpoint. Moreover, injection of ESCs may cause teratoma development or an immune rejection response. For these reasons, no clinical studies have been conducted in which ESCs were used as cell therapy for patients with heart disease.

iPSCs

In 2007, Yamanaka et al demonstrated PSC, which are similar to ESCs but derived from mature somatic cells using the reverse transcription factors Oct3/4, Sox2, Klf4, and c-Myc, and these were named iPSCs.²² iPSCs have a pluripotency similar to that of ESCs, and have rapidly emerged as a new source for stem cell therapy that overcomes the ethical problem of ESCs.

iPSCs can differentiate into a variety of cell lineages, including cardiomyocytes.²³ Nelson et al reported that cell therapy using mouse-derived iPSCs recovers cardiac function in an animal model of IHD.²⁴

Although iPSCs have shown positive cardiac regeneration results, they are disadvantageous because the generation yield that can be obtained using the 4 types of genes is extremely low, the efficiency of cardiomyocyte differentiation is poor, and the differentiated cells are a heterogeneous mixture of various types of cells including noncardiomyocytes.²⁵ Furthermore, iPSCs have the same problems as ESCs, such as teratoma development and the potential for an immune rejection response after transplantation.

Development of iPSCs is currently at the preclinical research stage, and iPSC generation methods are continuously being developed despite their short history.

Induced Cardiomyocytes and Direct Reprogramming Into Cardiomyocytes

Interestingly, recently reported studies have demonstrated that cardiomyocyte-like cells can be directly reprogrammed from postnatal cardiac or dermal fibroblasts using 3 cardiac developmental transcription factors: Gata4, Mef2c, and Tbx5 (GMT).²⁶ Furthermore, fibroblasts can be directly reprogrammed into cardiomyocytes using exogenously expressed pluripotency genes, such as *Oct4*, *Sox2*, and *Klf4*.²⁷ Despite such an amazing result, gene manipulation is still being conducted using viral vectors. Thus, controversy exists about the safety for clinical applications.

The latest results from Srivastava's team are a promising and important step toward cardiac regeneration. After local delivery of GMT-loaded retrovirus in mouse MI model, genetic lineage tracing showed infiltration of myocyte-like cells into the infarct border zone; these cells were confirmed to be descendants of cardiac fibroblasts. In addition, in vivo delivery of GMT decreased the infarct size and modestly attenuated cardiac dysfunction for up to 3 months after coronary ligation.²⁸

BMSCs

BMSCs are the best-characterized cells in terms of surface

antigens and growth properties *in vitro* and *in vivo*. Various studies have reported that treating heart disease with BMSCs has a positive effect on recovery of cardiac function.^{29–31}

A recent systematic review showed that the incidence of mortality or morbidity in 33 randomized clinical trials (1,765 participants) of patients undergoing BMSC therapy for acute MI was not significantly different from those undergoing a more standard therapy. Despite the high degree of heterogeneity observed, stem cell treatment improved the left ventricular ejection fraction (LVEF) significantly during a short-term follow-up (weighted mean difference [WMD], 2.87; 95% confidence interval (CI), 2.00–3.73). This moderate improvement in LVEF was maintained over the long-term follow up of 12–61 months (WMD, 3.75; 95% CI, 2.57–4.93).³²

Although some positive reports have indicated that BMSCs could be useful for patients with heart disease, dispute continues concerning their differentiation into cardiomyocytes, because of the lack of definite ground for determining that core cells can exert an effect on cardiac function.

Additionally, BM is not a homogeneous population; its cells have various lineages, including hematopoietic stem cells (HSCs). Some experimental evidence has suggested that diverse isolated HSC populations with c-kit⁺/Lin[−] and c-kit⁺/Sca-1⁺/Lin[−] improve cardiac function.^{33,34} Furthermore, according to the COMPARE-AMI trial results, CD133⁺ HSCs also improve LV function after transplantation.³⁵ Despite these promising results, debate continues regarding cell differentiation into cardiomyocytes.^{36,37}

Endothelial progenitor cells (EPCs), a type of BMSC, are progenitor cells with the ability to differentiate into endothelial cells (ECs). Various studies involving the administration of EPCs have shown positive effects on improving damaged heart function,^{38,39} but the EPCs did not differentiate into cardiomyocytes after transplantation.⁴⁰ However, it was evident that administering EPCs resulted in improved cardiac function. Such an improvement in cardiac function was probably related to the role of EPCs in promoting angiogenesis and supplying sufficient nutrients and oxygen for the survival and division of host cardiomyocytes, as well as replacing novel cardiomyocytes with endogenous stem cells, and also by exerting an additional positive effect on the survival of the replaced cardiomyocytes through a paracrine effect. Nevertheless, the definition of EPCs is still unclear for marker-based isolation, and efforts are necessary to overcome this problem.

MSCs

MSCs are advantageous because they can be isolated from a variety of tissues, including BM, adipose tissue, and cord blood. MSCs can differentiate into the mesenchymal lineages, including cardiomyocytes, skeletal myoblasts, chondrocytes, and adipose tissue.^{41–43} However, the rate of MSC differentiation into cardiomyocyte *in vivo* is very low.⁴⁴ After being transplanted into the body, MSCs show a paracrine effect by secreting various cytokines to promote survival, growth, or differentiation of other cells in the area of the MI, and this is considered the major function of MSCs for treatment efficacy.⁴⁵ Moreover, MSCs are immune privileged and have an immunosuppressive function. Because of these special abilities, MSCs can be used as an important source for stem cell-based cell therapy when heterogeneous transplantation is being performed.⁴⁶

However, most of the current studies on adipose-derived MSCs use the mononuclear cell layer, which contains many types of MSCs, rather than using a selected type. Expression of CD166, CD44, CD106, and Stro-1 has generally been used as a marker, but phenotype definitions and cellular characteristics

are so diverse that MSCs often show different characteristics depending on their source or donor. Another disadvantage is that the proportion of MSCs that differentiate directly into cardiomyocytes and those that actually survive for a long period is very low, as is the case with other types of stem cells.

Myoblasts

The first research on cell-based cardiac muscle regeneration that used myoblasts began with transplantation of myoblasts to treat IHD. Myoblasts are present in the basal membrane of the muscle niche and have the ability to regenerate muscle. They show strong resistance against ischemia, and have the ability to differentiate into myotubes in the body.⁴⁷ Thus, myoblasts received immediate recognition as a cell-based therapeutic agent for cardiomyocyte regeneration. Some experiments involving the administration of myoblasts have reported improved LV function.^{48,49}

However, despite such positive effects, transplanted myoblasts are unable to transdifferentiate into cardiomyocytes.⁵⁰ Moreover, follow-up results of the MAGIC trial reported that myoblasts transplanted into patients with ischemic cardiomyopathy caused serious side effects, such as arrhythmias.⁵¹

CSCs

As already described, cardiomyocyte regeneration using BMSCs and myoblasts has been rather weak. Thus, additional research is necessary. CSCs that can regenerate the heart exist in cardiac tissue, based on previous research, and several groups have identified a population of CSCs in the adult heart.

Stem Cell Antigen (Sca)-1⁺ Cells Sca-1 is a cell surface antigen used to isolate HSCs. However, some evidence suggests that Sca-1⁺ cells can be isolated from the adult heart, and can transdifferentiate into beating cardiomyocytes when cultured under specific differentiation conditions.^{52,53} Transplanted Sca-1⁺ cells home into the infarct border zone, and differentiate into cardiomyocytes, which express cardiac markers such as Nkx2-5 and GATA4. Furthermore, Wang et al reported that transplanting a Sca-1⁺/CD31[−] CSC population into an animal model improved LV function by angiogenesis.⁵⁴

c-kit⁺ Cells c-kit⁺ cells differentiate into cardiomyocytes, ECs, and smooth muscle cells (SMCs) *in vitro*, and contribute to regeneration of the myocardium after *in vivo* transplantation.⁵⁵ According to results from the SCIPIO study, transplantation of c-kit⁺ cells improved LV function.⁵⁶ The c-kit⁺ cell cardiac marker expression patterns are similar to those of Sca-1⁺ cells and a minority express Nkx2-5, GATA4, and Mef2c.

Despite these positive results, a study showed a lack of cardiomyocyte regeneration capability of c-kit⁺ cells in the adult heart.⁵⁷ Furthermore, the developmental origin of c-kit⁺ cells is unclear, because a BM transplantation experiment suggested that many c-kit⁺ cells in the adult heart are of BM origin.⁵⁸ Furthermore, c-kit is expressed not only in CSCs but also in BM-derived cell populations.³¹

Islet (Isl)-1⁺ Cells Isl-1 plays an important role in diverse organ development, including the heart.⁵⁹ Isl-1⁺ cells also exist in the cardiomyocyte niche, and Laugwitz et al have isolated Isl-1⁺ CSCs.⁶⁰ Several studies have suggested that the lack of Isl-1 hampers cardiac developmental potential.^{61–64} Recent studies have suggested that Isl-1⁺ cells contribute to all major cell types in the murine heart.^{59,60,65,66} Isolated Isl-1⁺ cells proliferate and differentiate into cardiomyocyte, SMC, and EC lineages when they are transplanted.⁶⁶ Isl-1⁺ cells are different from Sca-1⁺ and c-kit⁺ cells because they do not express other stem cell surface markers such as Sca-1 and c-kit.

Cardiac Side Population (SP) Cells Cardiac SP cells have

Table. Proposed Summary of the Advantages and Disadvantages of Various Stem Cells for Cardiac Regeneration

	ESC	iPSC	BMSC	MSC	MB	CSC
Differentiation capability						
Cardiomyocyte	Possible	Possible	Controversial	Possible	Controversial	Possible
EC	Possible	Possible	Possible	Possible	No report	Possible
SMC	Possible	Possible	Possible	Possible	No report	Possible
Other cell types	Possible	Possible	Controversial	Possible	No Report	Controversial
Cell source	Limit	Diverse	Limit	Diverse	Limit	Limit
Immune rejection response	Exists	Exists	No exist if autologous	No exist if autologous	No exist if autologous	No exist if autologous
Functionally and electrically synchronized after transplantation	Possible	Possible	Impossible	Possible	Impossible	Possible
Paracrine effect	Exists	Exists	Exists	Exists	Exists	Exists
Isolation markers	Diverse	Diverse	Diverse	Diverse	Diverse	Diverse
Isolation purity	Extremely low	Extremely low	Low	Low	Low	Low
In vitro amplification capability	Limit	Limit	Limit	Limit	Limit	Limit
Teratoma generation	Possible	Possible	No	No	No	No
Remarks	Ethical problems	Using viral vectors	–	–	–	–

ESC, embryonic stem cells; iPSC, induced pluripotent stem cells; BMSC, bone-marrow derived stem cells; MB, myoblasts; CSC, cardiac stem cells; EC, endothelial cells; SMC, smooth muscle cells.

been isolated using the vital dye exclusion method with Hoechst 33342 or Rhodamine 123, and the cells that extensively effluxed Hoechst 33342 were named SP cells. SP cells have been found in various organs, including BM, skeletal muscle, adipose tissue,⁶⁷ and the heart.⁶⁸ SP cells express Sca-1 and can be transdifferentiated into cardiomyocytes to improve LV function.⁶⁹ These stem cells express Nkx2-5, GATA4, and Mef2c after differentiation into cardiomyocytes. SP cells in the heart recover damaged heart function by migrating to the damaged area after the damage has occurred. However, the differentiation mechanism has yet to be elucidated.

Cardiosphere-Derived Cardiac Cells (CDCs) CSC populations have clonogenic ability and form spheroid aggregates in culture (ie, cardiospheres).⁷⁰ CDCs can differentiate into ECs, SMCs, and cardiomyocytes.⁷¹ Various studies have suggested that CDCs improve LV function,^{72,73} but they have a disadvantage, in contrast to other stem cells, in that considerable time is required to produce the cardiospheres. Thus, efforts are necessary to overcome this problem. Additionally, CDC-derived cardiac cells are contaminated with other cell types, such as cardiac fibroblasts. This disadvantage could be overcome by using cell surface markers.

Epicardium-Derived Cells (EPDCs) Interestingly, several studies have reported that EPDCs are found in the adult mammalian epicardium.⁷⁴ Limana et al identified that c-kit⁺/CD34⁺/CD45[–] stem cell types exist in the human and the murine epicardium, and that they can be differentiated into cardiomyocyte and vascular lineages.⁷⁵ Furthermore, Wilms tumor 1⁺ (Wt1⁺) cells can also be transdifferentiated into cardiomyocytes.⁷⁶ More recently, Wt1⁺ EPDCs, primed with thymosin β 4, were transdifferentiated into cardiomyocytes, which functionally incorporated host cardiomyocytes.⁷⁷ However, the differentiation potential of EPDCs is still controversial. Thus, further development is required with cellular surface markers to isolate EPDCs.

As described earlier, CSCs isolated by such diverse methods may have no relationship in terms of differentiation into cardiomyocytes. However, they have the common advantage of being derived from the heart; thus, they could exert a positive effect as a novel cell therapy.⁷⁸ However, CSCs have the

disadvantage that they are isolated from biopsy tissue, which may cause functional impairment, particularly for those isolated from older patients because of the aged cells. However, studies are ongoing in many aspects to overcome these problems.

Problems of Cardiac Regeneration Using Stem Cells and Measures to Overcome Limitations

A variety of stem cell sources can be used to overcome cardiovascular disease, but their advantages and disadvantages have to be clearly distinguished (Table). The primary consideration in the development of stem cell therapeutic agents to overcome cardiovascular diseases should be given to research on methods for improving stem cell survival and long-term engraftment after administration.⁷⁹ Stem cells must be administered in large amounts to show normal function after transplantation, because only an extremely small portion of transplanted stem cells undergo division.

Another consideration is discovering stem cells that have the potential to recover a damaged heart. Although a variety of stem cells have the potential for cardiac regeneration, some researchers have reported that they failed to exert a direct effect on regeneration and functional improvement of the heart.^{37,50,80} Therefore, efforts are needed to develop and analyze specific stem cells for cardiac regeneration.

A third consideration is standardizing the stem cell administration method and dosage. The outcome of treating cardiovascular diseases using stem cells is not so optimistic. The stem cell dosages that have shown positive effects on regenerating damaged heart were all different, and a meta-analysis resulted in very low values with respect to the functional recovery of the LV after stem cell administration.⁸¹

Last, currently available stem cell administration methods involve injecting through a blood vessel or directly into cardiac muscle,⁸² but the safety of these methods has not been tested. Therefore, an urgent consideration is the establishment of standardized methods to overcome this problem.

Conclusions

Based on the results of previous studies, it is clear that various stem cells can exert positive effects on cardiac regeneration. Nevertheless, many barriers must be overcome for these cells to be useful as a therapeutic agent to clinically treat patients. It will be important to develop cell therapeutic agents in which the strong point of each type of stem cell is maximized. However, development of a combined treatment strategy that uses all of the positive aspects of the stem cells, as has been described, would lead to the development of a next-generation stem cell therapeutic agent that has more positive effects on cardiovascular disease.

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