

## How do resident stem cells repair the damaged myocardium?

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**Author contributions:** Hayashi E drafted the text; Hosoda T finalized the paper.

**Supported by** The Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (C), No. 25461118

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**Received:** July 28, 2014

**Peer-review started:** July 29, 2014

**First decision:** September 16, 2014

**Revised:** September 25, 2014

**Accepted:** October 23, 2014

**Article in press:** December 16, 2014

**Published online:** January 26, 2015

cell infusion, are explored and discussed in this article. Some of them are related to the intrinsic property of the resident stem cells, such as direct differentiation, paracrine action, and immunomodulatory function, whereas others involve environmental factors, leading to cellular reverse remodeling and to the natural selection of "juvenile" cells. It has now been demonstrated that cardiac stem cells for therapeutic purposes can be prepared from tiny biopsied specimens of the failing heart as well as from frozen tissues, which may remarkably expand the repertoire of the strategy against various cardiovascular disorders, including non-ischemic cardiomyopathy and congenital heart diseases. Further translational investigations are needed to explore these possibilities.

**Key words:** Regeneration; Heart failure; Cell therapy; C-kit; Cardiac stem cells; Cellular reverse remodeling; Miracrine

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**Core tip:** The autologous transplantation of cardiac stem cells appeared to be safe and surprisingly effective for a small group of patients with chronic ischemic cardiomyopathy. Their specific feature as resident stem cells, the interaction with the surrounding tissue, and the natural selection during the cell culture process potentially contributed together to the outstanding consequences.

### Abstract

It has been a decade since the monumental discovery of resident stem cells in the mammalian heart, and the following studies witnessed the continuous turnover of cardiomyocytes and vascular cells, maintaining the homeostasis of the organ. Recently, the autologous administration of c-kit-positive cardiac stem cells in patients with ischemic heart failure has led to an incredible outcome; the left ventricular ejection fraction of the cell-treated group improved from 30% at the baseline to 38% after one year and to 42% after two years of cell injection. The potential underlying mechanisms, before and after

Hayashi E, Hosoda T. How do resident stem cells repair the damaged myocardium? *World J Stem Cells* 2015; 7(1): 182-185 Available from: URL: <http://www.wjgnet.com/1948-0210/full/v7/i1/182.htm> DOI: <http://dx.doi.org/10.4252/wjsc.v7.i1.182>

### INTRODUCTION

The advancement of emergency medicine enabled us to

rescue many patients with acute myocardial infarction, but they may eventually develop and suffer from chronic heart failure later in life. Accordingly, the demand of alternative approaches to chronic ventricular dysfunction is increasing dramatically. For decades, the mammalian heart was recognized as a postmitotic terminally differentiated organ, in which the number of cardiomyocytes was believed to be constant throughout the life span of organisms. Early this century, however, the existence of resident cardiac stem cells (CSCs) was unveiled<sup>[1]</sup>, questioning the validity of the long-lasting theory. The uninterrupted renewal of human myocardium in physiological and pathological circumstances has been depicted<sup>[2]</sup>, promoting our understanding of the self-renewing characteristic of the heart. This paradigm shift not only shook the biological dogma but revealed the possibility of an unprecedented therapeutic strategy for devastating diseases.

## CLINICAL APPLICATION OF CARDIAC STEM CELLS

By virtue of the explorations following the discovery of resident CSCs, the human heart has been characterized by the persistent renewal of its components, muscles and vessels. Moreover, by utilizing animal models, c-kit-positive CSCs are shown to regenerate the diseased myocardium of ischemic and non-ischemic origins<sup>[3-5]</sup>. Subsequently, in 2009 the first clinical use of autologous CSCs began; in this SCIPIO trial, the subjects with severe ischemic heart failure received an elective coronary artery bypass graft surgery, during which a tiny piece of the right atrial appendage was resected as the source of stem cells. Four months after the operation, only those with the left ventricular ejection fraction (LVEF) worse than 40% were included in the trial and randomly assigned to the control or cell-treated groups. While the cardiac function did not change in the control group, the LVEF dramatically improved in the treated patients, from 30% at the baseline to 38% after one year and to 42% after two years of cell administration<sup>[6,7]</sup>. The infarct size evaluated by an MRI remarkably reduced following the cell therapy, and the symptom improved as well. It should be noted that the major adverse cardiac event rate was unchanged with or without this therapeutic intervention<sup>[8]</sup>.

## DIRECT DIFFERENTIATION VS PARACRINE ACTION

A single intracoronary injection of one million CSCs, as described above, appeared to be safe and effective for 2 years or longer. How does this work? There are accumulated evidences suggesting that most cell types applied for heart failure, including skeletal myoblasts, bone marrow-derived cells, and cardiosphere-derived cells<sup>[9,10]</sup>, function through paracrine mechanisms<sup>[11-13]</sup>. In this regard, CSCs are found to work through the sequential processes of engraftment, proliferation, and direct differentiation into various cardiac cell types<sup>[14]</sup>, in addition to the paracrine action<sup>[15,16]</sup>. Actually,

a recent study showed that the injection of 200 million human mesenchymal stem/stromal cells (MSCs) and that of one million c-kit-positive CSCs into a two-week-old infarcted myocardium were similarly effective, revealing the great regenerative potential of resident stem cells<sup>[17]</sup>. Interestingly, the combined usage of these distinct cells resulted in a synergistic effect, formulating an attractive regimen for myocardial infarction.

## REGULATION OF IMMUNE RESPONSE

The environment of the host tissue is not always favorable for the transplanted cells to engraft, especially when allogeneic ones are used. Historically, MSCs were found to be immune privileged, due to their lack of major histocompatibility complex class II antigen. In fact, the endomyocardial injections of autologous and allogeneic bone marrow MSCs were similarly effective as a treatment for ischemic heart failure patients<sup>[18]</sup>. More recently, Di Trapani *et al.*<sup>[19]</sup> have demonstrated that certain stem cells of various sources, including CSCs, are able to regulate the immune response of the recipient<sup>[19]</sup>. Such properties may have a role *in vivo* in enhancing their regenerative ability, especially in inflammatory circumstances. These reports would support the enthusiasm for the “off-the-shelf” usage of allogeneic CSCs. The obvious advantages include minimizing the lot-to-lot variation of cell therapy and its applicability to the acute phase of the disease. Following the differentiation of engrafted CSCs *in vivo*, however, the immune system of the host may reject them, just like in the case with rat MSCs<sup>[20]</sup>. Although this particular study was carried out with rodents, stem cells of any given species would become immunogenic upon the acquisition of myogenic phenotypes. In the setting of allogeneic transplantation, therefore, the long-term benefit depends essentially on the paracrine action of the administered cells. Further evaluations would be needed to compare the advantages and disadvantages of respective therapeutic strategies.

## REVERSE REMODELING AT CELLULAR LEVEL

As stated above, while the administered cells may affect the circumstance, the microenvironment within the host would also influence the destiny of the exogenous cells. Based on the studies using immunosuppressed animals, human CSCs can create more cardiomyocytes than the lost myocytes of the recipient, within one month after injection<sup>[21]</sup>. Assuming a comparable growth behavior of the autologous CSCs, it might be unlikely that these cells divided continuously and frequently for more than 2 years. Instead, it would be reasonable to speculate that each differentiated progeny gradually matured to improve the global function of the organ. Lately, it was shown that microRNAs (miRs) can traverse gap junctions and influence the fate of the cells receiving these miRs. Specifically, miR-499, which is abundant in cardiomyocytes

and essentially absent in CSCs, can be transferred from myocytes to resident stem cells *via* gap junction channels, resulting in the enhanced differentiation of the primitive cells toward myocytic lineage<sup>[22]</sup>. Therefore, it can be presumed that the presence or absence of matured myocytes in a microenvironment determines the destiny of stem cells *in situ*. Actually, this theory is consistent with the previous observation in which CSCs engrafted in remote myocardium, full of surviving mature cells, progressively differentiate to become indistinguishable from its surrounding myocytes within five weeks<sup>[23]</sup>. On the contrary, at the infarcted/regenerated area, cardiomyocytes derived from injected CSCs tend to hold fetal/neonatal characteristics for the same period of time. Once committed to the myocytic lineage, CSCs themselves start to express miR-499, and the quantity increases as the differentiation process advances, which in turn may have an influence on neighboring immature cells through the mircrine mechanism. This cascade of progressive maturation may be called “cellular reverse remodeling”.

## CONSEQUENCE OF CELL EXPANSION

Another essential factor can be pointed out in the cell preparation procedure. The necessity of CSC cultivation prior to injection basically excludes the autologous implantation at the acute stage, and this is generally considered to be a major drawback of somatic stem cell treatments. This inevitable culturing step, however, may hold an advantageous aspect as well. Because rapidly multiplying “juvenile” cells grow faster than “senile” ones on the dish, this maneuver practically functions as a natural selection process. Conversely, when cells harvested from a body are used directly to treat an acute illness, the cell population may be heterogeneous by nature within the patients as well as among the preparations for various patients. Needless to say, it is unlikely that a longer culturing period gives a better outcome; there should be a certain threshold. Accordingly, CSCs should be characterized better, in order to pick the right timing and method for the cell preparation step.

## PERSPECTIVE

The clinical applications of resident CSCs began only five years ago. Due to the limited number of subjects and the relatively short observational period, we still do not yet know whether this therapy will improve hard endpoints such as the survival of patients. Also, the treatment was mainly applied to patients who required cardiac surgery. However, a recent research indicated that tiny pieces of the failing heart, approximately 5 mg in weight, are sufficient for CSC preparation<sup>[24]</sup>. Moreover, our group has lately succeeded in culturing CSCs from small surgical specimens kept frozen for longer than a year; the isolated cells possessed properties comparable to that of those derived from fresh tissues (unpublished observation). This is clinically relevant and important, in light of the

potential repetitive treatment of a single patient and/or applications to adult congenital heart diseases. We have to investigate further to see whether such repeated CSC injections and its usage for pediatric patients are safe and effective as expected. This endeavor will be able to salvage a large number of patients who do not have adequate therapeutic options at the present time.

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**P- Reviewer:** Aronow WS, Chawla M, Karaoz E **S- Editor:** Ji FF

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