Promotion of Cardiac Regeneration by Cardiac Stem Cells

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Research on myocardial regeneration is an exciting and promising area, which challenges the dogma that the heart is a nonregenerating organ. Recently, several methods of stem cell therapy have been developed. One method is to transplant cells into the infarcted area of the myocardium. Currently, clinical trials of autologous skeletal myoblast transplantation into the failed heart are underway and have been reported to improve the cardiac function. However, the mechanism of its efficacy is unknown, and there are some questions about the safety because myoblasts do not transdifferentiate into cardiomyocytes and may induce lethal arrhythmia. In this point, embryonic stem (ES) cells that can differentiate into cardiomyocytes are thought to be more promising. For patients experiencing extensive myocardial infarction or dilated cardiomyopathy, however, the effectiveness of cell transplantation is questionable. Bone marrow-derived cells have been reported to transdifferentiate into various types of cells in situ. Indeed, bone marrow-derived stem cells were reported to prevent left ventricular remodeling after myocardial infarction and improve cardiac function by their differentiation into cardiomyocytes. However, recent accumulating evidence has indicated that very few bone marrow cells, if any, transdifferentiate into cardiomyocytes. Cytokine therapy using G-CSF strongly prevents ventricular remodeling after myocardial infarction by antiapoptotic and angiogenic effects, but not by recruitment of bone marrow cells.

Over the past few years, adult hearts have been reported to contain the cardiac stem/progenitor cells such as c-kit+, Sca-1+, isl-1+, and side population cells. Because these cells have the ability to proliferate and differentiate into cardiomyocytes in vitro and in vivo, they might have the potential to regenerate the injured heart. However, there is almost no regeneration in the human heart after myocardial infarction, suggesting that cardiac regeneration accomplished by proliferation, migration, and differentiation of the cardiac stem/progenitor cells, is inhibited in vivo.

In this issue of Circulation Research, Urbanek et al address the possibility of cardiac regeneration by inducing migration and protection of cardiac stem cells and early committed cells (CSCs–ECCs) in a rodent myocardial infarction model. CSCs are defined by the expression of the stem cell-related antigens, c-kit, Sca-1, or MDR1. A fraction of CSCs, which expressed MEF2C (cardiac transcription factor), GATA6 (smooth muscle cell transcription factor), or Ets-1 (endothelial cell transcription factor) was named ECCs. Immunohistochemical analysis revealed that CSCs–ECCs express HGF receptor c-Met and IGF-1 receptor (IGF-R) and, under the treatment with their ligands, cultured CSCs–ECCs secreted HGF/IGF-1. When myocardial infarction was produced and human HGF/IGF-1 was locally injected, expression levels of murine mRNA and proteins for HGF/IGF-1 in infarcted tissue were increased. Activation of these growth factor signals was confirmed by phosphorylation of c-Met, IGF-R, and their downstream targets. Migration studies demonstrated that HGF promoted motogenic and invasive activity of CSCs–ECCs, whereas IGF-1 had little effect. Conversely, IGF-1 showed more antiapoptotic and proliferative effects on CSCs–ECCs compared with HGF.

Based on these in vitro findings, Urbanek et al examined whether HGF/IGF-1 stimulate migration, proliferation, and differentiation of CSCs–ECCs in the infarcted heart. They used highly sophisticated techniques to evaluate the migration of CSCs–ECCs. They found that cycling CSCs–ECCs exist in the atrioventricular groove so that retrovirus expressing enhanced green fluorescent protein (EGFP) was injected in this region. After EGFP was integrated into the CSCs–ECCs, myocardial infarction was made and subsequently HGF/IGF-1 were injected into the predicted pathway of migrating cells with gradient of their concentration. They examined ex vivo heart preparation by 2 photon microscopy and demonstrated migration of EGFP-positive cells toward the infarcted area through the interstitium of the heart. Furthermore immunohistochemical analysis showed that the locomotive EGFP-positive cells possess the characteristics of CSCs–ECCs. Consistent with their in vitro data, HGF but not IGF-1 had locomotive effects on CSCs–ECCs. Regenerated myocardium after the HGF/IGF-1 combined treatment was identified as BrdU positive cardiomyocytes and vessels. HGF/IGF-1 treatment increased the number of newly-formed cells, resulting in an increased volume of myocardium, improvement of cardiac function, and better survival. The BrdU-positive new myocytes isolated from regenerated myocardium are smaller than old cells, and these small cells exhibit better contractile function than cardiomyocytes isolated from spared myocardium.

The same group has reported that self-renewing, clonogenic, and multipotent cardiac stem cells exist in the various species, including human. However, in vivo kinetics of cardiac stem cells had been unknown because of the difficulty
of labeling and tracking of the cardiac stem cells in vivo. Urbanek et al overcome the difficulty by their extensive and skillful analyses and showed that exogenously-applied HGF induced migration of distant cardiac stem cells toward the infarcted area through the pathway defined by fibronectin. Despite the fact that many types of cytokines and growth factors, including HGF/IGF-1, were released in the ischemic myocardium, the resident stem cells cannot survive in infarcted myocardium, and the stem cells in the distant area do not take a part in regenerating heart tissue. The findings of this study suggest that the amount of the intrinsic factors are far less than the amount necessary to break the silence of the resident stem cells or protect them from apoptosis.

Although the study by Urbanek et al sheds light on intrinsic stem cell therapy by local administration of growth factors, it also raises several important questions that should be addressed by future studies. First, what determines the CSCs–ECCs quiescence in “cardiac niche”? In bone marrow niche, angiopoietin-1/Tie-2 signaling is critical for the maintenance of hematopoietic stem cell quiescence.16 Although it is not clear whether the similar mechanism is present in the heart, it is possible that signals to maintain CSCs–ECCs quiescence are antagonized by HGF/IGF-1 signaling. CSCs–ECCs coexpress HGF/IGF-1 and c-Met/IGF-R, and there is a positive feedback loop of HGF/IGF-1 signaling in CSCs–ECCs. Therefore, CSCs–ECCs may exit from the quiescence when challenged by higher doses of HGF/IGF-1, and the activated state of CSCs–ECCs may be maintained by the positive feedback loop of HGF/IGF-1 signaling. Identification of the mechanism that maintains CSCs–ECCs quiescence will be of particular importance, because inhibition of CSCs–ECCs quiescence can be a novel strategy to promote myocardial regeneration by enhancing CSCs–ECCs proliferation. Second, how does HGF induce migration of CSCs–ECCs to the infarct area? Among several signaling molecules activated by HGF, PI3-kinase (PI3K) seems to be critical for HGF-mediated cell migration, because PI3K is required for HGF-induced lamellipodia formation and subsequent migration in MDCK and C2C12 cells.17–19 Whether PI3K is also critical in CSCs–ECCs migration should be determined. In addition, it is possible that some negative regulators of CSCs–ECCs migration are expressed in the infarct area. Inhibition of such factors can be another strategy to promote myocardial regeneration. Third, what induces apoptosis of CSCs–ECCs in the infarct area? Small numbers of CSCs–ECCs migrate into infarct area without growth factor treatment. Understanding of the mechanism of the apoptosis would lead to more specific treatment.

In the study by Urbanek et al, animals were treated with growth factors 5 hours after coronary ligation. Whether administration of HGF/IGF-1 is also effective in the chronic stage after myocardial infarction, when ventricular remodeling is already established, should be investigated. Likewise, to determine whether growth factor treatment is also effective in diluted cardiomyopathy or other models of chronic heart failure with diffuse contractile dysfunction will be of great importance.

In summary, the study by Urbanek et al clearly demonstrates the therapeutic potential of CSCs–ECCs for myocardial regeneration. To address the questions raised by Urbanek et al would further advance our understanding of the stem cell system in the heart and provide important clues to the development of novel therapeutic strategies for heart diseases.

References


**Key Words:** cardiac stem cell, cardiomyocytes, regeneration, growth factor, myocardial infarction.
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