Optimizing Cell-Based Therapy for Cardiac Regeneration

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Long thought to be a terminally differentiated organ, recent findings suggest that the adult mammalian heart is a slowly regenerating organ and home to a resident population of cardiac progenitor cells (CPCs) that renew cardiomyocytes and have the potential to differentiate into multiple cell types within the myocardium. In spite of this, the regenerative capacity of the mammalian heart is inadequate compared with the resulting damage caused by ischemic episodes. In light of the challenges faced by resident progenitor cells, many studies have focused on delivery of exogenously prepared stem or progenitor cell types to the damaged heart. CPCs are an ideal candidate for cardiac cell-based therapy because they are programmed to differentiate into cardiomyocytes, endothelial cells, and smooth muscle cells, thus providing not only contractile benefit but also increased vascularization. However, the ischemic myocardium is a hostile microenvironment, and multiple factors contribute to prevent cardiac regeneration, including ischemia, inflammation, and fibrosis. Therefore, it is critical to provide a complementary approach that promotes CPC survival, proliferation, and differentiation.

In the study by Padin-Iruegas et al in this issue of Circulation, insulin-like growth factor 1 (IGF-1), an endogenous peptide that is activated in response to cardiac injury, is delivered to the heart using nanofiber tethering (NF-IGF-1) both alone and in combination with CPCs. In vivo administration of CPCs plus NF-IGF-1 led to significantly more CPC-derived cardiomyocytes that were larger, more differentiated, and incorporated electrically and mechanically into the host myocardium. This combination treatment also increased endogenous CPC proliferation and differentiation to cardiomyocytes, endothelial cells, and smooth muscle cells. Combination therapy led to significant improvement in both ventricular performance and morphological parameters compared with CPC or NF-IGF-1 treatment alone, emphasizing the therapeutic benefit of prolonged exposure of CPCs to IGF-1.

One of the biggest hurdles facing cell-based therapies in the ischemic heart is maintaining the survival of newly implanted cells. Reports have demonstrated cell survival rates broadly ranging from 1% to 32% 1 week after injection into injured hearts. Current thinking supposes 3 main causes of cell death: loss of matrix support, ischemia, and inflammation. Cell contacts with the extracellular environment and subsequent receptor engagement initiate protective signaling pathways that are important for survival. Preparing progenitor cells for therapeutic intervention inevitably leads to decreased adhesion-related survival signals and programmed cell death. Recent evidence suggests that inhibition of Rho-associated kinase can increase survival of human embryonic stem cells after dissociation, possibly by preventing anoikis, providing an opportunity to further improve cell viability through pharmacological means. More importantly, however, death also results from the ischemic milieu, characterized by increased reactive oxygen species production and mitochondrial dysfunction. This harsh environment is also highly inflamed after an ischemic episode. IGF-1 stimulates cell-protective mechanisms against oxidative stress and inflammation, thereby protecting CPCs from death. IGF-1 also facilitates survival of CPCs, possibly through local upregulation of vascular endothelial growth factor and consequent stimulation of angiogenesis. Because both CPCs and endogenous cardiomyocytes possess IGF-1 receptor, the expression of which is upregulated during myocardial repair after myocardial injury, locally applied IGF-1 should act on both CPCs and cardiomyocytes.

Another obstacle hampering cell-based cardiac repair is the relatively low number of endogenous stem/progenitor cells available in the heart. Previous work has demonstrated a plateau in the effectiveness of increasing the number of stem cells to be injected. Therefore, increasing in situ proliferation of CPCs should lead to better outcomes. In this work, Padin-Iruegas et al have shown that IGF-1 has cell-autonomous proliferative effects on CPCs. IGF-1 is an effective promoter of cell proliferation in pluripotent stem cells, including human embryonic stem cells. IGF-1 also promotes proliferation in human stem cell–derived cardiomyocytes and adult cardiomyocytes. The proliferative effect of IGF-1 in the cardiomyocyte lineage is unique in that progenitor cells may differentiate without surrendering their ability to proliferate. Work by Engert et al using the locally acting isoform mIGF-1 has demonstrated that IGF-1 stimulates the proliferation of myoblasts initially and promotes differentiation later in skeletal muscle. This property of IGF-1 appears to be in contrast to the effect of Wnt/β-catenin signaling on ISL1+ cells, another type of CPC, in that Wnt/β-catenin stimulates proliferation but inhibits differentiation of ISL1+ cells. In this sense, IGF-1 is an ideal growth factor for cell therapy in the heart.
The signaling mechanism competent for both proliferation and differentiation of CPCs is of great interest. In human embryonic stem cell–derived cardiomyocytes, IGF-1–induced proliferation is PI-3 kinase/Akt dependent but ERK independent. Activation of Akt in the nucleus causes proliferation of CPCs. Because IGF-1 enhances nuclear phospho-Akt in cardiomyocytes and because increased nuclear phospho-Akt is observed in the surviving myocardium in the study by Padin-Iruegas et al., if the same effect is seen in CPCs, it may mediate IGF-1–induced proliferation of CPCs. An important point, however, is that the NF-IGF-1–treated CPCs were larger than the untreated CPCs. Because nuclear Akt is thought to antagonize hypertrophy, additional signaling mechanisms should be activated in NF-IGF-1–treated CPCs, and further study is warranted to elucidate their role in mediating CPC proliferation.

Ensuring that progenitor cells differentiate into the desired cell type(s) is an equally important challenge. Treatment of CPCs with IGF-1 alone or in combination with hepatocyte growth factor gives rise to endothelial cells, smooth muscle cells, and cardiomyocytes. It would be interesting to test whether IGF-1 is sufficient to stimulate differentiation of CPC clones in a cell-autonomous fashion in vitro. Whether IGF-1 stimulates differentiation rather than proliferation depends on the signaling mechanism mediated through the C-terminal structure of the IGF-1 receptor, as well as the lack of insulin receptor substrate-1 phosphorylation in hematopoietic cells. Because nuclear activation of Akt increases the number of cardiomyocyte-committed CPCs, testing whether IGF-1 activates nuclear Akt is of interest. In skeletal muscle, Akt is involved in differentiation of satellite cells through p300 phosphorylation and subsequent chromatin remodeling. Recent work by Zhu et al. showed, however, that insulin-like growth factor binding protein-4 (IGFBP-4) plays an important role in mediating cardiomyocyte differentiation during development, when the cardiogenic effect of IGFBP-4 is inhibited by IGF-1 through IGFBP-4 sequestration. Thus, it is possible that IGF-1 may not stimulate all aspects of cardiomyocyte differentiation if CPCs use similar mechanisms to differentiate. Elucidating the downstream signaling mechanisms by which IGF-1 stimulates differentiation of CPCs may allow us to identify a better therapeutic intervention to facilitate CPC differentiation into cardiomyocytes. Padin-Iruegas et al. showed that many myocytes in the area of regeneration are BrdU positive. Thus, although they are functionally competent, they are not identical to existing cardiomyocytes. Clinically, it is important to track these new myocytes and to determine to what extent they continue to proliferate and to what extent IGF-1 treatment induces further differentiation of CPC-derived myocytes.

There are additional important effects of IGF-1 on endogenous progenitor cells that may translate to CPCs. Recent work has shown the necessity of IGF-1 for stem cell self-renewal. Furthermore, IGF-1 promotes the migration of mesenchymal stem cells from bone marrow and improves their localization to the site of infarct. The SDF-1: CXCR4 signaling axis is critical to paracrine signaling and homing of resident stem cells for cardiac repair and can be upregulated by IGF-1 (see the Figure).

The true novelty of the work by Padin-Iruegas et al. is the finding that IGF-1, when applied in the right location in the heart for a sufficient period of time, enhances cardiac regeneration by CPCs. Although injection of ex vivo–modified mesenchymal stem cells overexpressing IGF-1 may achieve the same goal, it requires ex vivo manipulation of the stem cell genome before injection, giving rise to the fundamental concerns of gene therapy, which may preclude the use of ex vivo manipulation for immediate clinical application. Nanofibers consisting of short peptides can be injected into the myocardium, where they reassemble to create a stable microenvironment and serve as a source of humoral factors. Prior work has shown that whereas IGF-1 tethered to nanofibers remained present 84 days later, untethered IGF-1 was no longer detectable 7 days after injection into the heart. This technique not only enhances survival and function of exogenous CPCs but also stimulates recruitment of endogenous...
CPCs and promotes angiogenesis to improve ischemia. Synergistic enhancement of cardiac regeneration by CPCs and NF-IGF-1 indicates that by optimizing the combination of the right cell population and the right humoral factor, one may further improve the efficiency of cell-based therapies.

This technology can easily be applied to additional growth factors/cytokines and even small molecules. An excellent system to discover novel and potentially important endogenous candidates that promote cardiac regeneration is the zebrafish. Zebrafish hearts have the remarkable capacity to regenerate when up to 20% of the ventricle is removed.26 A recent genetic screen for endogenous growth factors revealed 662 genes, including vegf, pdgf-a, igf2, and thymicin β4, that are differentially expressed during zebrafish heart regeneration.27 These targets may represent powerful endogenous factors that should be explored further if they indeed do translate to the mammalian heart. Using a small-molecule library screen for activators of naïve2.5, Sadek et al28 recently discovered a family of small molecules that can trigger cardiac gene expression in a variety of stem/progenitor cells, underscoring the potential for small molecules to promote differentiation of stem cells to cardiomyocytes for therapeutic use.

Recent progress shows that cardiomyocytes can be generated successfully from human induced pluripotent stem cells,29 indicating the possibility that we can make our own cardiomyocytes from fibroblasts. However, the fact that CPCs are genetically programmed to generate the exact constituents of the heart makes them more attractive as a source of cell therapy for the heart. With the recent establishment of a genetic method to purify another type of CPCs,30 we have an enhanced choice of CPCs for cell-based therapies. We expect that combinatorial efforts of molecular biology and bioengineering should further enhance the efficiency of heart regeneration and improve LV function in patients after myocardial infarction.

Acknowledgment

We thank Daniela Zablocki for critical reading of the manuscript.

Sources of Funding

This work was in part supported by US Public Health Service Grants HL059139, HL067724, HL069020, AG023039, AG027211, and HL91469.

Disclosures

None.

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**Key Words:** Editorials ■ myocardial infarction ■ signal transduction
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Circulation. 2009;120:831-834; originally published online August 24, 2009;
doi: 10.1161/CIRCULATIONAHA.109.887836

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