Cortical Bone–Derived Stem Cells
A Novel Class of Cells for Myocardial Protection

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A cute myocardial infarction (MI) remains a significant cause of mortality and morbidity worldwide, despite steady advances in our understanding and treatment of coronary heart disease. After MI, lost myocardium is replaced with scar tissue, leading to left ventricular (LV) remodeling and ultimately culminating with ischemic cardiomyopathy and congestive heart failure. We now know that the adult heart is not a postmitotic organ lacking capacity for self-renewal after injury. This paradigm shift occurred after identification of stem cell niches in the adult heart and isolation of cardiac progenitor cells, including c-kit+ cardiac stem cells (CSCs), side population cells, and cardiospheres. However, the endogenous proliferation of cardiac progenitor cells after MI is inadequate for full replacement of the large number of depleted cells. To overcome this problem, extensive efforts have been made in developing cell-based therapies to promote tissue repair by introduction of exogenous cells, such as bone marrow–derived mononuclear cells (BM-MNCs), bone marrow–derived mesenchymal stem cells (BM-MSCs), adipose tissue–derived mesenchymal stem cells, CD34+ stem cells, c-kit+ CSCs, and cardiosphere-derived cells, as evidenced by recent clinical trials.

For now, the BM-MNCs are the most widely used source of cells in cardiovascular regenerative therapy trials. However, despite some successful early-stage clinical trials, several recent studies involving the use of BM-MNCs have not been overwhelmingly positive. For example, in the placebo-controlled Transplantation in Myocardial Infarction Evaluation (TIME) trial in which investigators assessed whether differential timing of BM-MNCs delivery affected LV ejection fraction (LVEF) after acute MI (3–7 days), no significant differences between the BM-MNCs and placebo groups were observed for either primary or secondary end points. In fact, the TIME trial was the third study in a series of studies (Late-TIME, Effectiveness of Stem Cell Treatment for Adults with Ischemic Cardiomyopathy [FOCUS]-Cardiovascular Cell Therapy Research Network [CCTRN], and TIME) on BM-MNCs by the National Institutes of Health–sponsored CCTRN that have all met with negative results. These findings were independently confirmed by a subsequent study, Swiss acute MI, in which intracoronary delivery of BM-MNCs in the presence of an acute MI did not improve LVEF when cells were delivered at 5 to 7 days or 3 to 4 weeks post MI.

By contrast, other therapeutic cell types have shown suggestions of efficacy. In the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON) trial, patients with ischemic cardiomyopathy after previous MIs (years earlier) who were given either allogeneic or autologous BM-MSCs at different doses demonstrated modest improvements in terms of reversed LV remodeling, although this change did not translate into an improvement in LVEF.

In a separate trial called Cardiopoietic stem Cell therapy in heart failUIRE (C-CURE), investigators found that exposing BM-MSCs to a cardiogenic cocktail promoted the nuclear expression of myocytes-specific enhancer factor 2C, which led to improved LVEF and composite clinical score. Two recent clinical trials that used amplification of cardiac progenitor cells obtained from biopsied heart tissues followed by readministration to patients were performed. In the Cardiac Stem Cells in Patients with Ischemic Cardiomyopathy (SCIPIO) trial that used c-kit+ CSCs, patients had improved LVEF and smaller MI size at 1-year follow-up. The use of cardiosphere-derived cells in Cardiosphere-derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) was also shown to reduce infarct size in patients, although this was not accompanied by an improvement in LVEF.

In addition to these trials, Vrtovec et al reported that intracoronary CD34+ cell infusion in nonischemic dilated cardiomyopathy patients led to improved LVEF, exercise tolerance, and long-term survival. By labeling these CD34+ cells with 99mTc-hexamethylpropyleneamine oxime and performing single photon emission computed tomography imaging, the authors demonstrated a positive correlation between early imaging of CD34+ cell retention and late assessment of cardiac functional outcome.

The inconsistencies in clinical outcome using various stem cell types demonstrate the need for a more thorough re-evaluation of cell therapy strategies to eliminate any red herrings during preclinical stages. In addition, several other variables are crucial to determine the success of any cell therapy approach. First, the preparation and characterization of the various cell types have been shown to exert important effects on myocardial repair. For example, Seeger et al reported that heparin, typically used as an anticoagulant for intracoronary infusion of BM-MNCs, impairs the functional capacity of these cells by blocking stromal cell–derived factor 1 (SDF-1)/CXCR4 signaling, although the significance of this effect has been recently questioned. Second, the dose, timing, and manner of cell delivery are also crucial, and they are, in turn, complicated by the diseased state, the functional capacity of...
the cells, and the complex interactions between the cells and host myocardial microenvironment. To solve these problems, a better understanding of the basic biological mechanisms underlying beneficial effects of cell therapy is needed because knowledge of the mechanisms involved can offer a dynamic perspective of cardiac homeostasis and regenerative biology, eventually leading to improved cell therapy. Some of the hypothesized mechanisms include the following: secretion of paracrine factors, stimulation of neovascularization, activation of cardiac regeneration through recruitment of stem cells or proliferation of preformed cardiomyocytes, and transdifferentiation of transplanted stem cells.24–27

In this issue of Circulation Research, Duran et al28 present an extensive study describing cortical bone–derived stem cells (CBSCs). The authors show that cells isolated from the cortical bone tissue (rather than the bone marrow)29 are in a more primitive state, negative for most markers of the hematopoietic lineage, but are still capable of differentiating into osteoblasts, chondrocytes, and adipocytes in vitro as seen with MSCs. The authors also compared the potency of these CBSCs to c-kit+/Sca-1+ cardiac-derived stem cells (CDCs).10 In vitro, these 2 cell types were similar in terms of the type and amount of paracrine factors secreted (angiopoietin-1, β-fibroblast growth factor [FGF], hepatocyte growth factor, insulin-like growth factor-1, platelet-derived growth factor, stem cell factor, SDF-1, and vascular endothelial growth factor [VEGF]) and were capable of differentiating into cells expressing cardiogenic-specific proteins.28 In vivo, wild-type C57BL/6 mice receiving CBSCs had better survival during a 6-week period (77%) compared with those receiving sham saline (50%) or CDCs (66%). Although mice receiving either CBSCs or CDCs showed improved cardiac function and attenuated adverse cardiac remodeling, these changes were more pronounced in the CBSC-treated group. There was no significant difference in the areas at risk or ischemic size at 24 hours post MI for both groups compared with the sham saline condition. However, at 6 weeks post MI, chronic infarct size was significantly reduced in both groups, especially in the CBSC cohort.

To gain more insight into how these cells function in vivo as opposed to in vitro, the authors next performed immunostaining of the aforementioned 8 paracrine factors in both CBSC- and CDC-treated groups. Because CBSCs and CDCs were isolated from enhanced green fluorescent protein+ mice, containing analysis of both green fluorescent protein and individual paracrine factors revealed that CBSCs secreted only 2 factors (β-FGF and VEGF) on transplantation, with the VEGF expression persisting as long as 2 weeks post MI. By contrast, CDCs secreted 3 factors (β-FGF, VEGF, and angiopoietin-1) for a shorter period of time, only ≤24 hours. Interestingly, upregulation of VEGF, angiopoietin-1, β-FGF, and several other proangiogenic and antiapoptotic cytokines was also reported in a previous study involving transplantation of induced pluripotent stem cell–derived endothelial cells (iPSC-ECs) using microfluidic single-cell polymerase chain reaction analysis at 6 days after MI.30 An increase in neovascularization was observed in both CBSC- or CDC-treated hearts compared with saline-treated controls, although it should be noted that the majority of these blood vessels did not contain EGFP+ cells, indicating that they were derived via endogenous repair and not from the transplanted EGFP+ stem cells. Importantly, histological analysis demonstrated the presence of EGFP+ stem cell–derived adult cardiac myocytes that had normally striated α-sarcomeric actin networks and connexin43+ gap junctions throughout the border zone in CBSC-treated mice. By contrast, no EGFP+ cells with organized sarcomeres were detected in CDC-treated mice. Finally, when isolated for ex vivo analysis, these transplanted EGFP+ myocytes (CBSC group) displayed contractions and Ca++ transients that were indistinguishable from their endogenous EGFP+ myocytes, indicating that they had acquired a mature phenotype.

The authors suggest that CBSCs represent a bone-derived c-kit+/Sca-1+ stem cell population that can support the injured heart through direct transdifferentiation into adult cardiomyocytes and, to a lesser extent, vascular cells, as well as through paracrine factors. However, additional data might provide important clues into future applications of these CBSCs. For instance, since only 8 paracrine factors were selected for testing as proangiogenic factors, key molecules may still remain to be identified. In addition, since these CBSCs have been shown to possess immunomodulatory properties, it would be illuminating to study the efficacy of these cells when delivered in an allogeneic setting, which would model the recent POSEIDON trial.20 It is also interesting to note that although both CBSCs and CDCs secreted paracrine factors in the initial 24 hours, areas at risk and ischemic size in these groups were not significantly different compared with the control saline group, although at 6 weeks all cell–treated animals had smaller infarcts. Surprisingly, although CDCs stopped secreting any of those measured paracrine factor 24 hours later, as compared with the continued secretion of VEGF in CBSCs for ≤2 weeks, the improvement in LVEF and fractional shortening was similar in both groups at 1 week post MI, although improvement was greater in 6 weeks for the CBSC-treated mice. This suggests that the functions (if any) of the 8 measured paracrine factors are unrelated to immediate protection of area at risk, and produce delayed effects that require longer-term secretion. Of note, most of the molecules tested were targets of hypoxia-inducible factor-1, and previous gene therapy study has demonstrated that upregulation of hypoxia-inducible factor-1 leads to a sustained elevation of proangiogenic factors (coincidentally including β-FGF and VEGF measured in this study) at least for 2 weeks in a similar model.32 Thus, it may be worthwhile to consider combining gene and cell therapy to obtain greater synergistic effects.

Another particularly interesting aspect of this study was how the starting number of injected EGFP+ cells (40000 cells/heart) increased >3× (=130000 cells/heart), as measured by the estimated final number of EGFP+ mature/nonmature cardiomyocytes harvested 6 weeks post MI. The final number did not include other population of EGFP+ cells, such as vascular smooth muscle cells, vascular endothelial cells, and EGFP+ cells, that are smaller and lack either cardiac or vascular cell proteins. Significantly improved cardiac function was seen as early as 1 week post MI, which was impressive considering that the starting number of injected cells was lower than in other studies.33,34 These EGFP+ CBSCs were delivered into the hostile inflammatory microenvironment of acute MI, and yet
managed to survive, engraft, and proliferate robustly. By contrast, several other studies using longitudinal imaging analysis within similar transplanted animals have shown that most of the injected BM-MNCs, BM-MSCs, Sca-1+ CSCs, and adipose tissue–derived mesenchymal stem cells in similar settings die within 6 to 8 weeks after transplantation. 35-40 The aforementioned 300% increase in CBSC-derived new myocytes suggests that CBSCs either have a very high rate of retention after injection into the heart or may possess a remarkable proliferative and cardiac differentiation capacity, which have not been observed in several other adult stem cell types.

One of the proposed mechanisms conferring protection was partially attributed to the formation and maturation of CBSC–derived new myocytes, which is in accordance with the high proliferative/differentiation capacity described above. At 1 and 2 weeks post MI, EGFP+ cells at intermediate stages of myocyte differentiation were detected in all hearts analyzed (6/6). At 6 weeks post MI, mature EGFP+ cardiomyocytes were only detected in 5 of 8 hearts analyzed (=60%), despite a significant improvement in cardiac function compared with saline or CDC-treated groups. Hence, further investigation is warranted to explain the significant improvement in cardiac function, despite finding mature EGFP+ myocyte in only about half of the hearts. At the same time, the findings here showing that CBSCs are superior to CDCs in forming mature cardiomyocytes are intriguing. There appears to be a continuum of cells with some degree of cardiogenic potential: Oskouei et al42 reported that human c-kit+ CSCs were superior compared with MSCs in terms of improvement of cardiac function, cell engraftment, and also differentiation. As with all good experiments that generates more intriguing questions from its results, the Duran et al study has brought a new class of stem cells to the forefront for potential use in cardiac regenerative medicine, but much more research needs to be conducted to understand the biology of these cells fully. One fascinating approach would be to perform transcriptomic profiling on these CBSCs that would provide a thorough understanding of the genetic signature of these cells, which can then be added to existing resources. For example, a recent study by Dey et al42 identified molecular pathways specific for different cardiogenic stem and progenitor cells by comparing CSCs (c-kit+, Sca-1+, and side population) and BM-derived progenitor cells (BM-c-kit+ and BM-MSCs). Inclusion of these CBSCs would be invaluable for comparison. Elucidation of the molecular pathways behind this cohort of cells, which possess the secretory potential typically seen with MSCs along with myogenic potential typically associated with cells of cardiac origin, offers the possibility of including CBSCs as an exciting new player in the field of cardiac regeneration (Figure).

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Disclosures

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