Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen

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Shabbir A, Zisa D, Suzuki G, Lee T. Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. Am J Physiol Heart Circ Physiol 296: H1888–H1897, 2009. First published April 24, 2009; doi:10.1152/ajpheart.00186.2009.—Heart failure carries a poor prognosis with few treatment options. While myocardial stem cell therapeutic trials have traditionally relied on intracoronary infusion or intramyocardial injection routes, these cell delivery methods are invasive and can introduce harmful scar tissue, arrhythmia, calcification, or microinfarction in the heart. Given that patients with heart failure are at an increased surgical risk, the development of a noninvasive stem cell therapeutic approach is logistically appealing. Taking advantage of the trophic effects of bone marrow mesenchymal stem cells (MSCs) and using a hamster heart failure model, the present study demonstrates a novel noninvasive therapeutic regimen via the direct delivery of MSCs into the skeletal muscle bed. Intramuscularly injected MSCs and MSC-conditioned medium each significantly improved ventricular function 1 mo after MSC administration. MSCs at 4 million cells/animal increased fractional shortening by ∼40%, enhanced capillary and myocyte nuclear density by ∼30% and ∼80%, attenuated apoptosis by ∼60%, and reduced fibrosis by ∼50%. Myocyte regeneration was evidenced by an approximately twofold increase in the expression of cell cycle markers (Ki67 and phosphohistone H3) and an ∼13% reduction in mean myocyte diameter. Increased circulating levels of hepatocyte growth factor (HGF), leukemia inhibitory factor, and macrophage colony-stimulating factor were associated with the mobilization of c-Kit-positive, CD31-positive, and CD133-positive progenitor cells and a subsequent increase in myocardial c-Kit-positive cells. Trophic effects of MSCs further activated the expression of HGF, IGF-II, and VEGF in the myocardium. The work highlights a cardiac repair mechanism mediated by trophic cross-talks among the injected MSCs, bone marrow, and heart that can be explored for noninvasive stem cell therapy.

Trophic factors

ADVANCES in patient management and treatment have lowered death rates from heart disease over the last 30 years but have led to an increasing patient population living with heart failure. Unfortunately, the only therapy available to reverse the decline in cardiac function is heart transplantation. However, this option is available to very few patients due to a shortage of donor hearts. Late sequelae of immunosuppression and rejection further limit the efficacy of this approach (4). Recent interests in stem cell therapeutics have prompted preclinical and clinical studies on the feasibility and safety of stem cells for the treatment of heart disease (6, 46). Although mixed results have been documented without a clear consensus on the best cell for cardiac regeneration, the ease of large-scale cell expansion and immunoprivileged status of bone marrow mesenchymal stem cells (MSCs) are attractive features of adult stem cells (13, 48, 57).

Myocardial stem cell therapy often uses invasive cell delivery approaches such as intramyocardial injection or intracoronary infusion. Given that patients with heart failure are at an increased surgical risk, the development of a noninvasive cell delivery regimen is logistically appealing. A salient feature of MSCs is their ability to produce a plethora of trophic factors (7, 15), which may be harnessed for noninvasive stem cell therapy for heart failure. Indeed, documented cardiovascular beneficial effects of MSCs have largely been attributed to their paracrine actions independent of their differentiation potentials (14, 52, 56, 58). This recognition stems from the findings that efficiencies of myocardial recruitment and engraftment after local or systemic stem cell administration are typically too low to account for functional improvement. Our recent cell tracking study (32) estimated that only 1–2% of intracoronary-infused MSCs engrafted in the pig heart with no evidence of MSC differentiation into cardiomyocytes. Furthermore, since diseased tissue environments often exhibit pathological levels of ischemia, inflammation, and fibrosis, which can impair cell survival, the therapeutic delivery of stem cells to areas away from the damaged heart offers a novel concept.

The multiple trophic factors produced by MSCs are capable of attenuating tissue injury, inhibiting fibrotic remodeling, promoting angiogenesis, stimulating the recruitment and proliferation of tissue stem cells, or reducing inflammatory oxidative stress (7, 15, 34, 48). We hypothesize that MSCs, via secretion of these functionally synergistic trophic factors, are able to rescue the failing heart even when delivered away from the myocardium. Delivery of MSCs by intramuscular injection offers a feasible noninvasive strategy as skeletal muscle, being the most abundant tissue in the body, is amenable to repeated injection of large numbers of stem cells. Along this line, we have shown by PCR analysis that intramuscularly injected MSCs are trapped in the muscular bed with no detectable migration to other tissues (48). This cell injection regimen is used here to provide the ultimate proof that the trophic actions of MSCs underlie their cardiovascular therapeutic effects. Using a hamster heart failure model characterized by us and others (12, 16, 39, 45), we demonstrate, for the first time, that the noninvasive administration of MSCs or MSC-derived trophic factors via intramuscular injection effectively rescues the failing heart through intricate tissue cross-talk mechanisms. This noninvasive stem cell administration regimen, if validated clinically, is expected to facilitate future stem cell therapy for heart failure.

MATERIALS AND METHODS

Animals. F1B (normal) and TO2 (cardiomyopathic) male hamsters were obtained from Bio Breeders (Watertown, MA). All procedures

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and protocols conforms to institutional guidelines for the care and use of animals in research.

**Echocardiography.** Echocardiographic measurements were performed in a blind-folded fashion and were as described in our recent work (39).

**MSC culture and intramuscular implantation.** Porcine bone marrow MSCs were isolated as previously described (34, 59). To produce MSC-conditioned serum-free medium, MSCs were plated on a fibronectin-coated surface and grown to confluence. Cells were then washed thoroughly with HBSS and maintained in serum- and phenol-red free MEM for 24 h. The conditioned medium was harvested and filtered before use. For intramuscular implantation, MSCs (0.25, 1, or 4 million cells/animal) were resuspended in 0.8 ml HBSS and injected in equally divided doses into the left and right hamstrings of 4-mo-old TO2 hamsters. Control TO2 hamsters received the same volume of HBSS. Animals received a second intramuscular MSC implantation 2 wk later since preliminary cell injection trials indicated more prominent therapeutic effects with a repeated cell injection. For medium injection, TO2 hamsters received three weekly injections each of 0.8 ml of the conditioned medium for 4 wk.

**ELISA assay.** Circulating cardiac troponin I (cTnI) was assayed with a rat cTnI ELISA kit (Life Diagnostics) using plasma samples collected 1 mo after MSC administration. Assays of circulating mitogen-activated protein kinase (G/M-CSF) were performed using the MAP program (Rules-Based Medicine). Hepatocyte growth factor (HGF), VEGF, and IGF-II were analyzed by the following ELISA kits from R&D Systems: mouse HGF DuoSet (no. DY2207), rat VEGF DuoSet (no. DY564), and mouse IGF-II DuoSet (no. DY792). Heart tissues were homogenized in ice-cold lysis solution containing 0.1% Triton X-100 and 2 mM EDTA. Lysates were clarified, diluted to 1 mg protein/ml, and used for ELISA per the manufacturer’s instructions.

**Flow cytometry.** Peripheral blood mononuclear cells were isolated 3 days after MSC administration, and red blood cells were removed with lysis buffer (150 mM NH4Cl, 10 mM KHCO3, and 0.1 mM ethylenediaminetetraacetic acid) followed by an ice-cold wash and resuspended in normal saline. After being blocked with an Fc receptor blocker for 30 min, cells were labeled with phycoerythrin (PE)-conjugated CD133 (no. AC133, Miltenyi Biotec), PE-conjugated CD31 (no. 12-0311, eBioscience), and PE-conjugated c-Ki (no. 12-1171, eBioscience) antibodies. Flow cytometry was performed on ~25,000 cells, and data were analyzed using FCS Express (De Novo Software). Proper isotype-matched IgGs were used as controls. Dead cells were excluded by 7-aminocoumarin D counterstaining.

**Quantification of capillary and cardiomyocyte nuclear density.** Freshly excised tissues were immersed in OCT, frozen in liquid nitrogen, and stored at −80°C until use. Ventricular cross sections of 5 μm thick were obtained using a cryostat and fixed in an acetone-ethanol mixture (3:1) for 5 min. Sections were blocked with Serum-Free Protein Block (Dako) for 30 min. FITC-labeled Griffonia simplicifolia isocoten B2 (diluted 1:100) was incubated with the tissue sections overnight at 4°C. Cardiomyocytes were stained with a rabbit TnI antibody (no. sc15368, Santa Cruz Biotechnology) the next day for 3 h. The TnI antibody reacts with both cTnI and skeletal TnI of rodent and human origin. Sections were then incubated with a Texas red-conjugated anti-rabbit secondary antibody for 1 h and then mounted using Vectashield’s Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI; Vector Laboratories). Images were taken in 15–25 random fields using Zeiss’s Axioimager fluorescence microscope at ×200 magnification. Numbers of capillaries (FITC channel) and total nuclei (DAPI channel) were quantified by ImageJ software using the analyze particle feature. Noncardiomyocyte nuclei quantified from the merged images by their lack of TnI staining were subtracted from the total nuclei count to determine cardiomyocyte nuclear density. Black areas from images were subtracted using Photoshop-aided quantification of black pixels to calculate the total tissue area. Capillary and cardiomyocyte nuclear density were normalized to the total tissue area (in mm2).

**Quantification of apoptosis.** Analysis of apoptosis was performed on frozen sections prepared as described above using the ApopTag kit (Millipore) per the manufacturer’s instructions. TnI antibody was used to identify apoptotic myocytes, and analysis was performed similarly as described above. All apoptotic nuclei in each section were counted and normalized to total myocytes and nonmyocytes.

**Quantification of fibrosis and cardiomyocyte diameter.** Masson trichrome-stained sections were used for fibrosis analysis and cardiomyocyte diameters. Fibrosis was performed by Photodshop-aided quantification of image pixels. The blue color range was selected to represent fibrotic areas. At least 15 random fields at ×200 magnification were assessed for each slide by 3 independent examiners with 1 examiner being blinded. Artificial spaces (white clear areas) from images were subtracted using Photoshop-aided quantification of white pixels to calculate the total tissue area. The ratio of fibrotic areas to total tissue areas was calculated as a percentage of the fibrotic areas. For the quantification of cardiomyocyte diameters, at least 350 random cardiomyocytes were measured for each animal using AxioVision LE software’s measurement tool (Carl Zeiss).

**Quantification of c-Kit-positive, Ki67-positive, and phospho-histone H3-positive cells.** Paraformaldehyde-fixed, paraffin-embedded heart sections of 5 μm thick were used for c-Kit, Ki-67, and phospho-histone H3 (p-HH3) staining. Antibigen retrieval was done by steaming in 10 mM citrate (pH 6) for 30 min followed by permeabilization in 0.2% Triton X-100 for 20 min. Sections were blocked with normal saline solution supplemented with 0.025% Tween-20 and 2% nonfat milk

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MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.
powder for 30 min and incubated with diluted primary antibody overnight. The primary antibodies used were as follows: c-kit antibody (no. A4502, Dako), Ki67 antibody (no. RM-9106, Thermo Scientific), and p-HH3 antibody (no. 07-145, Millipore). Myocytes were stained with mouse cardiac troponin T (cTnT) antibody (no. MS-295, Thermo Scientific) the next day for 1 h. The cTnT antibody reacts with cTnT of multiple species. Sections were then incubated with Alexa 647-conjugated anti-rabbit and Alexa 488-conjugated anti-mouse secondary antibodies for 30 min and then mounted in Vectashield’s Mounting Medium with DAPI. Sections were analyzed as described above using Zeiss’s Axioimager fluorescence microscope at ×200 magnification.

Real-time quantitative RT-PCR. RNA isolation and quantitative RT-PCR protocols were performed as previously described (34). β2-Microglobulin was used as the reference gene for calculations. Injected MSCs were quantified by pig-specific 16S rRNA primers. A standard curve was created by generating a serial dilution curve by plotting the threshold cycles of the 16S rRNA gene against a known number of MSCs. The primer sequences are shown in Table 1.

Statistical analysis. Data are expressed as means ± SE. Comparisons were based on an unpaired Student’s t-test. P values of <0.05 were considered significant.

RESULTS

The hamster heart failure model. The TO2 hamster strain harbors a genetic defect in the δ-sarcoglycan gene that causes dilated cardiomyopathy leading to congestive heart failure clinically identical to that occurring in the general category of human heart failure (12, 16, 45). Functional deterioration of the TO2 heart is accompanied by prominent myocyte loss, inflammation, fibrosis, and calcified lesions (44, 45). We (39) have shown that the left ventricular (LV) ejection fraction (LVEF) and fractional shortening (FS) of the TO2 hamster heart decline by ~25% and 35%, respectively, at 4 mo of age. The MSC therapeutic trials described here were performed using 4-mo-old TO2 hamsters.

Noninvasive MSC delivery for heart failure. Preclinical and clinical studies of myocardial stem cell therapy often use invasive cell delivery approaches such as intramyocardial injection or intracoronary infusion. Given that patients with heart failure are at an increased surgical risk, it is logistically appealing to explore a noninvasive cell delivery approach. MSCs, via their ability of trophic factor production, may be harnessed toward achieving this goal. Using both histological and PCR detection methods, we (48) have recently shown that intramuscularly injected MSCs are largely trapped in the musculature with no detectable cell migration to other tissues including the heart. This noninvasive cell injection regimen takes advantage of the fact that skeletal muscle is the largest tissue of the body and is amenable to repeated injections, which is difficult to achieve with myocardial cell delivery routes. To address the feasibility of intramuscular injection of MSCs for heart failure, we first carried out cell dosage experiments comparing injections of 0.25, 1, and 4 million MSCs into the hamstring muscles of TO2 cardiomyopathic hamsters. Blinded echocardiography was performed 1 mo after cell injection and showed that all three MSC dosage groups significantly improved ventricular function and attenuated chamber dilation (Fig. 1, A and B). The 4 million cell dosage group clearly exhibited the most prominent functional improvement, as indicated by an ~40% increase in FS and ~10% decrease in LV diastolic diameter. The 4 million cell dosage group also caused showed an ~80% increase in systolic wall thickening (data not shown).

Since MSC migration to the heart was undetectable after the intramuscular injection (48), the observed functional improvement must have been mediated by trophic factors. To provide the evidence, MSC-conditioned medium was used to determine whether the administration of cell-free medium would similarly rescue the failing heart. The medium was administered by multiple injections into the hamstring muscle, and echocardi-
ography was performed after 1 mo. This experiment showed that MSC-conditioned medium was again effective in improving ventricular function and decreasing dilation compared with the control medium (Fig. 1, C and D). These functional experiments thus demonstrated the feasibility of noninvasive MSC therapy for heart failure using the convenient intramuscular injection route.

**Active regeneration of the failing heart.** Histological experiments were further performed to ascertain that the functional improvement caused by intramuscularly injected MSCs was associated with myocardial tissue regeneration. We found that capillary and myocyte nuclear density in the MSC treatment group was ~30% and ~80% higher than the control injection group, respectively (Fig. 2, A–C). The higher myocyte nuclear density can be contributed by newly regenerated myocytes, which are typically smaller (37, 48). Figure 2D indeed shows that the mean cross-sectional myocyte diameter was smaller in the MSC-treated group (15.8 ± 0.9 μm) than in the control group (18.2 ± 0.3 μm). The frequency histogram demonstrated a shift toward smaller myocyte diameters in the MSC group, suggesting the prominent presence of newly regenerated myocytes (Fig. 2E). To provide additional evidence along this line, we quantified two specific markers associated with cell cycle activity: Ki67 and p-HH3 (18, 33). Quantitative analyses showed that the expression of Ki67 (Fig. 3, A–C) and p-HH3 (Fig. 3, D–F) were increased by approximately twofold in the MSC group and that increased expression of the cell cycle markers could be detected in both myocyte and nonmyocyte populations. Thus, cardiac functional improvement after intramuscular injection of MSCs is mediated by active myocardial regeneration.

**Downregulation of apoptosis and tissue injury.** Since many trophic factors produced by MSCs possess antiapoptotic function (7, 15), the observed myocardial tissue regeneration could also be contributed by reduced apoptosis. Histological analysis of myocardial tissue sections revealed that MSCs decreased apoptosis of myocytes and nonmyocytes each by ~60% (Fig. 4, A and B), suggesting that trophic factors promoted cell survival in the myocardium. Consistent with this finding, circulating cTnI levels were decreased by ~60% (MSC group vs. saline control group; 1.8 ± 0.25 vs. 4.49 ± 0.9 ng/ml; Fig. 4C), reflecting a significant attenuation of myocardial tissue injury.

**Attenuation of pathological fibrosis.** The TO2 cardiomyopathic hamster heart exhibits progressive fibrosis marked by the elevated expression of collagens, matrix metalloproteinases (MMPs), and tissue inhibitor of metalloproteinases (TIMPs) (11, 44, 45). Examinations of histological sections revealed greatly diminished LV fibrosis and leukocyte infiltration after MSC treatment (Fig. 5A). Fibrotic areas in the TO2 saline control group were ~12%, in contrast to the ~1% fibrotic areas in the normal F1B hamster heart. MSC injection caused an ~50% decrease in fibrosis in the TO2 heart (Fig. 5B). Since the balance between collagen synthesis and degradation is mediated by MMPs and TIMPs and is of crucial relevance in maintaining myocardial structural integrity (49), we further...
used quantitative RT-PCR to assess the expression of these molecular players. Figure 5C shows that the control TO2 heart exhibited an elevated expression of collagens, MMPs, and TIMPs, as shown previously (11), and that MSC administration reversed the abnormal expression profiles of collagens, MMPs, and TIMPs. Thus, the noninvasive delivery of MSCs, through trophic activities, rescued the failing heart by improving ventricular function, promoting myocardial tissue regeneration and survival, and attenuating pathological fibrosis.

*Increased circulating and myocardial trophic factors.* We next sought to identify the therapeutic mechanisms mediated by the intramuscularly injected MSCs. The multiple trophic factors produced by MSCs are known to possess functionally synergistic and redundant activities that are beneficial to the heart (7, 15, 34). These trophic factors may be initially released from intramuscularly injected MSCs and subsequently affect the expression of growth factors in the myocardium. Several major growth factors and cytokines present in the plasma and heart tissue homogenates were analyzed by ELISA 1 mo after MSC administration. These immunoassays revealed increased circulating levels of HGF, LIF, and G/M-CSF in the MSC treatment group (Fig. 6A). Elevated expressions of HGF, IGF-
II, and VEGF in the myocardium were further identified by both ELISA (Fig. 6B) and quantitative RT-PCR (data not shown), indicating that the trophic activities of MSCs could amplify the expression of host growth factor genes in the myocardium.

Mobilization of bone marrow progenitor cells. Mobilization of bone marrow progenitor cells plays an important role in tissue repair (30). Among the multiple MSC trophic factors, HGF, VEGF, G/M-CSF, stem cell factor (SCF), IGF, and stromal-derived factor (SDF)-1 are known to be able to mobilize bone marrow progenitor cells (19, 23, 24, 28, 29, 31).

Along this line, we (34, 59) have previously demonstrated that the MSCs used in the present study express IGF-2, LIF, G/M-CSF, SDF-1, and VEGF. We therefore investigated whether bone marrow progenitor cells might be mobilized in response to injected MSCs. Flow cytometric analysis of peripheral blood indeed showed that MSCs significantly increased circulating progenitor cells expressing c-Kit, CD31, or CD133 surface markers (Fig. 7). Increased capillary and myocyte densities, as shown in Fig. 2, could be mediated by myocardial recruitment and the subsequent differentiation of circulating progenitor cells, some of which have been found to express the c-Kit marker (3).

Myocardial c-Kit-positive progenitor cells. Mobilized bone marrow progenitor cells are thought to participate in tissue repair through tissue homing mechanisms (19, 21, 30). Increased capillary and myocyte densities, as shown in Fig. 2, could be mediated by myocardial recruitment and the subsequent differentiation of circulating progenitor cells, some of which have been found to express the c-Kit marker (3).

Mobilized bone marrow progenitor cells might be mobilized in response to injected MSCs. Flow cytometric analysis of peripheral blood indeed showed that MSCs significantly increased circulating progenitor cells expressing c-Kit, CD31, or CD133 surface markers (Fig. 7), which have been shown to originate from the bone marrow compartment and contribute to tissue repair (21). A contribution by injected MSCs to these circulating progenitor cells can be ruled out because we and others have shown that intramuscularly injected MSCs are trapped in the musculature with no detectable migration (10, 48) and that MSCs do not express c-Kit (CD117), CD31, and CD133 markers (1, 59).

Myocardial c-Kit-positive progenitor cells. Mobilized bone marrow progenitor cells are thought to participate in tissue repair through tissue homing mechanisms (19, 21, 30). Increased capillary and myocyte densities, as shown in Fig. 2, could be mediated by myocardial recruitment and the subsequent differentiation of circulating progenitor cells, some of which have been found to express the c-Kit marker (3). To explore this possibility, we further examined whether the myocardium might harbor elevated pools of c-Kit-positive progenitor cells after MSC administration. Immunostaining revealed an approximately twofold increase after MSC administration in ventricular c-Kit-positive cells (Fig. 8, A and B). This increase was further corroborated by quantitative RT-PCR analysis of myocardial c-Kit expression (Fig. 8C). Thus, MSC-mediated mobilization of bone marrow progenitor cells is coupled with increased myocardial c-Kit-positive progenitor cells. Taken together, the noninvasive cell therapeutic regimen for heart failure takes advantage of the powerful trophic activities of MSCs, resulting in functional improvement and myocardial regeneration.
DISCUSSION

The present study demonstrates a novel noninvasive MSC therapeutic regimen for heart failure based on an intramuscular delivery route. Intramuscularly injected MSCs or MSC-conditioned medium improved ventricular function, promoted myocardial regeneration, attenuated apoptosis and fibrotic remodeling, recruited bone marrow progenitor cells, and induced the myocardial expression of multiple growth factor genes. These findings highlight the critical cross-talks between injected MSCs and host tissues, culminating in effective cardiac repair for the failing hamster heart.

Advantages of intramuscular MSC delivery. Patients with heart failure are at an increased surgical risk. While most stem cell trials have used intracoronary infusion or intramyocardial injection for cell delivery, these delivery methods are invasive, often clinically unsuitable, and can introduce harmful scar tissue, arrhythmia, calcification, or microinfarction in the heart (5, 60–62). Systemic delivery by intravenous infusion of MSCs has been found to cause entrapment of MSCs in the lungs (2). An important issue to consider is whether the engrafted stem cells may become electromechanically coupled with resident cardiomyocytes. Myoblasts, for instance, do not exhibit optimal electrophysiological integration upon myocardial engraftment, leading to postimplantation arrhythmogenesis (38). Successful clinical applications may be more feasible with a noninvasive approach, delivering MSCs to areas away from the damaged heart. Given the powerful trophic effects of MSCs, delivery of MSCs via an intramuscular route may be a
superior noninvasive strategy, allowing the repeated administration of large numbers of cells and circumventing entrapment and dilution by other tissue. In addition, since we and others (9, 35, 48) have shown that MSC treatment ameliorates muscular dystrophy, intramuscular injection of MSCs is expected to be well suited for treating muscular dystrophy patients with cardiomyopathies.

Cardiac repair mediated by trophic mechanisms. While early preclinical studies have suggested therapeutic mechanisms mediated by stem cell transdifferentiation or fusion (42, 55), it has become apparent that these mechanisms do not occur in sufficiently high frequency to account for the observed functional improvement after stem cell administration (15). Our cell tracking study (32) estimated that only 1–2% of intracoronary-infused MSCs engrafted in the pig heart, and yet this low efficiency of cell engraftment was able to significantly improve function in the porcine hibernating myocardium (50). We note that although systemic delivery of MSCs by intravenous infusion caused cell entrapment in the lungs (2), this cell delivery strategy was found to improve cardiac function in rats with acute myocardial infarction (40). These findings are consistent with increasing evidence suggesting that the cardiovascular beneficial effects of stem cell therapy are largely due to the actions of trophic factors or paracrine mediators (14, 53, 56). In addition, studies have attributed trophic activities of myoblasts and endothelial progenitor cells as critical cardio-protective mechanisms (20, 43). Our demonstration here that intramuscularly injected MSCs and MSC-conditioned medium are both therapeutically effective for treating hamster heart failure provides the ultimate proof for the critical role of trophic factors in stem cell therapy. While various single growth factor therapeutic regimens have been attempted for FGF, HGF, IGF, and VEGF, with encouraging results (36, 41, 47, 51), the MSC therapy is unique in its engagement of functionally synergistic and redundant trophic factors (7, 15) that may be required for the activation of the endogenous stem cell repair mechanism and a more sustained therapeutic effect.

Molecular cross-talks between MSCs and host tissues. Mobilization of bone marrow progenitor cells plays an important role in tissue repair (30). The MSCs used here have been shown to express trophic factors such as HGF, LIF, G/M-CSF, SDF-1, and VEGF (34, 59), which are capable of mobilizing bone marrow progenitor cells. Along this line, the administration of G/M-CSF has been proposed as a potential new therapy for myocardial infarction (22), and intramuscular injection of LIF plasmid DNA has been found to be cardioprotective (63). We indeed detected elevated levels of circulating HGF, LIF, and G/M-CSF in MSC-treated animals, and, consistent with this finding, circulating c-Kit-positive, CD31-positive, and CD133-positive bone marrow progenitor cells were increased after MSC administration. Although unrestrained MSC secretions may cause an abnormally abundant mobilization of progenitor cells, this effect of MSCs is unlikely to be sustained because we observed a progressive loss of injected MSCs (48). The mobilized progenitor cells can repopulate the myocardium, as shown here by increased myocardial c-Kit-positive progenitor cells, and participate in endogenous cardiac repair mechanisms. Notably, we have obtained evidence that this cell mobilization mechanism becomes impaired in aged TO2 hamsters, which may explain at least in part why the MSC therapeutic regimen fails to rescue the aging heart (data not shown). This molecular cross-talk between injected MSCs and the bone marrow compartment thus illustrates the dynamic and functionally relevant signaling cascade involved in stem cell repair. The signaling cascade depicted here can further activate the myocardial expression of HGF, IGF, and VEGF genes, highlighting an additional cross-talk circuit between MSCs and the myocardium. Similar to this finding, Cho et al. (8) demonstrated that the myocardial expression of several growth factor genes, including HGF, IGF, and VEGF, was upregulated after intramyocardial stem cell implantation. Tateno et al. (54) found that implanted stem cells stimulated muscle cells to produce angiogenic factors that resulted in neovascularization. Our finding here is consistent with previous reports (27, 41) demonstrating that HGF administration could improve cardiac function in TO2 cardiomyopathic hamsters. Work is in progress to characterize the role and response of host tissues after MSC administration.

Exploring the immunomodulatory properties of MSCs. MSCs are thought to possess unique immunomodulatory properties that can be explored for nonautologous or xenogeneic stem cell-based therapeutics (17, 57). The trophic action of MSCs can decrease the host production of inflammatory cytokines and induce T cell anergy. The immune phenotype of
culture-expanded MSCs is widely described as major histo-
compatibility complex (MHC) class I positive, MHC class II
negative, CD40 negative, CD80 negative, and CD86 negative,
which is regarded as nonimmunogenic, suggesting that MSCs
are capable of trespassing species defense barriers. The use of
MSCs in allogeneic and xenogeneic transplantation can reduce
the incidence and severity of graft versus host disease (57).
In this aspect, (48) they have recently demonstrated that intramus-
cularly injected human and porcine MSCs are well tolerated by
TO2 dystrophic hamsters and that the MSC treatment leads to
prominent skeletal muscle regeneration and attenuates oxidative
stress without inflaming the host immune system. Given
that stem cell function and potency can be impaired by aging
and disease (25, 26), the use of nonautologous human MSCs
isolated from healthy donors offers a major advantage since
these adult stem cells can be routinely expanded in culture and
thoroughly tested in advance for clinical applications.

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GRANTS

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