Enhanced IGF-1 expression improves smooth muscle cell engraftment after cell transplantation

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Enhanced IGF-1 expression improves smooth muscle cell engraftment after cell transplantation. The functional benefit of cell transplantation after a myocardial infarction is diminished by early cell losses. IGF-1 enhances cell proliferation and survival. We hypothesized that IGF-1-transfected smooth muscle cells (SMCs) would enhance cell survival and improve engraftment after cell transplantation. The IGF-1 gene was transfected into male SMCs and compared with SMCs transfected with a plasmid vector (vector control) and nontransfected SMCs (cell control). IGF-1 mRNA (n = 10/group) and protein levels (n = 6/group) were higher (P < 0.05) at all time points (n = 3, 7, and 14 days) compared with controls. VEGF was also increased in parallel to enhanced IGF-1 expression. IGF-1-transfected cells demonstrated greater cell proliferation, stimulated angiogenesis, and decreased caspase-3 activity after simulated ischemia and reperfusion (P < 0.05 for all groups) at 10^6 cells from three groups were implanted into the scar. One week later, IGF-1-transfected SMCs had increased myocardial IGF-1 and VEGF levels, increased Bcl2 expression, limited cell apoptosis, and enhanced vessel formation in the myocardial scar compared with the two control groups (P < 0.05 for all groups). The proportion of SMCs surviving in the implanted region was greater (P < 0.05) in the IGF-1-transfected group than in the vector or cell controls. Gene enhancement with IGF-1 improved donor cell proliferation, survival, and engraftment after cell transplantation, perhaps mediated by enhanced angiogenesis and reduced apoptosis.

Insulin-like growth factor-1; gene therapy; myocardial infarction; apoptosis

GENE AND CELL THERAPY has offered the promise of myocardial regeneration after a myocardial infarction. Angiogenic gene therapy to increase perfusion and function after a myocardial infarction has been evaluated in animals and humans (17, 23, 26). Although some studies have suggested improved perfusion, other studies failed to show a functional benefit (26). In addition, angiogenic gene therapy will not improve heart function in the absence of viable cardiomyocytes in the ischemic regions.

Cell transplantation has resulted in viable functioning muscle in damaged and scarred myocardium (7, 15, 18). Human and animal studies have demonstrated that muscle cells engrafted in damaged myocardial regions prevented cardiac dilatation, preserved ventricular function, and delayed heart failure (7, 8, 15, 18). The improvement in regional function was found to be proportional to the number of cells implanted (21). Because few cells survived implantation into ischemic myocardium (20), increasing cell survival should improve the functional benefit of cell transplantation. Cell engraftment has been shown to be improved by inducing a more profound angiogenesis in the ischemic region where the cells were injected (25). Cell engraftment should also be improved by interventions that prevent cell injury during implantation (34).

Combining gene and cell therapy should have a synergistic beneficial effect on the transplanted heart. If the cells were transfected in culture, the transplanted cells should express the gene product in the transplant avoiding many of the side effects of viral vectors when the gene was systemically delivered. Gene transfection should enhance cell engraftment by improving cell survival. Transfected with VEGF have been shown to induce a more profound angiogenic response than vector-transfected or unmodified control cell transplantation (32). Unfortunately, VEGF induced a capillary endothelial response but not an arteriogenic response (32).

IGF-1 not only induced angiogenesis but also stimulated cellular hyperplasia and suppressed apoptosis (3–5, 14, 16, 29, 31). Therefore, IGF-1 might offer substantial advantages over VEGF in enhancing cell engraftment after transplantation. In addition, increased expression of IGF-1 should have direct benefits on the failing heart. For example, IGF-1 overexpression in mice limited ventricular dilatation and wall stress after coronary ligation (6). Exogenous administration of IGF-1 in animals with heart failure improved hemodynamics and preserved cardiac systolic function (21). IGF-1 also provided a direct positive inotropic effect on cardiomyocytes (11). Recent clinical trials have reproduced some of these experimental benefits suggesting that enhanced IGF-1 expression could provide a new avenue of therapy for patients at risk of heart failure.

In this study, we evaluated the efficacy and possible mechanisms by which IGF-1 gene enhancement increased the survival and engraftment of smooth muscle cells (SMCs) in the injured myocardium.

METHODS

Experimental Animals

Male and female Lewis rats (200–250 g, Charles River Canada) were used as cell donors and recipients, respectively. All animal procedures were carried out with the approval of the Animal Care Committee of The Toronto General Research Institute and in compliance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. **PCR primer sequences of GAPDH, IGF-1, and VEGF genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Expected Size</th>
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<tr>
<td>GAPDH</td>
<td>Forward 5'-TGAAGCTGAGTCAACGGATTTGGT-3'</td>
<td>983 bp</td>
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<tr>
<td></td>
<td>Reverse  5'-CATGTGGGCCATGAGGTCCACCAC-3'</td>
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</tr>
<tr>
<td>IGF-1</td>
<td>Forward 5'-TACTCCGTAGCTCCAGCTTC-3'</td>
<td>482 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GGGTCTTCCTAAAGCTCGTCCTTCATGTTGT-3'</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td>Forward 5'-ACGAAGTTGGAATTTCCATGGA-3'</td>
<td>287 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GCTCTAATTTTGGGTGCTC-3'</td>
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Fig. 1. Cultured smooth muscle cells in their third passage (A, ×100) were stained with a monoclonal antibody against smooth muscle α-actin (B, ×200, green arrows point to positively stained cells and red arrows point to negatively stained cells). Light (C, ×40, and E, ×200) and ultraviolet (D, ×40, and F, ×200) microscopic views of the cells are shown on day 3 (C and D) and week 3 (E and F) after transfection with a plasmid containing the green fluorescent protein gene. The red arrows point to the green fluorescent cells. The transfection efficiency was 20 ± 5%, and the gene product persisted for at least 3 wk.
A. J. D’Ercole (University of North Carolina, Chapel Hill, NC). PUC18 plasmid was used as a vector control. For transfection, SMCs (2 × 10^6 cells/dish) were cultured for 24 h and then incubated with Superfect Transfection Reagent (60 μl, QIAGEN) containing IGF-1 gene plasmid DNA (10 μg) (IGF-1 group) or vector plasmid DNA (10 μg) (vector control) in 0.3 ml of serum-free medium for 2 min at room temperature. In the cell control groups, the cells were incubated with 0.3 ml serum-free medium under the same conditions. Culture medium (3 ml) was added into the culture dishes and incubated for additional 3 h at 37°C in an atmosphere of 5% CO₂. The transfection reagent was washed out with PBS, and the cells were then cultured. Transfection efficiency was estimated by transfecting the SMCs with a plasmid containing the green fluorescent protein gene under the same conditions.

**In Vitro Gene Expression**

SMC mRNA levels of IGF-1, VEGF, and GAPDH genes (an internal standard) in IGF-1, vector control, and cell control groups were measured on days 3, 7, and 14 after gene transfection (n = 10/group) using RT-PCR (Table 1) assay as we previously described (13). In brief, 1 μg of total cellular RNA was used for the evaluation of genes (QIAGEN RNaseasy and one-step RT-PCR kits), and PCR products were analyzed. Densitometry of the band of each gene was measured, and the ratio of growth factor gene to the GAPDH gene was used to provide a semiquantitative estimate of gene expression.

**In Vitro Growth Factor Protein Levels**

IGF-1 and VEGF protein levels were evaluated in cultured cells and conditioned culture medium from the three groups (n = 6/group) by a slot blot assay as we described previously (13). On days 3, 7, and 14 after gene transfection, SMCs were scraped from culture dishes and sonicated in 600 μl protein solution (50 mM Tris, 0.5 μg/ml leupeptin, 0.5 μg/ml peptatin, 0.5 μg/ml aprotinin, and 100 mM PMSF). After centrifugation, the supernatant was collected for protein assay. The conditioned medium was collected after the cells had been incubated in 10 ml medium for 72 h to measure secreted growth factor levels. On days 3, 7, and 14 after gene transfection, 1 ml of conditioned medium was collected for measurement.

Ten micrograms of total protein from each sample was applied to positively Hybond membranes (Amersham; Mississauga, Ontario, Canada). IGF-1 or VEGF proteins (Sigma) were used as standards at concentrations of 50, 25, 12.50, 6.25, 3.13, 1.56, 0.78, and 0.39 ng. Membranes were hybridized with a monoclonal antibody against IGF-1 or VEGF (Sigma) overnight at 4°C. After being washed, the membranes were incubated with goat anti-mouse IgG for 1 h, labeled with ECL (Amersham), and then imaged. The growth factor levels were calculated from the standard curve.

**Fig. 2.** IGF-1 mRNA levels (A, as a ratio to GAPDH) increased for at least 14 days after transfection. Intracellular (B) and extracellular (C) IGF-1 protein levels were similarly increased in the IGF-1-transfected cells compared with the vector and cell control groups (⁎P < 0.05).

**Fig. 3.** VEGF mRNA levels (A), intracellular VEGF protein levels (B), and extracellular VEGF protein levels (C) were greater in IGF-1-transfected cells compared with the vector and cell control groups (⁎P < 0.05).
FGF-1 GENE-ENHANCED CELL THERAPY

In Vitro Cell Proliferation

SMCs (2 \times 10^6 cells/dish) from the three groups were cultured. On days 3, 7, and 14 after transfection, cell number (n = 6/group per time point) was counted with a cell counter (Beckman Coulter). The cell number at each time point was averaged, and the increase in cell number between days 3 and 14 was calculated as an index of cell proliferation.

In Vitro Ischemia Injury

Seven days after gene transfection, 2 \times 10^6 cells of each of the three groups (n = 12/group) were exposed to 1.6 ml deoxygenated PBS solution (pH = 7.3–7.4, 300 mosM, and PO2 = 0 mmHg) for 2 h in a sealed chamber to simulate ischemia as we described previously (9). The cells were reperfused for 30 min with 8 ml normoxia PBS. Two milliliters of 4% trypan blue dye was added to six dishes in each group to evaluate cell injury sufficient to prevent dye exclusion. In the other six dishes per group, the number of cells was counted, and caspase-3 activities were measured by a Caspase-3 Colorimetric Assay (Chemicon).

In Vitro Angiogenesis

To determine the angiogenic potential of the transfected cells, we evaluated the capillary formation by endothelial cells induced by cell homogenates from each group. Endothelial cells (YPEN-1 cell line, American Type Culture Collection, 5 \times 10^4 cells/9.6-cm² well) were seeded onto growth factor-reduced Matrigel (n = 6/group). The 2 \times 10^6 cell homogenates (400 μl) derived from IGF-1-transfected cells, vector-transfected cells, or control cells were added to the endothelial cell cultures. After 18 h, the endothelial cell cultures were photographed under a phase-contrast microscope. The length of the capillary tubes was measured using arbitrary units employing Scion Image software (1 cm = 4.47 arbitrary units) and expressed as millimeters per 0.6 mm².

Myocardial Scar Generation

Female rat hearts were cryoinjured as described previously (15). In brief, rats were anesthetized with intramuscular ketamine (22 mg/kg body wt), followed by an intraperitoneal injection of pentobarbital (30 mg/kg body wt). The anesthetized rats were intubated, and positive pressure ventilation was maintained with room air supplemented with oxygen (2 l/min) using a Harvard ventilator (model 683; South Natick, MA). The rat hearts were exposed through a 2-cm left lateral thoracotomy. Cryoinjury was produced on the left ventricular free wall (LVFW) by freezing the myocardium for 15 s for 5 times and then for 1 min for 12 times using a 0.8 × 1.2-cm liquid nitrogen probe. The muscle layer and skin were closed with 3-0 vicryl sutures. The rats were postoperatively monitored for 4 h. Intramuscular Penlog XL (0.3 ml, 150,000 U/ml benzathine penicillin G and 150,000 U/ml procaine penicillin G) and subcutaneous buprenorphine (0.01–0.05 mg/kg body wt) were given after surgery.

Cell Preparation for Transplantation

After cell culture medium was removed, SMCs were washed with 4 ml of PBS for three times. The cultured cells were detached from the cell culture dish and from each other by adding 3 ml of 0.05% trypsin-EDTA in PBS and incubating at 37°C for 3 min. Ten milliliters of cell culture medium were added, and the cell number was counted. Cell suspensions containing 2 \times 10^6 IGF-1-transfected cells, vector-transfected cells, or untransfected cells were centrifuged and resuspended in 40 μl serum-free culture medium for transplantation.

Cell Transplantation

Three weeks after scar generation, the animals were randomly divided into three groups. The rats were anesthetized and ventilated as previously described in Myocardial Scar Generation. The myocardial scar was exposed through a midline sternotomy. A cell suspension (2 \times 10^6 cells) was injected into the center of the scar with an insulin syringe. The injection site was sealed with a purse string suture. Cyclosporine was not administered to any of the animals because we have previously demonstrated that syngeneic cell transplantation is associated with very little rejection and excellent engraftment (10, 28). The incision was closed with 3-0 vicryl sutures. The rats were postoperatively treated in the same way as previously described in Myocardial Scar Generation.

One week after cell transplantation, the transplanted area was harvested for measurement of IGF-1 and VEGF levels (n = 6/group) as described above. Cell survival was detected using Y chromosome real-time PCR (n = 6/group). For histological studies, the hearts were fixed in formalin. TdT-mediated dUTP nick-end labeling (TUNEL) staining was employed to identify apoptotic cells (n = 6/group), and blinded microscopy was used to calculate blood vessel density (n = 6/group).

Bcl2-to-β-Actin Levels in Scar Area

Bcl2 protein levels in the transplanted area (n = 6/group) were measured by a slot blot assay (monoclonal anti-mouse Bcl2, Sigma). Levels of β-actin were estimated with a monoclonal anti-human β-actin antibody (Sigma) as an internal standard. The ratio between Bcl2 and β-actin was used as an index of implanted cell apoptosis.

TUNEL Staining

The cells to be transplanted were labeled with Cell Tracker Green 5-chloromethylfluorescein diacetate (Molecular Probes; Eugene, OR) by incubating the cells with 20 μM of it in serum-free medium for 30 min. One week after cell transplantation, the rat hearts were harvested, fixed for 12 h, and sectioned into 10-μm-thick slides. Samples from

Fig. 4. More vascular structures were found when homogenates from IGF-1-transfected cells (A, ×100) were added to endothelial cell cultured on growth factor-reduced Matrigel for 18 h than homogenates from vector-transfected (B, ×100) or untransfected cells (C, ×100). The mean length of vascular structures was greater (D) in the IGF-1-transfected group compared with the other two groups (*P < 0.05).
the infarct region were treated with toluene and rehydrated through graded alcohol to water. After pretreatment with 0.4% pepsin in 0.01 N HCl, pH 2.0 for 5 min at 42°C, the sections were incubated in a solution containing dATP, dCTP, dGTP, biotin-11-dUTP, and Kle- now DNA polymerase in a moist chamber at 42°C for 1 h. After being rinsed in PBS, sections were incubated in streptavidin conjugated to Cy-3 for 30 min. Slides were counterstained for 5 min in 4,6-diaminidino-2-phenylindole (DAPI) to stain the nuclei and covered. The samples were photographed and assessed with a Nikon Eclipse TE200 microscope. The surviving implanted cells had a green fluorescence, the apoptotic and necrotic cells had a red or yellow fluorescence, and the nuclei containing DAPI had a blue fluorescence.

Vessel Density in Scar Area

Vascular density in the implanted region of the hearts (n = 6/group) was evaluated 1 wk after cell transplantation. The fixed tissues were sectioned and pretreated as described in TUNEL Staining. After pepsin treatment, endogenous peroxidase and biotin activities were blocked with 3% hydrogen peroxidase and an avidin/biotin blocking kit, respectively, for 10 min. The tissue sections were incubated with antibodies against von Willebrand factor at 1:2,000 and smooth muscle α-actin antibody at 1:200 for 1 h (DakoCytomation; Mississauga, Ontario, Canada) as we previously described (10, 32). After being washed, the sections were incubated with secondary antibodies conjugated with peroxidase for 30 min, followed by horse-radish peroxidase-conjugated ultrastreptavidin label reagent. The sections were developed with freshly prepared NovaRed solution for 5–10 min and counterstained with hematoxyline. Five fields from each section were randomly selected, and the number of blood vessels was counted in each field by a blinded observer using a Nikon Eclipse TE200 microscope. The number of blood vessels per high power field (0.6 mm²) was averaged to reflect the vascular density.

Cell Survival Quantification

The number of surviving transplanted male cells in the female recipients was estimated by the real-time PCR technique to quantify the number of Y chromosomes (20). Genomic DNA (n = 6/group) was isolated from the transplanted area for each of the three groups. Two microliters of DNA were used as a template for quantitative real-time PCR of the Y chromosome SYBR gene as a measure of the number of surviving male cells using the AB PRISM 7900HT Sequencing Detection System. The standard curve was created by mixing 0, 10, 100, 1,000, 10,000, 100,000, and 1,000,000 male SMCs together with female heart tissues.

Statistical Analysis

All data are expressed as means ± SE and were analyzed with SAS software (SAS Institute; Cary, NC). The three groups were compared by ANOVA. For variables measured at multiple times, two-way
ANOVA was employed to simultaneously assess the effects of time and group assignment. If the $P$ value associated with the $F$-ratio was statistically significant ($P < 0.05$), then a multiple-range test (Duncan’s or Tukey’s) was employed to specify the difference.

RESULTS

In Vitro Studies

Cell preparation and gene transfection. SMCs isolated from the rat aorta were expanded in culture (Fig. 1A). At least three passages were required to obtain adequate cell numbers for cell transplantation, and, therefore, passage 3 cells were used exclusively for the in vitro and in vivo components of this study. The purity of the SMCs at passage 3 was 94 ± 4% as identified by antibodies against smooth muscle $\alpha$-actin (Fig. 1B). More than 90% of the cells continued to stain positively 7 days after gene transfection. The plasmid gene was introduced into cultured SMCs with a transfection efficiency of 20 ± 5% (Fig. 1C), and the gene was constitutively expressed for at least 3 wk (Fig. 1D).

The expression of IGF-1 was significantly elevated in the gene-enhanced SMCs compared with both vector and cell controls (Fig. 2). Specifically, IGF-1 mRNA expression was greater ($P < 0.05$ for all groups, $n = 10$/group) in the gene-enhanced cells as early as 3 days after transfection, and the increase was sustained for at least 14 days after transfection (Fig. 2A). Corresponding to the gene expression pattern, both intracellular (Fig. 2B) and secreted (Fig. 2C) protein levels of IGF-1 were greater in the gene-enhanced cells compared with vector and cell controls as early as 3 days after transfection ($P < 0.05$ for all groups, $n = 6$/group). IGF-1 mRNA and protein expression were not different between vector and cell controls at any time point.

Because IGF-1 is a potent growth factor with diverse downstream effects, we next assessed the ability of IGF-1 gene enhancement to upregulate VEGF gene expression. IGF-1 transfection increased VEGF gene expression (mRNA levels) as well as intracellular and extracellular VEGF protein levels compared with vector and cell controls, which were not different from each other (Fig. 3). The differences in mRNA ($P < 0.01$ for all groups, $n = 10$/group) and protein levels ($P < 0.01$ for all groups, $n = 6$/group) were evident at 7 and 14 days after transfection and indicated an important relationship between the expression of these two growth factor genes.

Angiogenesis. Because IGF-1 gene enhancement increased VEGF expression, the ability of these cells to stimulate angiogenesis was evaluated 7 days after gene transfection. Greater capillary formation was found in the IGF-1-transfected group (Fig. 4) compared with both control groups ($P = 0.005$ and $P = 0.001$, $n = 6$/group). In contrast, there was no difference in the stimulation of angiogenesis between vector and cell control groups ($P = 0.345$).

Cell proliferation and survival. Although two million cells were seeded into culture dishes, cell numbers in IGF-1 and vector groups were 1.28 ± 0.08 x 10^6 and 1.27 ± 0.08 x 10^6 cells 3 days after transfection, which were significantly lower than control group, 2.58 ± 0.19 x 10^6 cells ($n = 6$/group). On days 7 and 14, cell numbers in the IGF-1 group increased to 2.87 ± 0.16 x 10^6 and 10.20 ± 1.07 x 10^6 cells, respectively. In the vector group, the cell numbers increased to 2.56 ± 0.36 x 10^6 and 7.98 ± 1.04 x 10^6 cells, respectively. In the control group, the cell numbers increased to 4.01 ± 0.27 x 10^6 and 9.91 ± 1.11 x 10^6 cells, respectively. The rate of SMC expansion in culture was increased ($P = 0.013$ and $P = 0.042$, $n = 6$/group) in the IGF-1-transfected cells (8.9 ± 1.1 x 10^6 cells) compared with both the vector-transfected (6.7 ± 1.0 x 10^6 cells) and cell control (7.3 ± 1.1 x 10^6 cells) groups from day 3 to 14 after gene transfection.

We then assessed whether IGF-1 gene enhancement limited SMC apoptosis after ischemia and reperfusion. After reperfusion, more cells survived (as indicated by fewer trypan blue-stained cells) after IGF-1 transfection compared with both vector and cell control groups ($P = 0.001$ and $P = 0.001$, $n = 6$/group; Fig. 5, A–D). Caspase-3 activity in the IGF-1-transfected cells was lower ($P = 0.001$ and $P = 0.001$, $n = 6$/group; Fig. 5E) than in either control groups.

In Vivo Studies

Cell transplantation and gene expression. IGF-1-transfected cells, vector-transfected cells, and control cells were injected into myocardial scar tissue. Cell transplantation resulted in engraftment in all three groups at 1 wk after cell transplantation. In the transplanted region, hearts that received IGF-1-transfected cells had higher protein levels of IGF-1 ($P = 0.048$ compared with vector control and $P = 0.015$ compared with cell control, $n = 6$/group; Fig. 6A) and VEGF ($P = 0.003$ and $P = 0.001$, $n = 6$/group; Fig. 6B).

Assessment of apoptosis and angiogenesis after cell transplantation. One week after cell transplantation, there were no implanted cells in the nontransplanted myocardial scar tissue.
Fewer apoptotic cells were found by TUNEL staining in the myocardial scar transplanted with IGF-1 transfected cells (Fig. 7C) compared with both controls (Fig. 7D and E). In addition, the ratio of Bcl2 to β-actin in the transplanted area was greater (Fig. 7F) in the IGF-1-transfected group (*P < 0.05).

Assessment of engraftment. Although the number of cells injected (2 x 10^6 cells) was the same in all three groups, more extensive engraftment was achieved with the IGF-1-transfected cells than the vector or cell controls. One week after cell transplantation, the number of surviving cells with Y chromosomes as detected by the real-time PCR was greater (P = 0.012 and P = 0.004, n = 6/group) in the IGF-1-transfected group compared with the two control groups (Fig. 9).

DISCUSSION

Both experimental animal and preliminary clinical investigations have demonstrated that cell transplantation improved global (15, 28, 33) and regional (7, 8, 21, 27) function after myocardial injury. In support of the causative role for the transplanted cells in mediating these benefits, the extent of functional improvement was proportional to the number of cells injected (21). Unfortunately, only a small percentage of the injected cells survived and engrafted in the infarct region, and it is believed that cell loss significantly limited the benefits of this experimental therapy (20). The mechanisms for cell loss were multifactorial. A significant proportion of cells were injured during injection while others likely perished as a result of ischemia or apoptosis in the injured myocardium. Muller-Ehmsen and colleagues (20) found that the length and orientation of the needle influenced survival after implantation. Sakakibara and colleagues (25) showed that pretreatment of the infarct region with fibroblast growth factor improved perfusion, cell engraftment, and functional recovery. We (32) have previously reported that VEGF gene enhancement increased the angiogenesis associated with cell transplantation. In the present study, we found that IGF-1 gene enhancement of donor cells increased angiogenesis, stimulated cell proliferation, and suppressed apoptosis. Although this study provided preliminary evidence of efficacy, subsequent studies in a large animal infarcted model will be required to determine whether IGF-1 gene transfection will improve perfusion and augment functional recovery.

We selected the IGF-1 gene to enhance cell transplantation. IGF-1 is a potent growth hormone capable of inducing cell proliferation, limiting apoptotic cell death, and attenuating maladaptive extracellular matrix remodeling in the failing heart.
Because cell transplantation and the administration of exogenous IGF-1 provided independent benefits for the failing heart in previous studies (14, 15), in this study we hypothesized that the combination of IGF-1-enhanced SMC transplantation would have a synergistic effect on improving cardiac performance of the infarcted heart. The advantage of using genetically modified implanted cells to overexpress IGF-1 in the myocardium compared with the administration of exogenous IGF-1 protein or gene are two-fold. First, gene-enhanced cell transplantation enabled a localized and sustained delivery of IGF-1 to the infarct area. Second, the gene-enhanced cells released VEGF and possibly other factors, which produced a more profound angiogenesis than those associated with the delivery of VEGF or IGF-1 alone.

The results of our study indicated that IGF-1-transfected SMCs were capable of a robust production and local secretion of IGF-1 both in culture and after cell transplantation into myocardial scar tissue. The in vitro studies indicated that enhanced IGF-1 expression decreased caspase-3 activity, whereas the in vivo studies showed that the ratio of Bcl2 to β-actin levels increased 1 wk after cell transplantation. These data suggested that the IGF-1 gene enhanced engraftment possibly by limiting cell apoptosis. Enhanced IGF-1 expression in culture also resulted in a more rapid cell expansion. Although its beneficial effect in vivo has not been identified, the rapid cell expansion should facilitate clinical cell transplantation therapies. While the present study demonstrated that the IGF-I gene was expressed for 2 wk in vitro and 1 wk in vivo, further studies will be required to determine the optimal duration of gene expression to enhance the transplant efficiency in preventing heart failure.

IGF-1 gene transfection might have further improved cell engraftment by inducing a more profuse angiogenic response than the transplantation of unaltered cells. The IGF-1 and VEGF gene expression were coupled in cultured SMCs (1, 19), and this dual regulation resulted in a more robust secretion of angiogenic factors with IGF-1 gene enhancement. We found that the secreted VEGF was bioactive because the transfected cell homogenates stimulated cultured endothelial cells to form vascular-like structures in vitro. In addition, blood vessel density in the myocardial scar tissue implanted with IGF-1-transfected cells was greater than both control groups. Given the intimate relationship between vascular density or perfusion and cell survival, it was likely that the enhanced VEGF-
induced angiogenesis contributed to the improved engraftment of the IGF-1 transfected cells in the failing heart, which could improve heart function after a myocardial infarction.

Cell engraftment was confirmed by evaluating the survival rate of implanted cells using real-time PCR to measure the Y chromosome of the implanted male cells. The technique was described by Muller-Ehmsen and colleagues (20), who found that the signal was rapidly eliminated from the myocardium when lethally injured cells were injected into the heart. Real-time PCR may be the most sensitive and specific method of quantifying cell survival after transplantation. We found that 20% of the implanted cells survived for 1 wk, similar to the results previously reported (20). Importantly, IGF-1 transfection increased cell survival to 33%. Despite a transfection efficiency of only 20%, the transfected cells had an 11% greater survival, which represented a 50% increase in cell survival. Increasing the number of cells that survive implantation should improve cardiac functional recovery significantly according to the calculations proposed by Pouzet and colleagues (21). However, further studies will be required to determine the specific contribution of enhanced IGF-1 expression on cardiac structure and function after cell transplantation in failing hearts.

Skeletal myoblast transplantation has been shown to improve regional and global function (7, 8, 18, 21, 27), although the myoblasts did not beat synchronously with the recipient heart. Because beating muscle cells were not required, SMCs could be ideally suited to prevent cardiac failure for the following reasons (15). SMCs are easily obtained from a peripheral vein. The SMCs can be readily expanded in vitro, and their growth will be minimally affected by donor age. Also, SMCs respond to hemodynamic stresses by hypertrophy and hyperplasia (15, 33), which will strengthen the infarct. Given these unique properties, we selected SMCs in this study to modify the remodeling process after a myocardial infarction. In addition, SMCs have been previously demonstrated to engraft in the myocardial scar, induce angiogenesis, modify matrix remodeling, increase elasticity, and improve global ventricular function (15, 33). Enhancement of the benefits of SMC transplantation with gene transfection has not been previously assessed.

Although this study suggested that IGF-1 enhancement improved cell engraftment, more definitive studies will be required to determine whether these modified cells can improve cardiac structure and function of the failing heart. We evaluated some of the mechanisms through which cell engraftment might be improved, but we did not provide conclusive evidence that any single mechanism was essential to improve cell survival. For example, the incremental benefit of limiting apoptosis compared with enhancing angiogenesis will only be determined in future studies. Beneficial effects of transfection of IGF-1 gene into other cell types, such as endothelia cells or myoblasts, on myocardial regeneration also need to be studied. Pretreatment of the ischemic myocardium by IGF-1 transfection might further enhance transplanted cell survival. Although the effects of cell transplantation have been demonstrated to extend beyond the injured region (32, 33), the benefit of gene-enhanced cell transplantation to the nonimplanted area will require subsequent investigations.

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GRANTS

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