Stem Cell Factor Gene Transfer Improves Cardiac Function After Myocardial Infarction in Swine

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Background—Stem cell factor (SCF), a ligand of the c-kit receptor, is a critical cytokine, which contributes to cell migration, proliferation, and survival. It has been shown that SCF expression increases after myocardial infarction (MI) and may be involved in cardiac repair. The aim of this study was to determine whether gene transfer of membrane-bound human SCF improves cardiac function in a large animal model of MI.

Methods and Results—A transmural MI was created by implanting an embolic coil in the left anterior descending artery in Yorkshire pigs. One week after the MI, the pigs received direct intramyocardial injections of either a recombinant adenovirus encoding for SCF (Ad.SCF, n=9) or β-gal (Ad.β-gal, n=6) into the infarct border area. At 3 months post-MI, ejection fraction increased by 12% relative to baseline after Ad.SCF therapy, whereas it decreased by 4.2% (P<0.004) in pigs treated with Ad.β-gal. Preload-recruitable stroke work was significantly higher in pigs after SCF treatment (Ad.SCF, 55.5±11.6 mmHg versus Ad.β-gal, 31.6±12.6 mmHg, P=0.005), indicating enhanced cardiac function. Histological analyses confirmed the recruitment of c-kit+ cells as well as a reduced degree of apoptosis 1 week after Ad.SCF injection. In addition, increased capillary density compared with pigs treated with Ad.β-gal was found at 3 months and suggests an angiogenic role of SCF.

Conclusions—Local overexpression of SCF post-MI induces the recruitment of c-kit+ cells at the infarct border area acutely. In the chronic stages, SCF gene transfer was associated with improved cardiac function in a preclinical model of ischemic cardiomyopathy. (Circ Heart Fail. 2015;8:167-174. DOI: 10.1161/CIRCHEARTFAILURE.114.001711.)

Key Words: angiogenesis ▪ gene therapy ▪ myocardial infarction ▪ paracrine factor

Ischemic cardiomyopathy is one of the major causes of heart failure (HF). It is characterized by poor perfusion, chronic loss of cardiomyocytes, scar formation, and adverse ventricular remodeling. Recently, cell therapy has received significant attention because of its potential for regenerating cardiomyocytes and replacing scar tissue with new cardiomyocytes. Despite the initial expectations that cardiomyogenesis would involve by introducing exogenous stem cells into the ischemic heart, most studies have failed to show transdifferentiation of injected cells into cardiomyocytes. It is now accepted that paracrine effects play a major role in the documented functional improvements after cell therapy in HF patients. Preclinical investigations have identified several paracrine factors that contribute positively to cardiac repair after myocardial infarction (MI), such as vascular endothelial growth factor, stromal cell-derived factor-1, insulin like growth factor-1, hepatocyte growth factor, and stem cell factor (SCF). SCF is a ligand of c-kit, and c-kit is a receptor tyrosine kinase. SCF binding to c-kit leads to receptor dimerization and activation of multiple downstream signaling pathways related to cell recruitment, differentiation, angiogenesis, and survival.

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More recently, Bolli et al showed that injecting c-kit+ stem cells in patients with ischemic cardiomyopathy resulted in dramatic improvement of left ventricular (LV) function in these patients. This study demonstrated the clinical importance of this cell type to the reparative process. Although their approach is capable of using the c-kit+ cells from the same patient (using the tissue obtained during the bypass surgery or from biopsy samples), ex vivo harvesting process limits its utility for acute to subacute disease applications. In these
settings, gene delivery may be useful as gene expression can
be upregulated relatively quickly.

Previous studies in rodents have provided evidence that
SCF increases the number of c-kit+ stem cells in the injured
heart and contributes to improved cardiac function and sur-

dv1, 7, 12, 14, 15 These studies have also identified valuable
insights, including the interaction between SCF and c-kit+
stem cells, mechanism of cardiac repair, and origin as well
as type of the c-kit+ cells involved. The important ques-
tion remaining is whether SCF treatment is also efficacious
in human ischemic heart diseases because there are large
physiological and structural differences between rodents and
humans. Therefore, the goal of our study was to validate the
therapeutic efficacy of SCF gene transfer in a clinically rele-
vant animal model of ischemic HF. We hypothesized that local
overexpression of membrane-bound human SCF at the infarct
border zone after MI will improve cardiac function in a swine
model of MI. Using invasive and noninvasive means of LV
functional and structural assessments, we report the beneficial
effects of SCF gene transfer on post-MI heart with focus on the
LV hemodynamics.

Methods

Study Protocol

The experimental protocols complied with the National Institutes
of Health, Guide for the Care and Use of Laboratory Animals, and
standards of United States regulatory agencies, as well as Position
of the American Heart Association on Research Animal Use.16 Protocols
were approved by the Institutional Animal Care and Use Committee
at the Icahn School of Medicine at Mount Sinai. A total of 22 female
Yorkshire pigs were enrolled in our chronic study. An MI was created
day 0, and the gene transfer was performed 1 week after the MI cre-
ation (1 Wk). The animals were caged housed in the Mount Sinai ani-
mal facility and underwent cardiac function assessments at 6 weeks
(6 Wk) and 3 months (3 Mo) post-MI. The study was performed in a
randomized manner and 3-dimensional echocardiographic (3DE) im-
ages were analyzed by a blinded investigator. We aimed to show im-
proved LV ejection fraction (EF) in the SCF treatment group assessed
by left ventriculography, which we have previously shown to accu-
trately estimate systolic dysfunction after MI.17 Accordingly, hemody-
namic measurements with a pressure catheter, left ventriculography,
and 3D measurements were performed at 1 Wk (immediately before
gene transfer), 6 Wk, and 3 Mo. Additionally, pressure–volume loop
relationships were assessed at 1 Wk and 3 Mo. In addition to the main
chronic study, adenovirus encoding for SCF with green fluorescent
protein (Ad.SCF, 2.0×10¹³ viral genomes, n=4) and β-gal with green
fluorescent protein (Ad.β-gal, 2.0×10¹³ viral genomes, n=2)-injected pigs
were euthanized at 1 week after the virus injection to evaluate the
efficacy of adenoviral gene transfer.

Animal Model and Gene Transfer

An MI was created as previously described with minor modifica-
tion.18 Briefly, pigs (18–21 kg) were sedated with Telazol (tiletamine/
zolazepam; 8.0 mg/kg) and Buprenorphine (0.6 mg) and anesthetized
with propofol (8–10 mg/kg/hour). Arterial access was obtained by
puncturing the femoral artery and a coronary balloon was delivered
to the proximal left anterior descending artery. Complete occlusion
of the left anterior descending artery was confirmed by angiogram
and maintained for 60 minutes. The balloon was then deflated and
an embolic coil was implanted just distal to the first diagonal branch.
Animals were allowed to recover after the confirmation of hemody-
namic stability. Gene transfer was performed 1 week after the MI cre-
ation by direct intramyocardial injection. Under sterile conditions
and inhalational isoflurane (2.0% to 2.5%) anesthesia, a left thoracotomy
was performed in the fourth intercostal space. Ad.SCF or Ad.β-gal
was delivered into the border area of the LV infarct and normal tissues
through an opened pericardium where a total of 15 to 20 injections
(50 μL/site) were made ≈0.5 cm apart, although avoiding the epicar-
dial vessels. After virus injections, the chest was closed and blood and
air were removed via a vacuumed thoracic drain.

Animals

Four of the 22 pigs for chronic study died within 24 hours after the MI
creation. Three pigs with a large amount of bloody pericardial effusion
were excluded because of the major influence for assessing baseline
cardiac function and difficulty to differentiate infarct border because
of abundant fibrocollagenous tissue around the infarct. Therefore, 9
pigs injected with Ad.SCF and 6 pigs with Ad.β-gal were included in
the chronic study. During the follow up, 1 pig each from Ad.SCF
group (11%) and Ad.β-gal group (17%) died because of HF.

Statistical Analysis

All values are reported as mean±standard deviation except where in-
dicated. Study sample size was determined by using an alpha value of
0.05, a power of 80% to detect a 5% absolute increase of left ventricular
ejection fraction (LV EF) in the assumption of 3% standard deviation
with 0.67 controls per treatment animal. Comparison between the 2
groups was performed by Student’s t test. Comparisons between repeat-
ed values at 1 Wk and at 3 Mo were done by repeated measures analy-
sis of variance to test the statistical significance of the treatment/time
interaction. Intra-class correlation coefficient was analyzed to assess
the agreement of 2 repeated measurements for 3DE volume analysis. A
P value of <0.05 was considered statistically significant.

Results

Gene Transfer Efficacy and c-Kit+ Cell Recruitment

To examine the subacute effects of SCF gene transfer within
the myoicardium, 6 extra pigs were euthanized 1 week after the
virus injection aside from the chronic study. Successful gene
transfer was inferred from green fluorescent protein expres-
sion along the needle track after the injections (Figure 1A).
In the pigs injected with Ad.SCF, clusters of c-kit+ cells were
found at the injection sites (median 340 cells/mm², 25th to
75th percentile; 131–1277 cells/mm²; Figure 1B), whereas
almost no c-kit+ cells were detected in the control pig heart.
Western blot analysis confirmed the increased expression of
SCF at the injection sites in the pigs treated with Ad.SCF
(Figure 1C).

Improvement of Cardiac Function

There was a 12% relative increase in LV EF at 3 Mo (absolute
cchange, +4.9%) in the Ad.SCF group compared with before
gene transfer, whereas it decreased by 4.2% (absolute change,
−1.6%; P=0.004) in the Ad.β-gal group (Figure 2; Table 1).
With respect to the 3DE analysis, image quality was subobjec-
tional in 13% of the data sets because of the lung overlap pre-
sumably from postsurgical lung adhesions. However, volume
data were assessable in all the measurements with intraclass
correlation coefficient of 0.992. Repeated measures analysis
of variance revealed statistically significant improvements of
LV EF after Ad.SCF therapy (Table 2) in 3DE analysis as
well. Furthermore, stroke volume index assessed by 3DE
also increased significantly after SCF treatment (Table 2).
Pressure–volume loop analyses exhibited similar end-systolic
pressure–volume relationship between the groups at 1 Wk;
however, the slopes in the Ad.SCF group were steeper at 3
Mo (Table 3, Figure 3). Though the slope of the end-systolic pressure–volume relationship did not reach a statistically significant difference, preload-recruitable stroke work, another load-independent measure of cardiac contractility, was found to be significantly higher after Ad.SCF treatment (Table 3). Other parameters of systolic function were consistent with these findings, including higher change in $dP/dt$ maximum (Figure 2), higher LV maximum pressure, and increased cardiac index (Table 2), but they did not reach statistical significance. In contrast, diastolic functional parameters, including $dP/dt$ minimum, tau, and end-diastolic pressure–volume relationship did not exhibit any noteworthy trends between the treatment groups (Table 2).

### Remodeling and Infarct Size

The growth of the animals was similar in both experimental groups. The size of the heart was not different between the groups at the time of gene transfer or at follow-up (Table 1 and 2). Reflecting the size of the heart, ventricular weight to body weight ratios at 3 Mo did not differ significantly between the groups. Likewise, the scar size was similar in both groups (Figure 4A). Wall motion score index, the best available estimate of infarct size in vivo with echocardiography, showed a modest correlation to the actual scar size at 3 Mo ($r=0.66$, $P=0.007$). Wall motion score index was similar between the groups at 1 Wk, which suggests similar scar size before the gene transfer (Figure 4B).

![Figure 1](image1.png) **Figure 1.** Gene transfer efficacy and recruitment of c-kit+ cells 1 week after gene transfer. A, Infected area is clearly distinguishable by GFP expression. Blue, DAPI-stained nuclei; green, GFP. B, Confocal image represents c-kit membrane staining (red). Clusters of c-kit+ cells were found at the injection sites after SCF gene transfer. Blue, DAPI-stained nuclei; red, c-kit; green, α-sarcomeric actin. C, SCF, PCNA, and P-H3 expression by Western blotting. Expression of SCF ($P=0.001$) as well as proliferation proteins (PCNA, $P=0.08$; P-H3, $P=0.008$) were increased in the SCF treated pigs.*$P<0.05$. GFP indicates green fluorescent protein; PCNA, proliferation cell nuclear antigen; P-H3, phospho-histone h3, and SCF, stem cell factor.

![Figure 2](image2.png) **Figure 2.** Percent change of functional and volumetric parameters from 1 week (before gene transfer) to 6 weeks and to 3 months. Ad.SCF group showed significant improvement in EF ($P=0.004$) and trends towered improved $dP/dt$ max ($P=0.06$) at 3 month. There were no statistically significant differences in EDVI ($P=0.95$), ESVI ($P=0.35$), and SVI changes ($P=0.11$) evaluated by left ventriculogram. *$P<0.05$, †$P=0.06$. EDVI indicates end-diastolic volume index; EF, left ventricular ejection fraction; ESVI, end-systolic volume index; SVI, stroke volume index; and $dP/dt$ max, $dP/dt$ maximum.
that a few cardiomyocytes are undergoing DNA replication. cell types undergoing cell cycling. Figure 

To further characterize the subacute effect of SCF gene transfer, we performed immunohistochemical analyses on infarct border tissue 1 week after the gene transfer. To inquire whether the c-kit+ cells are of bone marrow origin, these cells were costained with CD45 (Figure 6A). There were a few isolated cells both positive for c-kit and CD45; however, most of the clustered c-kit+ cells were negative for CD45, as well as vascular endothelial growth factor receptor 2 (Figure I in the Data Supplement). Because Western blot analyses revealed increased proliferation markers in the Ad.SCF group, we used double labeling techniques to identify specific cell types undergoing cell cycling. Figure 6B clearly shows that a few cardiomyocytes are undergoing DNA replication. Proliferating cell nuclear antigen staining revealed that ~1 out of 5 proliferating cell nuclear antigen positive cells were cardiomyocytes. To evaluate the effect of SCF on cell survival, TUNEL staining was performed. A significant decrease of TUNEL-positive cardiomyocytes was detected after Ad.SCF therapy (Figure 6C, P<0.001).

**Discussion**

The present study demonstrated that the overexpression of membrane-bound human SCF by adenoviral gene transfer in the subacute phase of MI improves cardiac systolic function 3 months after MI. Using a porcine model of MI induced LV dysfunction, we validated the efficacy of SCF gene therapy in a clinically relevant model of HF. Our results are consistent with the positive effects of SCF treatment on cardiac function observed in small animals and supports the possible translation of this therapeutic approach to the clinic.

The improvement in LVEF was significantly better after Ad.SCF treatment assessed by left ventriculography, and this observation was confirmed by blinded analyses of 3DE. Although they did not reach statistical significance, higher dP/dt maximum and steeper slope of end-systolic pressure–volume relationship after Ad.SCF treatment support the conclusion of improved cardiac function through this gene therapy–based cytokine therapy. Moreover, preload-recruitable stroke work increased significantly in the Ad.SCF group. Preload-recruitable stroke work is shown to be independent of heart size,20 more reproducible compared with end-systolic pressure–volume relationship, and afterload independent.21 Thus, we think it is valid to rely on preload-recruitable stroke work as a key indicator of SCF gene transfer efficacy because the heart size differs between individual animals. Furthermore, afterload may differ depending on the severity of HF, as well as sensitivity to the anesthetic agents between the animals.

### Table 1. Baseline Left Ventriculogram Data and dP/dt max

<table>
<thead>
<tr>
<th></th>
<th>Ad.β-gal</th>
<th>Ad.SCF</th>
</tr>
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<tbody>
<tr>
<td>EF, %</td>
<td>42.4±6.2</td>
<td>40.2±5.1</td>
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<tr>
<td>EDVI, mL/m²</td>
<td>115.9±30.7</td>
<td>106.6±14.8</td>
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<td>ESVI, mL/m²</td>
<td>67.4±22.6</td>
<td>64.2±15.1</td>
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<tr>
<td>SVI, mL/m²</td>
<td>48.3±10.2</td>
<td>42.3±3.0</td>
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<tr>
<td>dP/dt max, mm Hg/s</td>
<td>1666±373</td>
<td>1778±147</td>
</tr>
</tbody>
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*dP/dt max indicates dP/dt maximum; EDVI, end-diastolic volume index; EF, ejection fraction; ESVI, end-systolic volume index; and SVI, stroke volume index.

Table 2. Temporal Transition of Hemodynamic and 3D Echocardiographic Parameters

<table>
<thead>
<tr>
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<th>1 Wk</th>
<th>6 Wk</th>
<th>3 Mo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>20.8±1.3</td>
<td>20.8±1.2</td>
<td>28.2±2.3</td>
<td>28.0±2.0</td>
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<tr>
<td>Pressure catheter</td>
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<tr>
<td>P max, mm Hg</td>
<td>93.1±10.4</td>
<td>91.3±10.5</td>
<td>99.1±3.9</td>
<td>105.5±23.0</td>
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<tr>
<td>EDP, mm Hg</td>
<td>19.6±5.0</td>
<td>19.7±6.8</td>
<td>18.5±6.1</td>
<td>14.8±3.9</td>
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<tr>
<td>dP/dt min, mm Hg/s</td>
<td>-1196±232</td>
<td>-1174±338</td>
<td>-1395±419</td>
<td>-1475±620</td>
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<tr>
<td>tau, ms</td>
<td>64.5±17.3</td>
<td>61.0±18.7</td>
<td>73.3±8.3</td>
<td>71.3±17.7</td>
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<tr>
<td>HR, beats per minute</td>
<td>90.9±23</td>
<td>82.6±26</td>
<td>61.3±12.5</td>
<td>72.3±18.8</td>
</tr>
<tr>
<td>Right heart catheter</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CI, L/min/m²</td>
<td>4.3±1.0</td>
<td>3.6±0.9</td>
<td>3.3±0.4</td>
<td>3.5±0.8</td>
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<tr>
<td>3D echocardiography</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>EF, %</td>
<td>50.1±9.5</td>
<td>44.7±2.7</td>
<td>47.4±9.5</td>
<td>48.7±8.7</td>
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<tr>
<td>EDVI, mL/m²</td>
<td>124.8±12.1</td>
<td>117.2±14.9</td>
<td>160.8±18.5</td>
<td>141.4±34.9</td>
</tr>
<tr>
<td>ESVI, mL/m²</td>
<td>61.5±8.8</td>
<td>65.0±8.9</td>
<td>84.4±16.6</td>
<td>74.2±28.8</td>
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<tr>
<td>SVI, mL/m²</td>
<td>62.3±7.8</td>
<td>52.7±7.8</td>
<td>78.7±22.4</td>
<td>66.8±13.1</td>
</tr>
</tbody>
</table>

CI indicates cardiac index; dP/dt min, dP/dt minimum; EDP, end-diastolic pressure; EDVI, end-diastolic volume index; EF, ejection fraction; ESVI, end-systolic volume index; HR, heart rate; Pmax, maximum pressure; and SVI, stroke volume index. P values are from repeated measures ANOVA.
We postulate that the functional improvement in our study is in part caused by enhanced angiogenesis and increased cell survival. Pigs treated with SCF showed increased angiogenesis at 3 Mo. The presence of small vessels with multiple layers of smooth muscle cells suggests functional angiogenesis. Increased vasculogenesis at 3 Mo after Ad.SCF treatment also indicates the important role of SCF in the formation of capillaries. In addition, although keeping in mind a small sample number, higher relative coronary flow at the infarct border (Figure II in the Data Supplement) supports functional angiogenesis by SCF treatment and the potential for increased cardiac perfusion. SCF overexpression also resulted in decreased apoptotic cardiomyocytes 1 week after the gene transfer as demonstrated by decreased TUNEL-positive cells.

To our knowledge, this is the first demonstration of SCF gene therapy efficacy in a large animal model of HF. Beneficial effects of SCF treatment have been previously shown in small animal models of MI. Systemic injection of SCF and granulocyte colony-stimulating factor after MI improved cardiac function together with other beneficial effects in mice. A single local injection of SCF protein into the peri-infarct with intravenous injections of c-kit+ cells increased homing of c-kit+ cells in a mouse MI model; however, failed to show functional improvement. In that study, the amount of c-kit+ cells found at the SCF injected sites was small, and the authors concluded that it was not enough to improve cardiac function. In contrast, repeated administration of SCF and stromal cell-derived factor-1 gene using a microbubble destruction method in rats improved cardiac function, increased angiogenesis, and decreased infarct size. Likewise, sustained expression of SCF by gene transfer or by injection of SCF-overproducing mesenchymal stem cells successfully improved cardiac function in rodents.

Our study is consistent with these previous reports, establishing the translation of SCF treatment from rodents to large animals. However, despite the dramatic reduction of infarct size detected in the smaller animals, we did not find a significant difference in the infarct size nor in the weight of the heart after Ad.SCF injection in swine. This may derive from the disparity in size; the pig heart is about a thousand times heavier, whereas the amount of virus used was only 5 to 10 times more than in the rat study.

Gene therapy and stem cell therapy are both new emerging fields in cardiac treatment. Our study allows for comparison of gene therapy to recent reports of cell-based SCF delivery. Although increased SCF levels and improved cardiac function were confirmed after stem cell injection into the myocardium, we chose gene transfer as a means of treatment in our study because augmenting the level of a specific paracrine factor can be achieved by gene therapy. The combination of various paracrine factors in cell therapy may provide synergic effects by involving multiple pathways; however, this complicates interpretation of the results, and the effects can sometimes be deleterious. For example, intra-coronary stem cell injection with granulocyte colony-stimulating factor improved cardiac function, but was accompanied by an increased risk of restenosis after coronary stenting. In contrast, gene transfer activates only the pathways involving the specified factor. Moreover, gene therapy has an advantage of potentially achieving a higher level of targeted protein expression compared with stem cell injection. Previously, Fazel et al reported tumor development after the injection of mesenchymal stem cells engineered to overexpress SCF. The authors concluded that the combination of mesenchymal stem cells with SCF overexpression was the probable cause, although a prolonged cell culture period may have affected the cell quality. Our method does not require exogenous cell injection and may be able to circumvent such complications with natural or modified exogenous cells.

Despite the improvement in systolic function, diastolic function did not differ between Ad.SCF and Ad.β-gal treatments. This may be attributed to the delivery method and the choice of vector. We found adhesions of the pericardium in all of the pigs, which may have impeded ventricular expansion, and varying degrees of inflammatory response were found at the infarct in both groups. Both adenovirus and direct intramyocardial injection have been reported to induce inflammation. Thus, although we have demonstrated the beneficial effects of SCF gene therapy for ischemic HF, a different route of gene delivery and a different vector may minimize the inflammatory response and maximize the effect of SCF gene transfer that we detected.

Encouraging results in early clinical trials that used c-kit+ cells have been recently reported. In the SCIPIO study, 20 patients who received intracoronary injections of c-kit+ cells had striking improvement in LVEF and significant reduction of infarct size.

Table 3. Pressure–Volume Loop Relationships

<table>
<thead>
<tr>
<th></th>
<th>1 Wk</th>
<th>3 Mo</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ad.β-gal</td>
<td>Ad.SCF</td>
</tr>
<tr>
<td>ESPVR Slope, mm Hg/mL</td>
<td>2.2±0.90</td>
<td>2.07±0.84</td>
</tr>
<tr>
<td>IV0 mL</td>
<td>−27.3±23.2</td>
<td>−22.1±27.7</td>
</tr>
<tr>
<td>PRSW Slope, mm Hg</td>
<td>35.4±12.4</td>
<td>31.5±5.9</td>
</tr>
<tr>
<td>EDPVR Slope, mm Hg/mL</td>
<td>0.69±0.46</td>
<td>0.88±0.42</td>
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</table>

ESPVR indicates end-diastolic pressure–volume relationship; PRSV, end-systolic pressure–volume relationship; PRSW, preload-recruitable stroke work; and IV0, volume–axis intercept.

P values are from repeated measures ANOVA.
≤12 months. The CADUCEUS trial used cardiosphere-derived cells that contain a mixed population, including c-kit progenitors, and also showed significant reduction in infarct size together with improved regional contractility. Furthermore, Hare et al reported a combination of c-kit cells and mesenchymal stem cells enhanced the effect of therapeutic efficacy. These studies highlight the promising potential of c-kit cells over other types of stem cells. Previous studies in rodents reported the migration of bone marrow–derived c-kit cells after SCF treatment in cardiac repair. Similarly, Fazel et al demonstrated that c-kit cells are increased after MI accompanied by increased SCF expression, and majority of c-kit cells found in the infarcted myocardium were from bone marrow using a bone marrow chimeric mouse. In contrast, our results do not support these findings because most of c-kit cells found at the injection sites were negative for CD45 as well as vascular endothelial growth factor receptor 2. The discrepancy may be explained by the differences in studied time points or type of species. This also highlights the importance of present study in large animals, which have much closer physiological profiles to human. Although the actual origin of c-kit cells in our study remains to be elucidated, these cells could be of cardiac origin as suggested by others. Thus, in addition to the recruitment of bone marrow–derived stem cells, SCF therapy may be activating the cardiac resident c-kit cells and enhancing the endogenous repair.

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Limitations
Our study in this large animal model has several limitations. The mechanism by which SCF induces functional improvement cannot be exactly assessed in this large animal model because the precise origin of the c-kit+ cells cannot be exactly assessed. Nevertheless, c-kit+ cell recruitment, apoptosis inhibition, and increased angiogenesis are consistent findings with previous studies in small animals, and we believe demonstrating the functional improvement in a large animal study is a critical step in translating SCF therapy to clinical application. Because our study design and the difficulty in endogenous cell labeling in large animals, the presence of cardiomyocyte regeneration was not demonstrated. Future study is required to determine the regenerative property of SCF gene therapy focusing on the fate of c-kit+ cells. Relatively short follow-up in view of treating patients is another common limitation of large animal studies. van der Spoel et al performed meta-analysis on the effect of cardiac stem cell therapy in large animals and reported that the effect of cell therapy declines after 8 weeks. In contrast, we found a consistent trend of improved systolic function at 6 Wk and at 3 Mo. It is notable that at these time points, the SCF overexpression is presumably minimal because of the transient nature of adenoviral gene transfer. However, longer term follow-ups are required to establish the full potential of SCF gene transfer as an efficacious approach to treat HF.

Conclusions
In a swine model of MI, gene transfer of SCF at the ischemic border area improved systolic function up to 3 months post-treatment. Our results advance the potential of SCF gene transfer as a future treatment option for ischemic HF.

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Disclosures
None.

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The importance of c-kit+ cells in cardiac regeneration has been established in clinical and preclinical studies. Stem cell factor (SCF) is the ligand for this progenitor cell population and contributes to cell migration, proliferation, and survival. In this study, we demonstrate the therapeutic efficacy of stem cell factor gene transfer in a large animal model of myocardial infarction-induced heart failure. We used gene transfer as a complementary approach to target this cell population by overexpressing the stem cell factor and successfully showed functional improvement when treating at the subacute phase of myocardial infarction. Specifically, stem cell factor gene delivery was accomplished via a recombinant adenovirus vector in a pig infarct border zone 1 week after the induction of a large anterior myocardial infarction. Stem cell factor gene transfer resulted in increased populations of c-kit+ cells at the gene delivery site shortly after the gene delivery, followed by increased cardiac performance at the chronic stages. Our results extend the previously reported efficacy of stem cell factor treatment in rodents to a more clinically relevant model, supporting the translation of this gene-based approach to cardiac regenerative therapy.
Stem Cell Factor Gene Transfer Improves Cardiac Function After Myocardial Infarction in Swine

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SUPPLEMENTARY MATERIAL

Supplementary Methods

Recombinant Adenoviruses

Ad.SCF was constructed using the AdEasy XL Adenoviral Vector System (Stratagene). The full length of SCF membrane isoform cDNA was cloned into the pShuttle-IRES-hrGFP-1 vector. The linearized shuttle vector was recombined in Escherichia coli strain BJ5183 with AdEasy, a serotype 5 first-generation adenoviral backbone. The recombinant viral backbones were transformed into 293 cells and grown at large scale. Adenoviruses were purified by double cesium chloride gradient. Ad-EGFP-β-gal (Ad.β-gal) adenovirus was used as a control. To confirm the predicted viability of recombinant viruses, in-vitro transfections of cultured cardiomyocytes were conducted for 8 hours at a multiplicity of infection 50 for Ad.β-gal and 100 for Ad.SCF for an additional 24 to 48 hours. A total of 1.5x10^{12} viral particles were injected per animal which was the equivalent of 1x10^{11} plaque forming units.

Cardiac function measurements

The pigs were prepared in the same manner as the MI creation. Under propofol anesthesia, percutaneous punctures were performed to obtain arterial and venous accesses. A 7-Fr Swan-Ganz catheter (Edwards-Lifesciences LLC, Irvine, CA USA) was advanced through the venous sheath up into the pulmonary artery, where cardiac output was measured by several
injections of a saline bolus until 3 or more stable measurements were obtained. A 7-Fr, 12-electrode, dual-field conductance catheter (Millar Instruments, Houston, TX) was advanced into the LV for assessment of LV pressure-volume relationships. Subsequently, an 11-Fr balloon catheter was advanced to the inferior vena cava for preload alterations. Data analysis was performed using iox2 (Emka Technologies, Falls Church, VA). The conductance catheter gain factor α was calculated as the ratio of conductance derived cardiac output to that measured by thermodilution. Parallel conductance was adjusted using the end-diastolic volume obtained from 3DE. Following the pressure-volume relationship measurement, left ventriculography was performed and analyzed as previously described (1).

A Philips ie-33 ultrasound system (Philips Medical Systems, Andover, MA, USA) was used to acquire echocardiographic data with a multi-frequency imaging transducer. Complete Doppler trans-thoracic echocardiographic studies were performed. Images were recorded during end-expiratory breath-hold in the standard LV apical view. 3DE datasets were acquired from 4 to 7 consecutive cardiac cycles in R-wave-triggered mode. Post-acquisition image analyses were performed offline using the Q-lab application (Phillips Medical Systems) by a single blinded investigator. LV volumes were calculated using 3D full-volume algorithms with semi-automated border detection. Analyses were performed for 2 different sequences and the average of 2 measurements was used for final data. The 3DE data were also used to evaluate changes in wall motion as flows. Wall motion score indices were
assigned depending on the segmental EF. A score of either 1 (normal regional EF>55%), 2 (hypokinesia: EF=33-55%), 3 (akinesia (EF=0-25%), or 4 (dyskinesia EF<0%) was given to each of the 17 segments and averaged.

**Histology**

At the end of the study, pigs were euthanized under deep anesthesia. Hearts were explanted and sectioned into 6 slices along the long axis. To quantify the infarction size by digital planimetry, 5 slices of heart tissue were immersed in 1% triphenyl tetrazolium chloride. The remaining slice was used for histological and protein analysis, and was divided into sections. Each sample was preserved in three different sub-samples respectively for histology, biodistribution, and immunohistochemistry: formalin, snap frozen, and snap frozen in optimum cutting temperature (OCT) compound. Frozen samples were sectioned into 3 layers: epicardium, myocardium, endocardium.

**Western blotting**

Cardiac tissue was minced and subsequently homogenized in radioimmunoprecipitation assay buffer containing a protease inhibitor cocktail (Sigma-Aldrich). Protein extracts (10 μg) were separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad), and probed with following antibodies specific for SCF (Cell signaling),phospho-histone H3 (Cell
signaling), and proliferation cell nuclear antigen (PCNA) (Cell signaling). Peroxidase-conjugated secondary antibodies (Sigma-Aldrich) and GAPDH (Sigma-Aldrich) protein loading control were also employed. Blots were developed with Super Signal West Pico (Pierce). Protein band densities were quantified by using quantity Image J software (NIH).

Immunohistochemistry

OCT-embedded blocks of myocardium were sectioned (8 μm) and mounted on positively charged microscope slides. These sections were fixed with 4% paraformaldehyde and treated with the antigen specific primary antibodies, followed by fluorescently labeled secondary antibodies specific for the primary antibody. Images were acquired using Zeiss LSM510 META confocal microscope. The heart sections from 1 week post gene transfer were treated with CD117, CD45, α-sarcomeric actin, PCNA, and VEGFR2 specific antibodies to evaluate their expression. Apoptosis rate was also assessed in the sections 1 week after SCF therapy using TUNEL technique by direct immunofluorescence with the ApopTag Red in situ apoptosis detection kit (Chemicon International Inc, Temecula, CA). Tissue sections were counterstained with Hoechst 33258 (1 μg/ml) (Sigma, St. Louis, MO). To quantify apoptosis, ten randomly selected microscopic fields per section were examined in the infarct border area of each section. The percentages of apoptotic cells were determined by counting the
TUNEL-positive nuclei relative to the total number of nuclei. Angiogenesis was assessed at 3Mo by measuring the number of smooth-muscle covered vessels (vessels/mm²) in the myocardial sections of infarct border area using a double staining technique for anti-α-SMA antibody and CD31 (both from Abcam, Cambridge, MA). Vasculogenesis was also evaluated by counting the density of capillaries (capillaries/mm²) in the infarct border area stained with isolectin IB4 (Sigma, St. Louis, MO). Only vessels <10 μm in diameter were taken into account to exclude venules and small arterioles.

Coronary flow measurement

After hemodynamic measurement, a guiding catheter was advanced and a coronary angiography was performed to confirm the status of coronary arteries. In two of Ad.SCF and one of Ad.β-gal pigs, regional coronary flows in border area and remote area were assessed. Regional coronary flow was measured as published previously(2). In brief, 2 x 10⁷ polystyrene fluorescent microspheres (15 μm; Interactive Medical Technologies, Irvine, CA) were injected into the left ventricle (LV). Reference blood was withdrawn from a femoral artery sheath using a specialized pump for 2 min at a rate of 2.9 ml/min (Harvard Apparatus, Holliston, MA). Distribution of fluorescent microspheres in the border zone and remote zone was quantified by flow cytometric analysis (Interactive Medical Technologies) and the numbers were compared.
Supplementary Figure 1. Co-staining of c-kit and VEGFR2 in the infarct border zone 1 week after the gene transfer. Only a few c-kit+/VEGFR2+ cells are found in the infarct border and majority of c-kit+ cells were negative for VEGFR2. Blue; DAPI-stained nuclei, red; c-kit, green; VEGFR2.
Supplementary Figure 2. Regional coronary flow measured by microspheres.

Relative coronary flow of infarct border area to remote area were compared (Ad.SCF n=2, Ad.β-gal n=1). The pigs treated with Ad.SCF had a higher relative coronary flow compared to the pig treated with Ad.β-gal.

Supplementary References