Human CD34+ Cells in Experimental Myocardial Infarction
Long-Term Survival, Sustained Functional Improvement, and Mechanism of Action

Jingxiong Wang,* Sui Zhang,* Brian Rabinovich, Luc Bidaut, Suren Soghomonyan, Mian M. Alauddin, James A. Bankson, Elizabeth Shpall, James T. Willerson, Juri G. Gelovani, Edward T.H. Yeh

Rationale: Human CD34+ cells have been used in clinical trials for treatment of myocardial infarction (MI). However, it is unknown how long the CD34+ cells persist in hearts, whether the improvement in cardiac function is sustained, or what are the underlying mechanisms.

Objective: We sought to track the fate of injected human CD34+ cells in the hearts of severe combined immune deficiency (SCID) mice after experimental MI to determine the mechanisms of action.

Methods and Results: We used multimodality molecular imaging to track the fate of injected human CD34+ cells in the hearts of SCID mice after experimental MI, and used selective antibody blocking to determine the mechanisms of action. Bioluminescence imaging showed that injected CD34+ cells survived in the hearts for longer than 12 months. The PET signal from the injected cells was detected in the wall of the left ventricle. Cardiac MRI showed that left ventricular ejection fraction was significantly improved in the treated mice compared to the control mice for up to 52 weeks (P < 0.05). Furthermore, treatment with anti-α4β1 showed that generation of human-derived cardiomyocytes was inhibited, whereas anti–vascular endothelial growth factor (VEGF) treatment blocked the production of human-derived endothelial cells. However, the improvement in cardiac function was abolished only in the anti-VEGF, but not anti-α4β1, treated group.

Conclusions: Angiogenesis and/or paracrine effect, but not myogenesis, is responsible for functional improvement following CD34+ cells therapy. (Circ Res. 2010;106:1904-1911.)

Key Words: human CD34+ cells ■ experimental myocardial infarction ■ molecular imaging ■ myogenesis ■ angiogenesis

Adult progenitor cells have been used to treat patients with acute myocardial infarction (MI) or chronic ischemic cardiomyopathy with variable success. 1-8 Nonlabeled autologous progenitor cells were used in these clinical trials. Hence, it was hard to track the fate of the transplanted cells in the treated hearts even postmortem. 3 In animal studies, it is easier to track the transplanted human progenitor cells via the xenogenic differences between the transplanted cells and the surrounding cardiac tissues. We have injected human CD34+ cells into the hearts of SCID mice after experimental MI and used HLA as a marker to identify the transplanted cells. 9-11 Others have used GFP, sex mismatch, or allelic differences to track the transplanted cells. 12 Such methodology is not dynamic and requires euthanasia of the animals to gain a single data point. To perform noninvasive monitoring of the transplanted cells, some laboratories have labeled the transplanted cells with iron oxide magnetic particles or superparamagnetic nanoparticles and followed them with MRI. 13,14 Activated macrophages, however, can engulf iron oxide–labeled stem cells, making it difficult to interpret the data. 15 Thus, it is important to develop more reliable way to track transplanted cells in animal studies.

The advancement of molecular imaging techniques provides tremendous potential to identify noninvasively the location, magnitude, and duration of cellular survival and fate of the transplanted cells. In principle, this approach requires the introduction of a reporter gene into the transplanted cells. A convenient reporter is firefly-Luciferase (f-Luc), an en-
zyme that cleaves d-luciferin and generate photon, which can be monitored by a bioluminescence detector. This method has been highly successful in small animal models because of the relatively short distance between the photon emitting cells and the detectors. However, this technique will not likely work in larger animals or humans because of tissue attenuation, scattering, and of the difficulty in localizing these signals inside larger bodies.

To track transplanted cells reliably in larger animals or in humans, we and others have developed PET reporter gene human herpes simplex virus type 1-thymidine kinase (HSV1-tk). HSV1-tk is essentially nontoxic in humans, and is currently being used in clinical gene therapy protocols as a “susceptibility” gene for treatment of cancer (in combination with ganciclovir). Importantly, the acquired sensitivity of HSV1-tk–transduced cells to ganciclovir provides an additional margin of safety because the genetically modified cells can be easily eliminated by treatment with ganciclovir. In this study, we constructed a triple fusion (TF) reporter vector encoding for enhanced-GFP (e-GFP, for cell selection), f-Luc (for bioluminescence imaging [BLI]), and HSV1-tk (for PET imaging). Using this reporter along with MRI and CT, we sought to determine the localization and persistence of the labeled CD34+ cells transplanted into living animals after experimental MI. Furthermore, we investigated whether these transplanted cells contributed to improvement in cardiac function, and what are the underlying mechanisms.

The mechanisms by which the transplanted progenitor cells benefit the cardiac function remain obscure. We have previously shown that adult human peripheral blood CD34+ cells injected into SCID mice transform into cardiomyocytes, endothelial cells, and smooth muscle cells in hearts injured by experimental MI.9 We have also demonstrated that most human-derived cardiomyocytes result from fusion of human CD34+ cells with mouse cardiomyocytes, whereas endothelial cells are directly differentiated from human CD34+ cells.11 Furthermore, fusion of CD34+ cells with cardiomyocytes is mediated by the interaction of VCAM-1 and α4β1, after which the fused cells re-enter the cell cycle and become new cardiomyocytes.10 The biological relevance of this finding is that myogenesis can be blocked by antibodies against VCAM-1 or α4β1 but not by antibody against vascular endothelial growth factor (VEGF). On the other hand, angiogenesis can be blocked by anti-VEGF, but not by anti-α4β1 antibodies. Thus, myogenesis can be distinguished from angiogenesis on the basis of selective antibody blockade and the relative contribution of myogenesis and angiogenesis to cardiac repair can be determined in the SCID mouse MI model.

Methods

Animals

All animal studies were approved by the Institutional Animal Care and Use Committees of the University of Texas-MD Anderson Cancer Center. Female SCID mice (C3H, The Jackson Laboratory, Bar Harbor, Me), weighing 16 to 20 g were used.

Construction of Lentivector

We excised a TF reporter gene encoding HSV1-tk, e-GFP, and f-Luc (termed as TGL),18,19 from pBluescript II SK+ using SaI and BamHI and ligated into pENTRIA (Invitrogen, Carlsbad, Calif) generating pENTRIA-nesTGL. This vector was then recombined with a self-inactivating lentiviral destination vector encoding the human ubiquitin promoter according to the specifications of the manufacturer (Invitrogen). Lentiviral packing, concentration, and titer were performed as previously described.20,21

Isolation and Transduction of CD34+ Cells

Human peripheral blood CD34+ cells were isolated as previously described.22 The isolated cells were transduced with lentiviruses at a multiplicity of infection of 50 as previously described.23

Induction of MI and Transplantation of Human CD34+ Cells Into the Mice

After anesthesia (3% isoflurane and oxygen) and mechanical ventilation, the left anterior descending artery was ligated. 10 minutes later, we injected 1×10^6 CD34+ cells in 25 μL of saline directly into the peri-infarcted areas. Control mice were injected with saline.

In Vitro and In Vivo BLI of f-Luc Gene Expression in CD34+ Cells

The cells were serially diluted and seeded on a 24-well plate; ρ-luciferin (Caliper LS, Alameda, Calif) was added at 1 μg/mL to the media and luminescent signal was measured using IVIS 200 (Caliper LS). In vivo BLI was determined 10 minutes after IP injection of ρ-luciferin (150 mg/kg). We manually defined regions of interest to measure signal intensities, expressed as photons/sec/cm²/4π (photon flux).

Small Animal MRI

A 7.0 T Biospec small animal scanner (Bruker Biospin Inc, Billerica, Mass) was used. Imaging gradients with 60 mm inner diameter were used with a 35 mm inner diameter linear birdcage-style volume resonator. T1-weighted anatomic reference images were acquired using a 3D fast low-angle shot gradient echo sequence. A retrospectively gated fast low-angle shot pulse sequence was used to acquire cardiac cine images exhibiting excellent contrast between bright blood and adjacent myocardium. To measure volumetric left ventricular ejection fraction (LVEF), at least 6 short axis images were scanned at 1 mm interval from the apex to the base of the heart. End-diastolic (ED) and end-systolic (ES) left ventricular volumes were obtained by the biplane area length method, and percent LVEF was calculated with the equation (ED−ES)/ED×100.21

Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BLI</td>
<td>bioluminescence imaging</td>
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<tr>
<td>ED</td>
<td>end diastolic</td>
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<td>e-GFP</td>
<td>enhanced green fluorescent protein</td>
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<tr>
<td>ES</td>
<td>end systolic</td>
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<tr>
<td>FACS</td>
<td>fluorescence-activated cell sorting</td>
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<td>f-Luc</td>
<td>firefly-luciferase</td>
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<td>HSV1-tk</td>
<td>human herpes simplex virus type 1-thymidine kinase</td>
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<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
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<td>MI</td>
<td>myocardial infarction</td>
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<tr>
<td>TF</td>
<td>triple fusion</td>
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<td>TGL</td>
<td>fused HSV1-tk, e-GFP, and f-Luc gene</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<tr>
<td>[18F]FDG</td>
<td>2-[18F]-fluoro-2-deoxy-D-glucose</td>
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<td>[18F]FEAU</td>
<td>[18F]-labeled 2'-deoxy-2'-fluoro-5-methyl-1-β-D-arabinofuranosyluracil</td>
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Micro-CT
A micro-CT (RS-9 tabletop CT scanner, General Electric Medical Systems, London, Ontario) was used. We took 360° at 1° increments, with each projection consisting of a 500-ms x-ray exposure. We then used a cone-beam back-projection reconstruction method, after normalizing and correcting the raw projection images for bad detector pixels. The CT value grayscale from the initial reconstructed raw data were then calibrated into Hounsfield units by sampling air, water, and bone material standards positioned within the field of view of the image.

Micro-PET
Micro-PET R4 scanner (Concorde Microsystems, Knoxville, Tenn) was used. PET imaging was performed 2 hours after intravenous administration of the radiolabeled nucleoside analog [18F]-labeled 2′-deoxy-2′-fluoro-5-methyl-1β-D-arabinofuranosyluracil ([18F]FEAU), which was synthesized with high specific activity as previously described.24 The data acquisition, image reconstruction, and [18F]FEAU-derived radioactivity quantification were performed as previously described.17

Coregistration of MRI, CT, and PET Images
To accurately coregister images, we used a chamber that was manufactured in-house. We registered PET to the reference anatomic CT through the coregistration chamber’s marker set, and this was further refined through a normalized mutual information cost function. We relied on an indirect MRI to CT registration process to register MRI and PET together. First, relatively high resolution MRI was registered to the reference CT via a normalized mutual information cost function.25–27 Independently, the diastole phase of a high planar- but low axial-resolution gated MRI data set was registered to the low resolution MRI of the same animal via a simpler correlation cost function.26 The geometric transformations (eg, PET to CT, CT to MRI, and MRI to gated MRI) were finally combined together to register gated MRI slices to PET images via the CT surrogate. Gated MRI slices thus provided the geometric transformations (eg, PET to CT, CT to MRI, and MRI to gated MRI) were finally combined together to register gated MRI slices to PET images via the CT surrogate. After data were retrieved from the scanners, all processing and visualization paradigms were achieved through an ad hoc hybrid combination of commercial platforms and in-house developments.

In Vivo Antibody Blocking of Myogenesis and Angiogenesis
We designed an experimental protocol (Figure 4A) based on our previous observation. Four groups of mice received anti-o4β1 or anti-VEGF antibodies or their respective isotype-matched control antibodies. The fifth group of mice received cells only, and the sixth group received neither cells nor antibodies. At the end of the study, hearts were removed and cellular subsets were analyzed with fluorescence-activated cell sorting (FACS) to ascertain the effects of antibody treatments. Antibodies were administered to the animals by IP injection at a concentration of 50 μg/mouse in 0.3 mL PBS 30 minutes before injected with CD34+ cells.

Preparation of Single-Cell Suspension From the Heart and Cell Staining for FACS
Single cell suspension was obtained by enzyme digestion. Isolated cells were washed, fixed, permeabilized, and incubated with monoclonal anticardiac troponin T (1:200, clone 1A11; Advanced Immunochemical), or with a polyclonal anti-VE cadherin (1:100, Bender MedSystems) for 30 minutes at 4°C. The secondary antibodies were conjugated with Alexa Fluor 633 (Molecular Probes), or conjugated with Alexa fluor 488, respectively. After 3 washes, all cells were incubated with antihuman HLA-ABC conjugated with PE (Cedarlane Laboratories) for 30 minutes before FACS.

Immunohistochemistry
5 -micrometer serial sections were collected on slides, fixed with 3.7% paraformaldehyde (pH 7.4) at 4°C for 5 minutes, rinsed in PBS 3 times, and blocked at room temperature for 30 minutes in PBS containing 5% horse serum. Slides were then incubated with primary antibodies at room temperature for 1 hour, rinsed 3 times, and incubated with the secondary antibodies at room temperature for 30 minutes. The slides were rinsed in PBS 3 times again and sealed with a mounting medium containing DAPI (Vector Laboratories); anticalcific troponin T (Abcam); anti-CD31 (Abcam). Secondary antibodies (IgG) were Alexa fluor 488–conjugated goat anti-mouse IgG; Alexa fluor 568 donkey anti-rabbit.

Statistics
Student t test was used to compare the means of 2 groups. When 3 or more means were compared, 1-way ANOVA followed by multiple comparisons among means was used. All data collection and analyses were performed in a blinded fashion.

Results
Efficiency of Lentiviral Transduction of CD34+ Progenitor Cells In Vitro
We used e-GFP to monitor TGL expression efficiency in transduced CD34+ cells. The average transduction efficiency was 38.2±4.3% at multiplicity of infection of 50. A linear correlation was observed between transduced cell numbers and photon flux (Online Figure I, available at http://circres.ahajournals.org).

In Vivo BLI Revealed Long-Term Persistence of Transplanted CD34+ Cells After MI
We performed in vivo noninvasive BLI in SCID mice injected with TGL-expressing CD34+ cells to monitor cell localization and survival (measured as bioluminescent activity) over 12 months in living animals subjected to MI. We observed a bioluminescent signal only in the chest overlying the hearts (Figure 1A), suggesting that transplanted CD34+ cells did not migrate to peripheral organs in sufficient numbers to be detected. The change in photon flux over time in hearts is shown in Figure 1B. Three days after injection, maximum photon flux was observed (2.98±0.31 ×10^4), this decreased to 7.20±0.13×10^4 by week 25, and maintained at that level for greater than 1 year (Figure 1B). Photon flux was 2 to 3 fold higher during the first 2 weeks after cell injection (P<0.01). This suggests that transplanted CD34+ cells had multiplied. Alternatively, the protein expression of f-Luc may have increased following transplantation. Importantly, no signal above the noise level was observed from other organs in both the experimental and the control mice, indicating that no teratoma was induced by the transplantation of the transduced CD34+ cells.

Coregistration of MRI, Micro-CT, and Micro-PET Images Confirmed the Localization of Transplanted CD34+ Cells
Through the coregistration of MRI/CT/PET images (Figure 2 and Online Video I), we can precisely localize the transplanted CD34+ cells in the perifunction area. PET was used to localize the TGL-expressing CD34+ cells using [18F]FEAU as the substrate for HSV1-tk. Phosphorylation of [18F]FEAU by HSV1-tk retained the radio-labeled PET probes inside the transplanted CD34+ cells and can be imaged by a PET scanner. As previously reported, nonspecific [18F]FEAU uptake was observed in the gallbladder and
kidneys. As shown in Online Video I, there was no PET signal in the cardiac region in a sham-operated mouse. However, PET signals were clearly visible in the cardiac region in a CD34⁺/H11001 cell transplanted mouse (Online Video II).

**Improvement in Cardiac Function in Infarcted Hearts After Transplantation of CD34⁺ Cells**

We used MRI to evaluate the therapeutic benefit of CD34⁺ cell transplantation into the infarcted hearts (Figure 3 and Online Video III). ES and ED volumes, measured for up to 52 weeks, were used to calculate the LVEF (Figure 3A). One day and 3 days after MI, the decreases in EF were similar in both the control and cell-treated groups (Figure 3B). These results suggest that our MI protocol was carried out consistently and achieved a similar degree of cardiac damage in both the control and the cell treated group. The EF began to diverge in a statistically significant way between the control and cell-treated group 1 week following MI and improvement in LVEF in the cell treated animals was sustained up to 52 weeks.

**Angiogenesis and/or Paracrine Effect Is Responsible for Functional Improvement**

To the mechanism of action, we used FACS to analyze cellular subsets in the hearts 2 months after MI. Figure 4B
The anti-VEGF treated hearts was similar to the hearts that did not receive cell therapy, whereas other groups showed a significant increase in the number of CD31+ vessels. Thus, our results suggest that angiogenesis and/or paracrine effect, but not myogenesis, is responsible for improving cardiac function following cell therapy.

Discussion

A variety of adult progenitor cells have been used as cellular therapy for acute MI. Kawamoto et al reported that CD34+ cells purified from peripheral blood mononuclear cells can preserve myocardial integrity and function after MI. Using similarly prepared human CD34+ cells in a mouse model of experimental MI, we demonstrated that transplanted CD34+ cells became cardiomyocytes, endothelial cells, and smooth muscle cells. Thus, CD34+ cells have the potential to be a clinically useful adult progenitor cells for cardiac repair. Recently, Losordo et al published the results of a phase I/IIa study using autologous CD34+ cells for intractable angina, showing clinical improvements in patients treated with CD34+ cells for up to 6 months. Our study demonstrates that human CD34+ cells persist in the heart for up to 12 months following injection into the peri-infarct zone of SCID mice, and furthermore, the improvement in LVEF is also preserved for at least 6 months. Thus, these results suggested that transplanted CD34+ cells may similarly persist in the clinical setting, a finding which may correlate with myocardial repair.

It is difficult to track the fate of injected cells in clinical studies. Hofmann et al have labeled non purified bone marrow cells with 2-[18F]-fluoro-2-deoxy-D-glucose ([18F]FDG) and infused them into the infarct-related coronary artery. Fifty to 75 minutes after cell transfer, 1.3% to 2.6% of [18F]FDG-labeled bone marrow cells were detected in the infarcted myocardium using 3D PET imaging. Whereas after transfer of [18F]FDG-labeled CD34-enriched cells, 14% to 39% of the total activity was detected in the infarcted myocardium. However, this method cannot be used to track the long-term fate of injected CD34+ cells.

Efficient expression of a given reporter is critical for in vivo tracking transplanted cells using live animal imaging techniques. This is particular critical for multimodality imaging using a fusion of multiple reporter genes because the fusion tends to decrease activity of each reporter. Lentivirus can effectively introduce and integrate reporter genes into the genome of CD34+ cells, achieving long-term and stable reporter gene expression. In this study, we constructed lentivectors expressing the TGL reporter gene driven by human ubiquitin promoter. Remarkably, the transduced cells with these vectors survived in the infarcted hearts for longer than 1 year. We have also confirmed that the transplanted CD34+ cells contribute to restoring cardiac function in the long-term. The profile of LVEF recovery is comparable with results reported elsewhere. These findings validate that multimodality molecular imaging is a powerful method for tracking progenitor cells in vivo.

BLI enabled us to determine the location, magnitude, survival, and especially the long–term time–spatial kinetics of the transplanted CD34+ cells. BLI, however, is not clinically applicable because of several limitations (see introduction).
To generate a clinically applicable molecular imaging protocol, we included the HSV1-tk gene in the TF reporter gene and used $[^{18}F]$FEAU-PET concomitant to BLI to monitor the transplanted CD34<sup>+</sup> cells. Micro-PET imaging displayed similar tempo-spatial kinetics for HSV1-tk gene expression as BLI. The coregistered MRI/CT/PET images provided us with detailed morphological picture of CD34<sup>+</sup> cell therapy. This paradigm can be applied clinically to track the fate of progenitor cell therapy in humans.

Previous studies have shown that several factors contribute to improvement in cardiac function, including the formation of new muscle cells and blood vessels from the transplanted cells. In addition, the secretion of cytokines from the transplanted cells can have a paracrine effect on improving cardiac function. Here, we showed that angiogenesis and/or paracrine effect, rather than myogenesis, is responsible for the functional improvement. Following the injection of 1 million CD34<sup>+</sup> cells to the perinfarct region, we have consistently observed that approximately 0.8% to 0.9% of total cardiac cells were HLA<sup>+<sup>/troponin T<sup>+</sup>, as determined by FACS analysis. Only anti-VEGF inhibited the formation of human-derived endothelial cells (HLA<sup>+/VE-cadherin<sup>+</sup>). Anti-VEGF, but not anti-α4β1, antibodies diminished the effect on the improvement in the LVEF caused by the injection of human CD34<sup>+</sup> cells. Treatment with anti-α4β1 or anti-VEGF antibodies did not affect LVEF following MI without cell therapy (Data are expressed in means±SE (n=4/group). **P<0.01; *P<0.05.

![Figure 4. In vivo antibody treatments inhibit myogenesis/angiogenesis and affect cardiac function induced by injection of CD34<sup>+</sup> cells into mice after MI. A, Schematic of the experimental design. All animals underwent baseline MRI scanning before the experiments. All animals, except those in the MRI control group, which were used to monitor LVEF without any perturbation, had experimental MI on day 0. Anti-α4β1, anti-VEGF, and the respective isotype control antibodies were injected IP 30 minutes before MI. The heart was removed 8 weeks after MI and analyzed by FACS. B, Anti-α4β1, but not anti-VEGF, antibodies inhibited the formation of human-derived cardiomyocytes (HLA<sup>+/troponin T<sup>+</sup>), as determined by FACS analysis. C, Only anti-VEGF inhibited the formation of human-derived endothelial cells (HLA<sup>+/VE-cadherin<sup>+</sup>). D, Anti-VEGF, but not anti-α4β1, antibodies diminished the effect on the improvement in the LVEF caused by the injection of human CD34<sup>+</sup> cells. E, Treatment with anti-α4β1 or anti-VEGF antibodies did not affect LVEF following MI without cell therapy (Data are expressed in means±SE (n=4/group). **P<0.01; *P<0.05.)
endothelial cells and the paracrine effect mediated by VEGF, abrogated the improvement in cardiac function further suggests that angiogenesis and/or paracrine effect play a critical role in the improvement in cardiac function in this murine model.

It was shown that anti-α4β1 antibody mobilizes cardiac progenitor cells from the bone marrow to enhance cardiac repair after MI. Thus, anti-α4β1 antibody treatment could be a double-edged sword that, on the one hand, blocks the formation of human-derived cardiomyocytes but, on the other hand, recruits mouse progenitor cells from the bone marrow to enhance cardiac repair. We think this is unlikely based on the data shown in Figure 4E. In this experiment, animals with experimentally induced MI did not receive cell therapy. Instead, they were treated with either anti-α4β1 or anti-VEGF antibody over a 2-month period and neither anti-α4β1 nor anti-VEGF antibody altered the drop in LVEF following MI. Thus, it is unlikely that in our study the anti-α4β1 antibody enhanced the LVEF that compensated for the drop in LVEF attributable to blocked myogenesis.

Recently, Wu and colleagues reported tracking of the fate of transplanted adipose tissue- and bone marrow–derived mesenchymal stem cells using BLI. However, the mesenchymal stem cells survived for less than 5 weeks after transplantation and did not contribute to functional recovery. In contrast, our present study showed that CD34+ cells persisted for up to 1 year and contributed to functional recovery. Furthermore, our study also provides insights into the mechanistic repair. Although antibody treatment can exert other effects, our FACs analysis and immunohistochemical studies suggest that our antibody treatment protocol can distinguish angiogenesis/paracrine effect from myogenesis, leading to mechanistic insights.

In conclusion, we coregistered MRI, micro-PET, micro-CT, and BLI images to successfully obtain anatomic and functional information on CD34+ cells transplanted in mice with experimental MI. We demonstrated that BLI is capable of long-term tracking of transplanted CD34+ cells and that we can use micro-PET, micro-CT, and MRI images to accurately localize transplanted CD34+ cells. Furthermore, we demonstrated that the long-term survival of the transplanted CD34+ cells contributed to improvement in cardiac function. Using selective antibody blocking, we also demonstrated that angiogenesis and/or paracrine effect accounts for the functional improvement.

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Disclosures

None.

References

Adult stem cells have been used to treat patients suffering from myocardial infarction or heart failure. However, it is not known how long these cells survive after transplantation and how these cells improve cardiac function. To address these questions, we transplanted human CD34+ cells, a type of adult stem cells that can be purified from blood, into the heart of immune-deficient mice that had sustained experimental myocardial infarction. These CD34+ cells improved cardiac function after transplantation.

Novelty and Significance

What Is Known?

- Adult stem cells have been used to treat patients suffering from myocardial infarction or heart failure.
- CD34+ cells, one type of adult stem cells, can generate new myocardium and blood vessels.

What New Information Does This Article Contribute?

- Human CD34+ cells survived for up to 1 year in the mouse heart following experimental infarction.
- Human CD34+ cells improved cardiac function after transplantation.
- Human CD34+ cells did this by forming new blood vessels or producing beneficial chemicals, but not by forming new myocardium.

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We also show that the transplanted cells effected this improvement by forming new blood vessels or producing beneficial chemicals but not by forming new myocardium. This study provides new insights into the mechanisms whereby one type of adult stem cells helps to repair the heart. We expect that our results will be helpful in improving the design of clinical trials to determine the best way to provide cell therapy to patients with heart disease.
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Online Figure I. In vitro characterization of lentivector encoding the TGL reporter gene. A, Schematic representation of the lentivector encoding the TGL reporter gene driven by hUbiq promoter. B, Flow cytometric histogram of CD34+ cells transduced with the lentivirus encoding the TGL reporter gene driven by the hUbiq promoter (48 hours post transduction). C, In vitro BLI of 1x10^5-8x10^6 TGL^+CD34^+ cells (48 hours post transduction). D, Correlation between luciferase activity and the number of CD34^+ cells.
Online Figure II. Frozen sections of the peri-infarct region of hearts from mice injected with CD34\(^+\) cells treated with different blocking and related isotype control antibodies were stained to examine the expression of cardiac troponin T and HLA to detect human CD34\(^+\) cell-derived cardiomyocytes. Images were 400X magnified.
Online Figure III. Frozen sections of the peri-infarct region of hearts from mice injected with CD34⁺ cells treated with different blocking and related isotype control antibodies were stained to examine the expression of CD31 and HLA to reveal human CD34⁺ cell-derived endothelial cells. Images were 400X magnified.
Online Figure IV. Frozen heart sections from mouse injected with/without CD34⁺ cells. Sections from the peri-infarct were stained with anti-CD31 to visualize the vessel density. Images were 400X magnified.
Online Figure V. Number of CD31⁺ vessels in peri-infarct region. The bars represent mean±SD of vessels in 5 frozen sections. For each section, vessel numbers in 6 fields (400X) were counted and averaged. Asterisks indicate significant difference (p<0.05).
Supplemental Legends for the Video Files

**Online Video I.** This movie was produced in the course of the validation of our multimodality approach at the earliest time point of our time series, when no specific \(^{18}\text{F}\)FEAU uptake is visible (uptake in the gall bladder is non specific and part of normal \(^{18}\text{F}\)FEAU physiology). The intent here is to demonstrate how our multimodality approach works and the information that can be extracted from the high-dimensional multimodality space. The movie shown sequentially, first, anterior to posterior coronal slices through the coregistered CT (gray) and PET (color); then, 3D CT (e.g., skeleton) and maximum intensity projection (MIP, in bright orange) of PET showed non specific uptake in gallbladder, kidneys and liver, as well as external markers used for PET to CT registration; after that, anterior to posterior coronal slices through the CT (gray) and 3D MRI (cyan), followed by a 3D combination of gated MR volume (green), PET MIP (bright orange), and CT (e.g., skeleton). 3D gated MRI is then played as a sequence in relation to the CT-derived skeleton, and finally, as a gated slice (gray) in relation with matching PET slices.

**Online Video II.** This movie is from the same data set as Fig. 3. The rotating micro-PET MIP (orange) clearly demonstrates the extent of the \(^{18}\text{F}\)FEAU uptake of the engraftment in relation to the CT anatomy (e.g., skeleton and some soft tissue).

**Online Video III.** Representative MRI images demonstrating the beating heart of a control mouse and a mouse transplanted with CD34\(^+\) cells. Data from baseline and 6 months after transplantation are shown.