Transdifferentiation of Human Peripheral Blood CD34\textsuperscript{+}-Enriched Cell Population Into Cardiomyocytes, Endothelial Cells, and Smooth Muscle Cells In Vivo

Edward T.H. Yeh, MD; Sui Zhang, MD, PhD; Henry D. Wu, MD; Martin Körbling, MD; James T. Willerson, MD; Zeev Estrov, MD

Background—Adult human peripheral blood cells have been shown to differentiate into mature cells of nonhematopoietic tissues, such as hepatocytes and epithelial cells of the skin and gastrointestinal track. We investigated whether these cells could also transdifferentiate into human cardiomyocytes, mature endothelial cells, and smooth muscle cells in vivo.

Methods and Results—Myocardial infarction was created in SCID mice by occluding the left anterior descending coronary artery, after which adult peripheral blood CD34\textsuperscript{+} cells were injected into the tail vein. Hearts were harvested 2 months after injection and stained for human leukocyte antigen (HLA) and markers for cardiomyocytes, endothelial cells, and smooth muscle cells. Cardiomyocytes, endothelial cells, and smooth muscle cells that bear HLA were identified in the infarct and peri-infarct regions of the mouse hearts. In a separate experiment, CD34\textsuperscript{+} cells were injected intravenicularly into mice without experimental myocardial infarction. HLA-positive myocytes and smooth muscle cells could only be identified in 1 of these mice killed at different time points.

Conclusions—Adult peripheral blood CD34\textsuperscript{+} cells can transdifferentiate into cardiomyocytes, mature endothelial cells, and smooth muscle cells in vivo. However, transdifferentiation is augmented significantly by local tissue injury. The use of peripheral blood CD34\textsuperscript{+} cells for cell-based therapy should greatly simplify the procurement of cells for the regeneration of damaged myocardium. (Circulation. 2003;108:2070-2073.)

Key Words: cells ■ muscle, smooth ■ endothelium ■ myocyte

Cell-based regeneration therapy has been widely touted as a novel means of repairing damaged heart after myocardial infarction (MI), chemotherapy-induced damages, or other injuries leading to heart failure. Various cell populations, such as embryonal stem cells, cord blood cells, and mesenchymal stem cells, have been suggested as a source for replacement therapy. It will be useful to identify a readily available cell source that does not require significant manipulation. Adult human peripheral blood stem cells have been shown to differentiate into mature hepatocytes and epithelial cells of the skin and gastrointestinal track.\textsuperscript{1} In the present study, we investigated whether human peripheral blood cells could transdifferentiate into cardiomyocytes, mature endothelial cells, or smooth muscle cells using the SCID mouse as a recipient.

Methods

Animals

Female SCID mice (CB-17) weighing 16 to 23 g were used (Charles River Laboratories, Wilmington, Mass). Two and a half million human peripheral blood CD34\textsuperscript{+} cells were injected into the left ventricle of 19 mice without surgical manipulation, and the heart was harvested at 24 hours (n=3), 4 days (n=3), 12 days (n=7), 30 days (n=3), or 60 days (n=3) after cell transplantation. Another group of 6 mice receiving experimental MI (n=3) or sham surgery (n=3) was injected with the same number of CD34\textsuperscript{+} cells through the tail vein and killed at day 60 after transplantation.

Induction of MI

Mice were ventilated with 100\% oxygen using a rodent ventilator (Inspira ASV, Harvard Apparatus, Inc). The chest was opened, a 7-0 silk suture (Ethicon, Inc, Johnson & Johnson Co) was passed with a tapered needle under the left anterior descending coronary artery 1 to 2 mm from the tip of the left atrium, and the 2 ends of the suture were tied to induce MI. Two million human peripheral CD34\textsuperscript{+} cells were injected into the tail vein 16 hours after MI was induced. Sham-operated mice received the same procedure except left anterior descending ligation.

Isolation of CD34\textsuperscript{+} Cells From Human Peripheral Blood

CD34\textsuperscript{+} fractionation was performed using immunomagnetic beads as previously described and contained more than 90\% CD34\textsuperscript{+} cells.\textsuperscript{2}
Tissue Harvesting
Hearts were removed, embedded in OCT, snap frozen in liquid nitrogen, and stored at −80°C. Mouse hearts in OCT blocks were sectioned, and 5-μm serial sections were collected on slides followed by fixation with 3.7% paraformaldehyde (pH 7.4) at 4°C for 5 minutes and stained immediately.

Immunofluorescence Staining
After rinsing slides with PBS 3 times, the slides were blocked at room temperature for 30 minutes in PBS containing 5% horse serum and incubated with primary antibodies at room temperature for 1 hour. The slides were rinsed 3 times and incubated with the secondary antibodies at room temperature for 30 minutes. Paired primary and secondary antibodies were used for double staining. The slides were rinsed again, and DAPI solution was applied for 5 minutes. Reagents used were anti–human leukocyte antigen (HLA)-ABC (BD Biosciences); anti–troponin T antibody (Santa Cruz Biotechnology) reacts against cardiomyocytes of human and mouse; anti-α-smooth muscle actin (Spring Bioscience) reacts against both human and mouse smooth muscle actin; and anti–VE-cadherin reacts against endothelial cells (Bender Medsystem). Secondary antibodies were Alexa Fluor 488-conjugated goat anti-mouse IgG (Molecular Probes) for anti-HLA and goat anti-rabbit IgG Rhodamine (Santa Cruz Biotechnology) for anti-smooth muscle actin and anti–VE-cadherin antibodies.

Results
Transdifferentiation of Human Peripheral Blood CD34+ Cells in the Injured Heart
We first assessed the specificity and cross reactivity of the antibodies against human HLA-ABC in cultured human smooth muscle cells and endothelial cells, mouse smooth muscle cells, and peripheral blood CD34+ cells using FACS analysis. No cross-reaction to mouse cells was found (data not shown). Also, there was no cross reaction between smooth muscle–specific α-actin and VE-cadherin antibodies, confirmed by immunofluorescence staining of cultured human vascular smooth muscle cells and human endothelial cells (data not shown).

Clusters of cells (Figures 1A through 1D) and single cells (Figures 1E through 1H) with the morphological appearance typical of cardiomyocytes were stained positively with both anti-human HLA-ABC and anti-cardiac troponin T in the peri-infarct area in all 3 mice that had sustained MI 60 days. However, no cardiomyocytes derived from human peripheral blood CD34+ cells were observed in the infarct zone. No transdifferentiated cardiomyocytes were found in tissue sections of sham-operated animals after cell transplantation (3 mice).

Blood vessels that stained with anti-HLA antibody were seen mainly in the infarct area. Double staining for HLA and smooth muscle α-actin indicated that human CD34-derived smooth muscle cells had participated in the neovascularization after acute MI (Figures 1I through 1L). Double staining of the blood vessels with anti-HLA and anti–VE-cadherin confirmed that human CD34+ cells transdifferentiated into mature endothelial cells (Figures 1M through 1P).

Minimal Transdifferentiation in the Heart Without Major Injury
To investigate the transdifferentiation potential of human blood CD34+ cells in animals without MI, CD34+ cells were injected intraventricularly into the left ventricle of SCID mouse. No transdifferentiation was found in the hearts at 24 hours, 4 days, 30 days, and 60 days after injection. However, in 1 of the 7 mice examined 12 days after injection, cardiomyocytes and blood vessels double stained with anti-HLA and cardiac troponin (Figures 2A through 2C) smooth muscle α-actin (Figures 2D through 2F) were observed.

Discussion
Attempts have been made to regenerate damaged myocardial tissue. Cells from a variety of sources have been used for the purpose. Many recent studies have focused on the use of embryonic stem cells and bone marrow–derived cells, including adult hematopoietic stem cells, mesenchymal stem cells, and bone marrow side population cells. CD34+ cells isolated from bone marrow and peripheral blood have been successfully used for regeneration of the blood vessels after experimental MI. A recent report described the differentiation of human CD34+ cells, when cocultured with rat cardiomyocytes, into the cardiomyocytes in vitro. However, there have been no reports on transdifferentiation of human peripheral blood–derived CD34+ cells into the cardiomyocytes in vivo.

Using human HLA as a marker for the donor cells, we demonstrate transdifferentiation of CD34+ cells into cardiomyocytes in vivo, although the frequency of the event is low in uninjured hearts. The cells derived from transplanted CD34+ cells demonstrate mature cardiomyocyte morphology and seem to be integrated into the myocardium of the peri-infarct area. The fact that no transdifferentiated cardiomyocytes were found inside the infarct zone is in agreement with the in vitro observation that contacts between the donor and host cells are necessary for transdifferentiation. CD34+ cells from the bone marrow and peripheral blood have long been recognized to contain endothelial progenitors. Consistent with these findings, double staining of the blood vessels with anti-HLA and anti–VE-cadherin documented the transdifferentiation of peripheral blood CD34+ cells into vascular endothelial cells. It has been reported that CD34+ cells in human blood contain a population of smooth muscle progenitor cells. Our data also demonstrate the potential of these cells to differentiate into vascular smooth muscle cells in vivo.

Terada et al demonstrated in vitro that bone marrow stem cells were able to fuse with embryonic stem cells and to adopt their phenotype. Recently, Wang et al and Vassilopoulos et al demonstrated that cell fusion was the major mechanism in regeneration of the damaged hepatocytes of mice. Because no chromosome analysis was performed in our study, we could not exclude the possibility that cell fusion is partly responsible for the phenotype conversion of the injected CD34+ cells, especially in myocytes where fusion of myotube is part of the differentiation process. However, the phenotypic conversion of the injected CD34+ cells into endothelial cells or smooth muscle cells may occur predominantly through transdifferentiation. Additional studies are in progress to differentiate between these possibilities.

In previous studies, donor cells were often labeled with the green fluorescence protein and β-galactosidase. These labeling techniques require extensive manipulation of the
Figure 1. Immunofluorescence staining images of transdifferentiated human peripheral blood CD34⁺ cells. Tissue sections (5 mmol/L) are from hearts harvested at 2 months from animals with experimental MI followed by the intravenous injection of CD34⁺ cells. Sections were stained for human HLA-ABC (A, E, I, and M), cardiac troponin T (B and F), smooth muscle–specific α-actin (J), and VE-cadherin (N). HLA-ABC was detected with Alexa Fluor 488-labeled secondary antibody, and non-HLA markers were detected with rhodamine-labeled secondary antibodies. Images C, D, G, H, K, L, O, and P are overlays of the double staining. Higher magnification of images C, G, K, and O (×200) are presented in images D, H, L, and P (×600), respectively. Nuclei were stained with DAPI. Scale bars=10 mm.
donor cells. Therefore, we have chosen to use human HLA molecule as the marker for the donor cells. The monoclonal antibody (W6/32) for detection is specific for a monomorphic epitope. This method, therefore, can be used in future studies on stem cell transplantation in which human cells are used as donors.

In our study, the frequency of transdifferentiation of human blood CD34⁺ cells is extremely low in uninjured hearts. Apparently, severe tissue damage plays a critical role in the event. This is in concordance with the commonly accepted notion that transdifferentiation of adult stem cells in the heart is a random and rare event. The low frequency of transdifferentiation we have observed in uninjured animals might be attributable to poor homing of the transplanted cells in the absence of injury.

Our findings are clinically relevant in that adult peripheral blood stem cells may be superior to other cell sources in cell-based therapy for myocardial regeneration. Their use obviates the painful procedure of bone marrow aspiration and the attendant anesthesia risks. In addition, autologous stem cell transplantation does not require long-term immune suppressive therapy. Thus, the use of autologous peripheral blood stem cells for myocardial regeneration is a promising alternative for the treatment of heart failure.

References
Transdifferentiation of Human Peripheral Blood CD34⁺-Enriched Cell Population Into Cardiomyocytes, Endothelial Cells, and Smooth Muscle Cells In Vivo
Edward T.H. Yeh, Sui Zhang, Henry D. Wu, Martin Körbling, James T. Willerson and Zeev Estrov

_Circulation_. 2003;108:2070-2073; originally published online October 20, 2003; doi: 10.1161/01.CIR.0000099501.52718.70
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/108/17/2070

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/