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Original Research Report

Transplantation of Human Undifferentiated Embryonic Stem Cells into a Myocardial Infarction Rat Model

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ABSTRACT

Human embryonic stem (hES) cells hold great therapeutic potential for cell transplantation. To date, it remains uncertain whether undifferentiated hES cells can differentiate into cardiac lineage in vivo during myocardial infarction. Here we provide the first report that undifferentiated hES cells can survive in rat hearts during myocardial infarction without the formation of teratoma using undifferentiated green fluorescent protein (GFP)-transgenic hES cells. Using a laser-capture microscope to dissect the GFP-positive cell area from the hES-injected hearts, we documented the expression of human cardiac-specific genes, including GATA-4, Nkx-2.5, and cardiac troponin I. Taken together, our results demonstrate that undifferentiated hES cells can be driven to the cardiac lineage under the local injured environment in the heart, which may provide a potential method for regenerating de novo myocardium to treat myocardial infarction.

INTRODUCTION

MYOCARDIAL INFARCTION (MI) is the leading cause of congestive heart failure and death in developed countries. The current pharmacotherapy and various interventional and surgical therapeutic methods for MI are limited in preventing deterioration of heart function, partially due to their inability to repair or replace damaged myocardium and regenerate new myocardium. Because functional restoration of the damaged heart presents a formidable challenge, it is imperative to develop new strategies for patients with MI (1,2).

The use of cellular therapy offers a promising approach for MI treatment. Different types of regenerating cells are currently being employed to determine their capacity for the treatment of MI in the clinic (3). Recent randomized controlled clinical studies have been conducted to analyze the efficacy of bone marrow-derived stem cell transplantation therapy after acute MI. Although some of these studies have showed significant improvement in cardiac function, others showed ambiguous results (4,5). Indeed, there is little evidence to show that bone marrow-derived stem cells are able to regenerate cardiomyocytes (4,5).

Although cardiomyocytes derived from human embryonic stem (hES) cells have been documented to integrate efficiently into of the hearts of host animals (3,6,7,8), the

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FIG. 1. Transplantation of GFP-transgenic hES cells into the myocardial infarcted heart. (**A**) Morphology of GFP-transgenic hES cultured on Matrigel-coated plate, viewed by phase-contrast (*left*) and fluorescence (*right*) microscopy, respectively. (**B**) Infarction of rat hearts induced by ligation of the left anterior descending coronary artery. The arrowhead indicates the site of human GFP-ES cell injection near the infarcted area (circled). (**C**) Hematoxylin & Eosin staining showing the infarction of rat hearts. Bar, 100 μm. (**D**) Reprehensive image showing the robust fluorescence in the injected area 2 months after GFP-hES cell injection. Bar, 100 μm.

possibility of undifferentiated hES cells as transplanted cell types for MI have not been examined. In the present study, using undifferentiated green fluorescent protein (GFP)-transgenic hES cells, we documented for the first time that undifferentiated hES cells can survive in the rat hearts during MI with human cardiac-specific gene expression.

MATERIALS AND METHODS

GFP-transgenic hES cell culture

GFP-transgenic hES cells were derived from WiCell H1 cells (passage 33) transfected with lentiviral vectors encoding enhanced green fluorescent protein (EGFP). The undifferentiation and pluripotency of these derived cells had been reported (9). The GFP-transgenic hES cells were expanded routinely on Matrigel with conditioned medium as described (10).

MI model and cell transplantation

MI in male Sprague-Dawley rats at 6 weeks of age was induced by ligation of the left anterior descending coronary artery, as previously described (11). Infarcted animals were grouped randomly into sham- and GFP-transgenic hES cell treatment. Successful occlusion was recognized by pallor of the anterior left ventricular free wall. Once the infarcted area stopped to increase (around 30 min), the culture medium (20 μl) without cells (sham) or 5×10^5 GFP-transgenic hES cells in 20 μl of medium were injected through a 25-gauge needle at the left ventricular base along the border of the left ventricular infarcted areas. No antiimmunological rejection drugs were given to rats. All animal experimental protocols were approved by the Animal Use and Care Committee of the Morehouse School of Medicine.

Laser-capture microdissection

GFP-positive areas in hearts of hES cell-injected group were isolated by the laser-capture microdissection (LCM) system (Leica Microsystems, Deerfield, IL) using a minor modification of a method described previously (12). Briefly, sections (10 μm thick) were cut using a cryostat (CM 1850, Leica Microsystems) and thaw-mounted onto uncharged glass slides. LCM was performed by cutting selected GFP-positive areas into HS-CapSure non-contact LCM film (Leica). Microdissected cell clumps were immediately processed for RNA isolation using the RNeasy micro kit (Qiagen, Valencia, CA) as described by the manufacturer.

Reverse transcription PCR

The cDNA was synthesized using Superscript III first-strand synthesis system (Invitrogen, Carlsbad, CA). The

TABLE 1. PRIMER SEQUENCES

Gene	*Sequence*	*Product size (bp)*
Oct-4	F: 5′-gaaggtattcagccaaacga-3′	216
	R: 5′-aaattctccagggttgcctct-3′	
Nanog	F: 5′-actccatgaacatgcaacct-3′	166
	R: 5′-actggataggcatcatggaa-3′	
GATA-4	F: 5′-tcctggaaagaagacgactg-3′	219
	R: 5′-gattttggagtgaggggtct-3′	
Nkx2.5	F: 5′-ggattttgcattcactcctg-3′	196
	R: 5′-agctcagtcccagttccaa-3′	
cTnI	F: 5′-actgaccctccaaacgcccc-3′	256
	R: 5′-cctctcgctccagctcttgc-3′	
18sRNA	F: 5′-ggaagggcaccaccaggagt-3′	317
	R: 5′-tgcagccccggacatctaag-3′	

F, Forward; R, reverse.

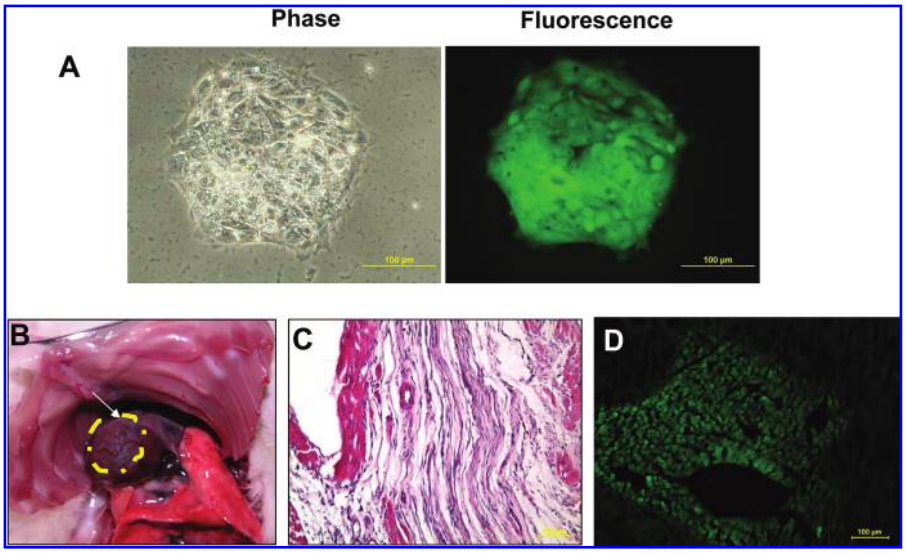
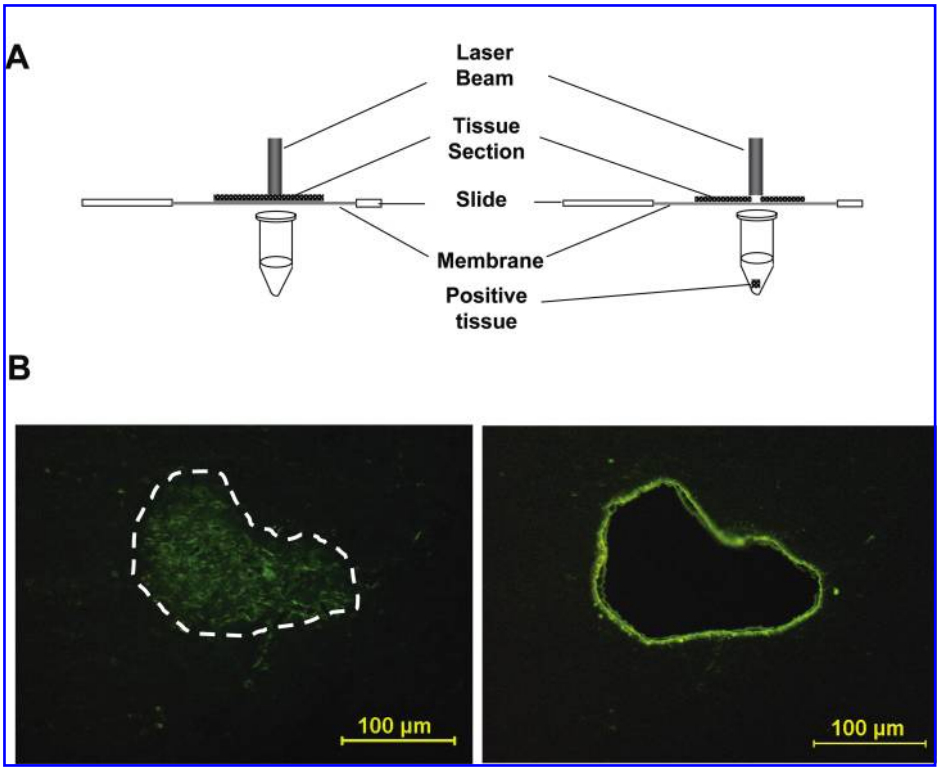


FIG. 1.



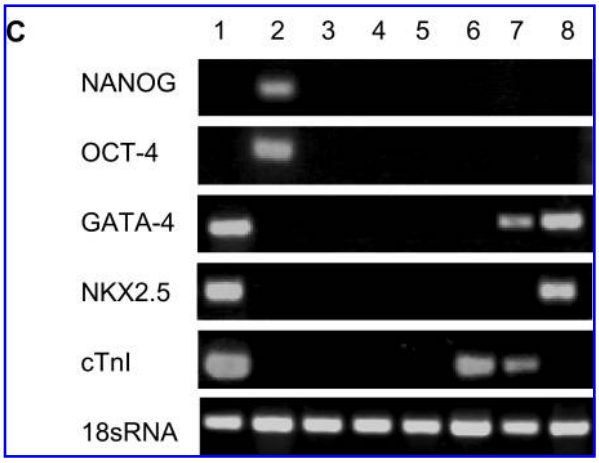


FIG. 2. Human cardiac-specific gene expression in the rat MI hearts injected with GFP-hES cells. (**A**) Schematic representation of the membrane mounted specimen in LCM. The left and right panels represent before and after LCM, respectively. (**B**) Representative fluorescent images showing before (*left*) and after (*right*) if LCM, respectively. Bar, 100 μm. (**C**) Human cardiac-specific genes are expressed in the GFP-positive areas dissected from rat MI hearts injected with GFP-hES cells by RT-RCR. Line 1, Human heart RNA purchased from Clontech (Cat#: 636532); line 2, undifferentiated GFP-transgenic hES cells; lines 3–5, sham-operated control rat hearts; lines 6–8, three MI rat hearts injected with undifferentiated GFP-transgenic hES cells.

levels of transcripts were determined by PCR. All primers were designed to be specific to human cardiac genes. The PCR primers used are described in Table 1.

RESULTS AND DISCUSSION

To date, regenerative medicine offers a promising approach for the treatment of MI. As the first step in addressing whether undifferentiated hES cells are a potentially therapeutical resource for the future MI treatment, we used hES cells with sustained GFP expression (9) to study the consequence of hES cell transplantation (Fig. 1A) in a rat model of MI. The GFP-transgenic hES cells were injected into the left ventricular base of the heart (Fig. 1B). As expected, fibrosis in the infarcted area of MI sections was observed after the MI (Fig. 1C). To evaluate the survival of hES cells, sections of the infarcted area were examined for GFP levels. Interestingly, there was robust green fluorescence in the area of rat hearts injected with hES cells (Fig. 1D), suggesting that hES cells can survive in the rat heart at least 2 months after MI. In addition, there was no teratoma formation observed in all tissues examined, including the heart, liver, and kidney (data not shown). Failure of teratoma formation may be due to the maintenance of proper signals provided by the injured environment that drives undifferentiated hES cells to directed differentiation without uncontrolled growth (13,14).

Among 11 hES cell transplantation rats, 5 had GFP fluorescence in the injected area of MI rat hearts. To determine whether cardiomyocytes were differentiated from the injected hES cells in the rat MI hearts, we dissected GFP-positive cell areas using a laser capture microdissection system (Fig. 2A,B). Intriguingly, there were human cardiac-specific gene including GATA-4, NKX2.5, and cardiac troponin I (cTnI) expressed in the GFP-positive samples from rat MI hearts injected with hES cells (lanes 6–8 in Fig. 2C). As expected, the sham control rat hearts did not express any human cardiac-specific gene (lanes 3–5 in Fig. 2C). In addition, there was no expression of undifferentiated hES cell-specific markers (e.g., Oct-4 or NANOG) detected in the transplantation group, suggesting that hES cells injected into the MI rat heart underwent differentiation.

Taken together, our results demonstrate for the first time that undifferentiated hES cells can be differentiated to the cardiac lineage under the local environmental signals in the heart. The signaling processes involved in directing ES cell differentiation to cardiomyocytes in the heart are only beginning to be studied actively. The continuation of these studies may provide a supplemental method for regenerating de novo cardiomyocytes to treat MI.

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