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Basic Study

Regenerative potential of mouse embryonic stem cell-derived PDGFR α ⁺ cardiac lineage committed cells in infarcted myocardium

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Abstract

BACKGROUND

Pluripotent stem cell-derived cardiomyocytes (CMs) have become one of the most attractive cellular resources for cell-based therapy to rescue damaged cardiac tissue.

AIM

We investigated the regenerative potential of mouse embryonic stem cell (ESC)-derived platelet-derived growth factor receptor- α (PDGFR α)⁺ cardiac lineage-committed cells (CLCs), which have a proliferative capacity but are in a morphologically and functionally immature state compared with differentiated CMs.

METHODS

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We induced mouse ESCs into PDGFR α ⁺ CLCs and α MHC⁺ CMs using a combination of the small molecule cyclosporin A, the rho-associated coiled-coil kinase inhibitor Y27632, the antioxidant Trolox, and the ALK5 inhibitor EW7197. We implanted PDGFR α ⁺ CLCs and differentiated α MHC⁺ CMs into a myocardial infarction (MI) murine model and performed functional analysis using transthoracic echocardiography (TTE) and histologic analysis.

RESULTS

Compared with the untreated MI hearts, the anterior and septal regional wall motion and systolic functional parameters were notably and similarly improved in the MI hearts implanted with PDGFR α ⁺ CLCs and α MHC⁺ CMs based on TTE. In histologic analysis, the untreated MI hearts contained a thinner ventricular wall than did the controls, while the ventricular walls of MI hearts implanted with PDGFR α ⁺ CLCs and α MHC⁺ CMs were similarly thicker compared with that of the untreated MI hearts. Furthermore, implanted PDGFR α ⁺ CLCs aligned and integrated with host CMs and were mostly differentiated into α -actinin⁺ CMs, and they did not convert into CD31⁺ endothelial cells or α SMA⁺ mural cells.

CONCLUSION

PDGFR α ⁺ CLCs from mouse ESCs exhibiting proliferative capacity showed a regenerative effect in infarcted myocardium. Therefore, mouse ESC-derived PDGFR α ⁺ CLCs may represent a potential cellular resource for cardiac regeneration.

Key words: Pluripotent stem cell; Embryonic stem cell; Cardiomyocyte; Myocardial infarction; Regeneration

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Core tip: We demonstrated that mouse embryonic stem cell-derived platelet-derived growth factor receptor- α ⁺ cardiac lineage-committed cells have proliferative capacity but are in a morphologically and functionally immature state compared with differentiated cardiomyocytes; these cells exerted a regenerative effect on infarcted myocardium.

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INTRODUCTION

Myocardial infarction (MI) and heart failure are the most common causes of death in patients with cardiovascular disease^[1]. Despite remarkable advances in therapeutic strategies for heart failure, such as novel drugs, ventricular assist device implantation, and heart transplantation, the burden of the disease remains high. Cardiac regeneration using stem cell therapy is an attractive therapeutic strategy to rescue damaged cardiac tissue^[2]. Among stem cell populations, pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), exhibit a higher efficacy in cardiomyocyte induction and expansion rate compared with adult stem cells^[2]. Indeed, previous large numbers of reports demonstrated functional improvement of damaged myocardium in murine, rodent, and porcine MI models that received PSC-derived cardiomyocytes (CMs)^[2-4].

However, the proliferative capacity of PSC-derived CMs is decreased after beating and terminal differentiation^[5]. Furthermore, there is no definite surface marker of differentiated PSC-derived CMs to facilitate purification^[6]. Recently, several studies have been conducted to identify a novel marker for cardiac progenitor or cardiac lineage-committed cells (CLCs), which are intermediate-stage cells between mesodermal cells and differentiated CMs with proliferative capacity^[7-10]. Our group previously established a novel class of cells from PSCs-platelet-derived growth factor receptor- α (PDGFR α)⁺ CLCs-induced using a combination of four specific modulators:

the mitochondrial permeability transition pore inhibitor cyclosporin A (CsA), the ROCK inhibitor Y27632, the antioxidant Trolox, and the activin A receptor type II-like kinase (ALK5) inhibitor EW7197 (collectively referred to here as CsAYTE)^[11]. This novel population of actively proliferating cells is cardiac lineage-committed but in a morphologically and functionally immature state compared with differentiated CMs^[11]. In the present study, we investigated the regenerative potential of mouse ESC-derived PDGFR α + CLCs in a murine MI model and compared their efficacy with differentiated CMs.

MATERIALS AND METHODS

Mouse ESCs and OP9 cell culture

EMG7 mouse ESCs, which have an α MHC promoter-driven enhanced GFP gene, E14Tg2a ESCs, and OP9 cells were generated as described previously^[12-14] and transferred to KAIST.

Generation of EMG7 mouse ESCs expressing tdTomato fluorescence

Lentiviruses were generated by transfecting FUtDW (Addgene plasmid 22478)^[15] with pMD2.G (Addgene plasmid 12259), pMDLg/pRRE (Addgene plasmid 12251) and pRSV-Rev (Addgene plasmid 12253)^[16] in 293T cells using jetPEI (Polypus-transfection). Supernatants were collected 48 h after transfection, filtered through a 0.45 μ m filter, and concentrated by Lenti-X concentrator (Clontech). Viral particles were resuspended in ESC medium with 4 mg/mL polybrene. EMG7 mouse ESCs were incubated in this medium for 24 h. Selection of ESCs was performed by FACS sorting.

Induction of mouse ESC-derived mesodermal precursor cells and CLCs

For the induction of Flk1⁺ mesodermal precursor cells (MPCs), ESCs were cultured without leukemia inhibitory factor (LIF, Millipore) and plated on a 0.1% gelatin-coated dish at a cell density between 1×10^3 and 1.5×10^3 cells cm² in the differentiation medium, which is α MEM (Invitrogen) containing 10% fetal bovine serum (FBS, Welgene), 0.1 mmol/L of 2-mercaptoethanol (Invitrogen), 2 mmol/L of L-glutamine (Invitrogen) and 50 U/mL of penicillin-streptomycin (Invitrogen). Medium was changed every other day for 4.5 d. At day 4.5, differentiated ESCs were harvested with 0.25% trypsin-EDTA (Invitrogen), and antigen retrieval was performed in the differentiation medium for 30 min in an incubator. Then, cells were washed using 2% FBS in phosphate buffered saline (PBS) and incubated with biotinconjugated anti-mouse Flk1 antibody (clone AVAS12a1, eBioscience) and anti-streptavidin MicroBeads (Miltenyi Biotec). Flk1⁺ MPCs were sorted by AutoMACS Pro Separator (Miltenyi Biotec). For induction of CLCs, sorted Flk1⁺ MPCs were plated onto the mitomycin C (AG Scientific)-treated confluent OP9 cells at a density of $5-10 \times 10^3$ cells cm² in the medium containing 3 μ g/mL of CsA, 10 μ mol/L of Y27632, 400 μ mol/L of Trolox, and 1 μ g/mL of EW7197 (CsAYTE)^[14,17]. The medium was refreshed every other day. Live images of differentiation process of CLCs and CMs were obtained using Axiovert 200M microscope (Carl Zeiss) equipped with AxioCam MRm (Carl Zeiss). Phase contrast images including beating CMs were obtained using an Infinity X digital camera and DpxView LE software (DeltaPix).

Flow cytometry analysis and cell sorting

The cells were harvested with 0.25% trypsin-EDTA or dissociation buffer (Invitrogen). To analyze live cells, antigen retrieval was performed in the differentiation medium for 30 min in an incubator and the cells were incubated for 20 min with the following antibodies: Allophycocyanin-conjugated anti-mouse PDGFR α (eBioscience, 17-1401, clone APA5, 1:100) and phycoerythrin/Cy7-conjugated anti-mouse Flk1 (BioLegend, 136414, clone AVAS12a1, 1:50). In live cell analysis and sorting, dead cells were excluded using 4,6-diamidino-2-phenylindole (DAPI, Sigma, D8417, 1:1000), and OP9 cells were excluded from Flk1⁺ MPC by gating in flow cytometry. The differentiated CMs were sorted using α MHC-GFP. Analyses and sorting were performed by FACS Aria II (Beckton Dickinson). Data were analyzed using FlowJo Version 7.5.4 software (TreeStar).

Animals

Twenty eight male 9-wk-old BALB/c nude mice were kept in the specific pathogen free before the experiment under a 12:12 h light/dark cycle with lights on at 8:00 AM. They were deprived of food for 18 h but permitted water ad libitum before surgery. Animal care and experimental procedures were performed to conform the NIH guidelines (Guide for the care and use of laboratory animals) and approved by the

Animal Care Committee of KAIST (KA2013-40).

Preparation of acute MI model in mouse and cell transplantation

All mice were anesthetized through an intraperitoneal injection of a combination of anesthetics (80 mg/kg ketamine, 12 mg/kg xylazine) before any procedures. After intubation, the mice were ventilated with room air (SomnoSuite™, Kent scientific). MI was induced by exposing the heart by left thoracotomy and permanently ligating the proximal portion of left anterior descending coronary artery with an 8-0 prolene thread under respiratory support. After ligating the proximal portion of left anterior descending coronary artery, infarction of the anterior wall of left ventricle was confirmed in each mouse by the presence of a pale anterior wall and myocardial hypokinesis. Immediately after ligation of coronary artery and the confirmation of infarction, 100 μ L PBS containing 1×10^6 PDGFR α ⁺ CLCs or α MHC⁺ CMs were intramyocardially injected with a 31-gauge (0.25 mm) insulin syringe into the 3 different sites along the borderline of the infarcted area.

Transthoracic echocardiographic analysis

Transthoracic echocardiography (TTE) studies were performed (VIVID 7 dimension system, General Electric-Vingmed Ultrasound) 15 d after MI surgery and cell implantation. Images were obtained using an i13L transducer (5.3-14.0 MHz, GE Healthcare) with high temporal and spatial resolution. Two-dimensionally targeted M-mode parameters were measured at a level of papillary muscle in parasternal short axis view during 6 consecutive cardiac beats. All measurements were performed in a blind fashion according to the guidelines of American Society for Echocardiography.

Histologic and morphometric analyses

Before sacrifice, mice were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). For hematoxylin and eosin (H and E) staining, samples were fixed overnight with 4% paraformaldehyde and embedded in paraffin after tissue processing. For immunofluorescence staining, samples were fixed in 4% paraformaldehyde, dehydrated in 20% sucrose solution overnight, and embedded in tissue freezing medium (Leica). Samples were blocked with 5% goat (or donkey) serum in 0.01% Triton X-100 in PBS and then incubated overnight at 4 °C with the following primary antibodies: Mouse anti- α -actinin monoclonal antibody (Sigma Aldrich, A7811, clone EA-53, 1:100) or rabbit anti- α -actinin polyclonal antibody (Abcam, ab68167, clone EP2529Y, 1:100), rabbit anti-Ki-67 polyclonal antibody (Abcam, ab15580, 1:200), mouse anti- α -SMA monoclonal antibody (Sigma Aldrich, A2547, clone 1A4, 1:500), hamster anti-CD31 monoclonal antibody (Millipore, MAB1398Z, clone 2H8, 1:400), and rabbit anti-GFP polyclonal antibody (Millipore, AB3080, 1:200). After several washes, the samples were incubated for 2 h at RT with the following secondary antibodies: Cy5-conjugated anti-mouse IgG (Jackson ImmunoResearch, 715-175-150, 1:1000) and Cy3-, Cy5-, FITC-conjugated anti-rabbit IgG antibodies (Jackson ImmunoResearch, 711-165-152, 711-175-152, 711-095-152, 1:1000). Then the samples were mounted with fluorescent mounting medium (DAKO) and immunofluorescent images were acquired using a Zeiss LSM780 confocal microscope (Carl Zeiss). To calculate capillary density, number of CD31⁺ endothelial cells was counted per random 0.5 mm² area in the infarcted myocardium at 15 d after cell implantation. To analyze the regenerative effects of host myocardium, number of Ki-67⁺/ α -actinin⁺ CMs was counted per 104 nuclei in the peri-infarcted area, ranged within 200 μ m from infarcted region, at 3 and 15 d after cell implantation. Images were analyzed using ImageJ software (<http://imagej.nih.gov/ij/>, 1.47V, NIH, United States).

Statistical analysis

Values are presented as mean \pm SD. For continuous data, statistical significance was determined with the Mann-Whitney *U* test between 2 groups and the Kruskal-Wallis test followed by Tukey's honest significant difference test with ranks or multiple-group comparison. Statistical analysis was performed with SAS 9.4 (SAS Institute Inc). Statistical significance was set at $P < 0.05$ or 0.01.

RESULTS

Implantation of PDGFR α ⁺ CLCs and α MHC⁺ CMs equally improves contractile function and structure in infarcted heart

To investigate the regenerative potential of PDGFR α ⁺ CLCs and α MHC⁺ CMs, cells were sorted, and approximately 1×10^6 of each were implanted into the left

ventricular myocardium after inducing acute MI. The results of the recipient groups were compared with those of MI hearts without implantation. Analyses were performed at 2 wk after implantation of cells (Figure 1A). To trace the implanted cells in the infarcted heart, we induced PDGFR α ⁺ CLCs from ESCs expressing tdTomato fluorescence (Figure 1B). As shown in Figure 1C, the implanted cells were mainly distributed along several myocardial cavities 1 h after implantation.

First, to evaluate the functional recovery of infarcted hearts after cell implantation, we performed TTE 14 d after implantation. Compared with that in untreated MI hearts, the anterior and septal regional wall motion was notably and similarly improved (see arrowheads in Figure 2A) in the MI hearts implanted with PDGFR α ⁺ CLCs (hereafter designated as MI+PDGFR α ⁺ CLCs) and α MHC⁺ CMs (designated as MI+ α MHC⁺ CMs). Moreover, the left ventricular internal dimension during systole of both MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs was approximately 12%–23% less compared with that of untreated MI hearts (Figure 2B). Both MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs also showed significant and similar improvements in systolic functional parameters, which included an ejection fraction increased by 20.0/15.6% and fractional shortening increased by 9.5/7.2%, respectively, compared with those of untreated MI hearts (Figure 2C). All TTE parameters are summarized in Table 1. These findings indicate that the implantation of PDGFR α ⁺ CLCs and α MHC⁺ CMs had similar beneficial effects in the functional recovery of acutely infarcted hearts. Next, to confirm whether the implanted cells were properly engrafted to the infarcted myocardium, we performed histologic analyses at 15 d after implantation. Overall, the gross sizes of MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs were smaller than that of untreated MI hearts (Figure 2D). Hematoxylin and eosin staining showed that untreated MI hearts had a thinner ventricular wall (0.19 mm) than did controls, while the ventricular walls of MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs were similarly thicker (0.47 mm and 0.39 mm, respectively) compared with that of untreated MI hearts (Figures 2E and F).

Integration, differentiation, proliferation, and survival of implanted PDGFR α ⁺ CLCs in the infarcted heart

Importantly, implanted PDGFR α ⁺ CLCs and α MHC⁺ CMs were visible as tdTomato⁺/ α -MHC-GFP⁺ cells aligned and integrated with host CMs (Figure 3A). Implanted PDGFR α ⁺ CLCs and α MHC⁺ CMs were mostly differentiated into α -actinin⁺ CMs, and they did not convert into CD31⁺ endothelial cells or α SMA⁺ mural cells (Figures 3B and C). Moreover, CD31⁺ blood vessels in the infarcted area increased by 2.1- and 1.8-fold in MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs at day 15 after implantation (Figures 4A and B), while the numbers of Ki-67⁺ CMs also transiently increased equally by 2.4-fold at day 3; no such increases were detected at day 15 in both groups (Figures 4C and D). Thus, in addition to integration of implanted MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs into the host myocardium, paracrine effects of MI+PDGFR α ⁺ CLCs and MI+ α MHC⁺ CMs appeared to be involved in the functional recovery of acutely infarcted hearts. Both types of implanted cells persisted up to 60 d after implantation (Figure 4E), which was the longest observation period in this study.

DISCUSSION

In the present study, we demonstrated the regenerative potential of mouse ESC-derived PDGFR α ⁺ CLCs in a murine MI model. Implantation of PDGFR α ⁺ CLCs and α MHC⁺ CMs equally improved the contractile function and structure in the infarcted heart. Notably, implanted PDGFR α ⁺ CLCs were well integrated with host CMs and mostly differentiated into CMs.

Various transcription factors and cell-surface markers of cardiac progenitors or CLCs have been identified in previous studies^[6]. Our group developed PDGFR α ⁺ CLCs induced by CsAYTE, which significantly enhanced the commitment of mesodermal cells to CLCs; in addition, the PDGFR α ⁺ CLCs can spontaneously further differentiate into CMs without additional manipulation or stimulation under *in vitro* conditions^[11]. However, there are few studies regarding the engraftment and regenerative potential of cardiac progenitors or CLCs compared with differentiated CMs after implantation under *in vivo* pathologic conditions^[18,19]. Takeda *et al*^[7] recently found that human iPSC-derived CM-fated progenitors from a subpopulation of kinase insert domain receptor (KDR)⁺ and PDGFR α ⁺ cells express CD82^[7]. Consistent with our findings, purified CD82⁺ cells gave rise to CMs under both *in vitro* and *in vivo* conditions^[7]. Interestingly, CD82⁺ cells showed considerably greater engraftment than differentiated vascular cell adhesion molecule 1 (VCAM1)⁺ CMs after transplantation

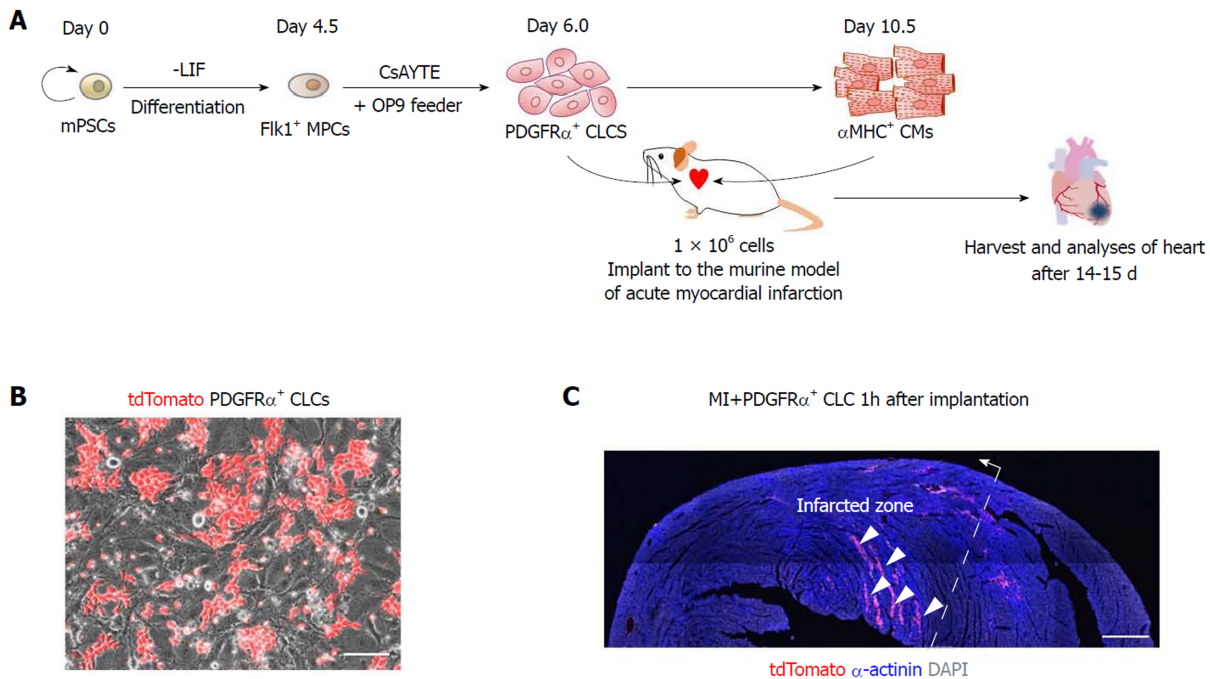


Figure 1 Implantations of platelet-derived growth factor receptor- α ⁺ cardiac lineage-committed cells and α MHC⁺ cardiomyocytes in the acute myocardial infarction murine model. **A**: Experimental scheme of implanting either platelet-derived growth factor receptor- α (PDGFR α)⁺ cardiac lineage-committed cells (CLCs) or α MHC⁺ cardiomyocytes (CMs) into acute myocardial infarction (MI) murine model. Analyses were performed at 2 wk after implantation of approximately 1×10^6 cells of PDGFR α ⁺ CLCs or α MHC⁺ CMs into the left ventricular myocardium of acute MI murine model; **B**: Live cell image showing tdTomato⁺ cells during induction of PDGFR α ⁺ CLCs from embryonic stem cells. Scale bars, 100 μ m; **C**: Representative confocal image showing implanted tdTomato⁺ PDGFR α ⁺ CLCs in the myocardial spaces (arrowheads) of the infarcted zone (dotted line and arrow), which was formed by ligation of coronary artery 1 h prior to the implantation. Scale bar, 500 μ m. CLCs: Cardiac lineage-committed cells; CMs: Cardiomyocytes; PSCs: Pluripotent stem cells; ESCs: Embryonic stem cells; LIF: Leukemia inhibitory factor.

to the subrenal space^[7]. These data indicated that the proliferative capacity of CLCs is higher than that of differentiated CMs under *in vivo* conditions. Furthermore, CD82⁺ cells primarily differentiated into CMs within infarcted hearts at approximately 95% efficiency; nevertheless, there were no data related to functional and structural recovery in the infarcted hearts^[7]. The LIM-homeodomain transcription factor ISL1 is the most well-known marker of cardiac progenitors, and recent studies demonstrated that ISL1⁺ cardiac progenitors also exhibit regenerative potential in the infarcted heart^[20,21]. Another developed strategy, direct reprogramming, was used to generate proliferative induced cardiac progenitors from fibroblasts with cardiac-specific transcription factors (Mesp1, Tbx5, Gata4, Nkx2.5, and Baf60c), and these reprogrammed cells were revealed to have regenerative potential in MI^[22,23]. Collectively, the previous and current data provide compelling evidence that cardiac progenitors or CLCs are potential cellular resources for cardiac regeneration.

However, our data failed to demonstrate the superior regenerative effect of proliferative PDGFR α ⁺ CLCs compared with differentiated α MHC⁺ CMs after implantation, consistent with a previous report^[19]. Although PDGFR α ⁺ CLCs exhibit more proliferative capacity than differentiated α MHC⁺ CMs, their expansion might be restricted owing to the limited space of the myocardium, especially in a small mouse model. Further experiments using large animal models, such as swine or non-human primates, might be necessary to confirm the regenerative effect of CLCs. In addition, the pathologic microenvironment of damaged heart might affect the proliferation and survival of implanted cells. Indeed, the previous and current data demonstrated that the engraftment of implanted CLCs and CMs was gradually decreased with time. Despite suboptimal engraftment and eventual death of the implanted cells in infarcted myocardium, the regenerative effect of implanted cells might result from differential paracrine effects^[24]. Recent data demonstrated the significant upregulation of promigratory, proangiogenic, and antiapoptotic gene expression in the infarcted myocardium of groups treated with CMs compared with groups treated with PSCs and the controls^[24]. Our data also revealed enhanced angiogenesis after implantation of PDGFR α ⁺ CLCs and α MHC⁺ CMs. Therefore, the previous and current data suggested that not only direct integration but also the paracrine effect of implanted CLCs and CMs contributes to cardiac regeneration^[24]. Further studies are needed to better understand the therapeutic mechanisms following transplantation of CLCs and to enhance engraftment.

Table 1 Echocardiographic parameters of Control, MI, MI+PDGFR α ⁺ CLCs, and MI+ α MHC⁺ CMs groups

| Group | LVIDd (mm) | LVIDs (mm) | IVSd (mm) | IVSs (mm) | LVPWd (mm) | LVPWs (mm) | LVEDV (mL) | LVESV (mL) | LVSV (mL) | LVEF (%) | FS (%) |
|---|---------------|--------------------------|--------------|--------------|---------------|---------------|--------------------------|--------------------------|--------------|--------------------------|--------------------------|
| Control (n = 7) | 4.46 ± 0.23 | 3.14 ± 0.14 | 0.76 ± 0.07 | 1.08 ± 0.05 | 0.76 ± 0.10 | 1.11 ± 0.07 | 0.22 ± 0.04 | 0.08 ± 0.01 | 0.14 ± 0.03 | 62.7 ± 2.52 | 29.2 ± 1.72 |
| MI (n = 7) | 5.57 ± 0.60 | 5.09 ± 0.54 | 0.67 ± 0.04 | 0.71 ± 0.07 | 0.74 ± 0.09 | 1.03 ± 0.17 | 0.42 ± 0.14 | 0.32 ± 0.10 | 0.10 ± 0.03 | 22.5 ± 2.91 | 8.61 ± 1.12 |
| MI+PDGFR α ⁺ CLCs (n = 7) | 4.96 ± 0.38 | 3.92 ^b ± 0.54 | 0.68 ± 0.10 | 0.87 ± 0.27 | 0.68 ± 0.06 | 0.99 ± 0.13 | 0.21 ^b ± 0.14 | 0.12 ^b ± 0.08 | 0.09 ± 0.06 | 42.4 ^b ± 4.38 | 18.1 ^b ± 2.54 |
| MI+ α MHC ⁺ CMs (n = 7) | 5.15 ± 0.56 | 4.38 ± 0.60 | 0.65 ± 0.04 | 0.69 ± 0.04 | 0.74 ± 0.07 | 1.08 ± 0.12 | 0.28 ^a ± 0.14 | 0.18 ^b ± 0.10 | 0.10 ± 0.05 | 38.1 ^b ± 6.86 | 15.8 ^b ± 4.79 |

^aP < 0.05 vs MI.^bP < 0.01 vs MI.

The parameters present as mean ± SD. LVIDd: Left ventricular internal diameter diastole; LVIDs: Left ventricular internal dimension systole; IVSd: Interventricular septal diastole; IVSs: Interventricular septal systole; LVPWd: Left ventricular posterior wall diameter diastole; LVPWs: Left ventricular posterior wall diameter systole; LVEDV: Left ventricular end diastolic volume; LVESV: Left ventricular end systolic volume; LVSV: Left ventricular stroke volume; LVEF: Left ventricular ejection fraction; FS: Fractional shortening; CLCs: Cardiac lineage-committed cells; CMs: Cardiomyocytes; MI: Myocardial infarction.

Proper electromechanical integration of PSC-derived CMs into host myocardium is crucial for preventing fatal arrhythmia after transplantation^[1]. In a recent study, Chong *et al*^[25] reported remuscularization of infarcted myocardium after injection of human ESC-derived CMs into non-human primate models of MI^[26]. These grafts formed electromechanical junctions with the host myocardium and beat in synchrony, but ventricular arrhythmias were noted after transplantation^[25]. Another recent study showed that monkey iPSC-derived CMs improved cardiac contractile function after transplantation into infarcted monkey hearts; nonetheless, the incidence of ventricular tachycardia was transiently but significantly increased^[27]. In our study, we could not evaluate the occurrence of ventricular arrhythmia because of the technical difficulties associated with the mouse model. Therefore, further studies using large animal models might be necessary to confirm the arrhythmogenic effect of proliferating CLCs compared with differentiated CMs after transplantation into infarcted heart.

In conclusion, PDGFR α ⁺ CLCs served as the potential donor population for cardiac regeneration, and our findings provide conceptual and technical advances in stem cell therapy for cardiac regeneration.

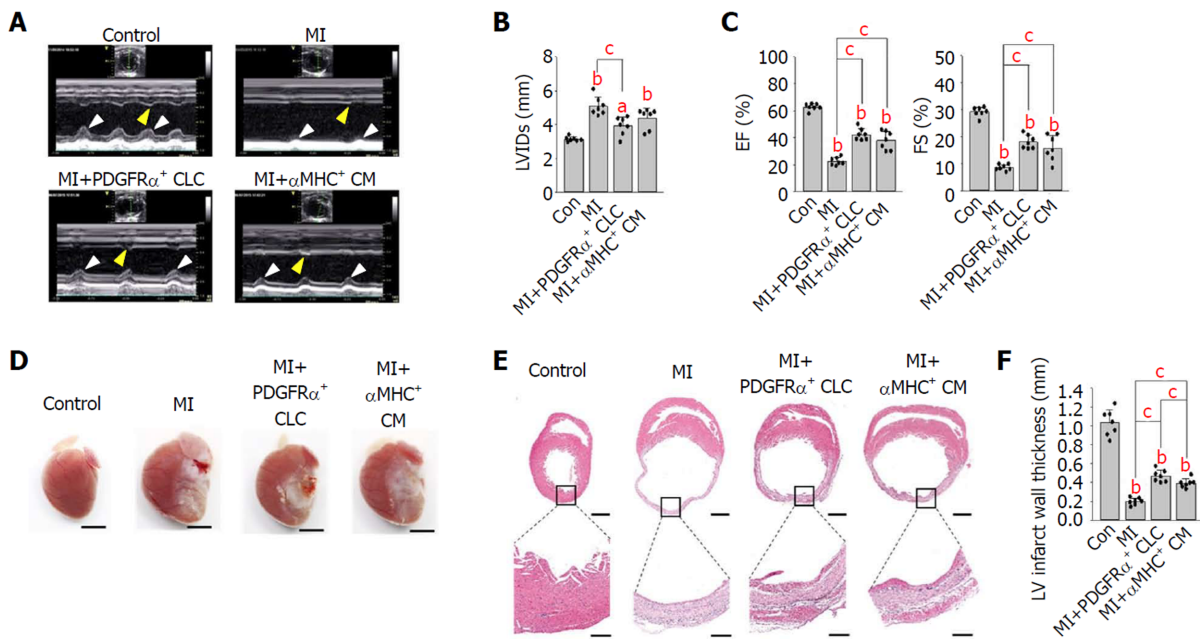


Figure 2 Implantations of platelet-derived growth factor receptor- α + cardiac lineage-committed cells and α MHC+ cardiomyocytes equally improves contractile function and structure in the infarcted heart. **A:** Representative M-mode transthoracic echocardiography views of control, myocardial infarction (MI), MI+ platelet-derived growth factor receptor- α (PDGFR α)⁺ cardiac lineage-committed cells (CLCs), and MI+ α MHC⁺ cardiomyocytes (CMs). Improved anterior (white arrowheads) and septal (yellow arrowheads) regional wall motion are observed in the left ventricles of MI+PDGFR α + CLCs and MI+ α MHC+ CMs; **B and C:** Quantifications of left ventricular internal dimension in systole (mm), ejection fraction (%) and fractional shortening (%). Each group, $n = 7$. ^a $P < 0.05$ and ^b $P < 0.01$ vs Con; ^c $P < 0.01$ vs MI; **D:** Gross images of hearts in control, MI, MI+PDGFR α + CLCs, and MI+ α MHC+ CMs. Scale bars, 2.5 mm; **E:** H and E staining of mid-sectioned hearts of control, MI, MI+PDGFR α + CLCs, and MI+ α MHC+ CMs. Scale bars, 1 mm and 50 μ m in the upper and lower panels, respectively; **F:** Quantifications of the thickness (mm) of left ventricle in the infarcted region. Each group, $n = 7$. ^b $P < 0.01$ vs Con; ^c $P < 0.01$ vs MI or MI+PDGFR α + CLCs. CLCs: Cardiac lineage-committed cells; CMs: Cardiomyocytes; MI: Myocardial infarction.

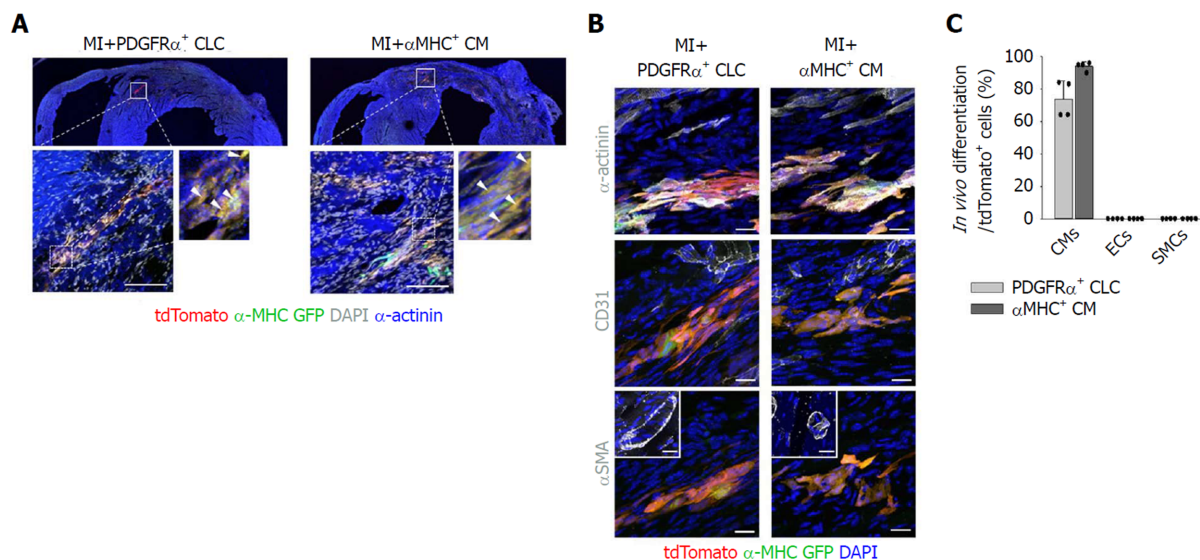


Figure 3 Integration and differentiation of implanted platelet-derived growth factor receptor- α + cardiac lineage-committed cells in the infarcted heart. **A:** tdTomato-tagged platelet-derived growth factor receptor- α (PDGFR α)⁺ cardiac lineage-committed cells (CLCs) or α MHC⁺ cardiomyocytes (CMs) were implanted into the infarcted myocardium and integration was confirmed by immunostaining. tdTomato⁺/ α -MHC-GFP⁺ cells (white arrowheads) are implanted PDGFR α + CLCs and α MHC+ CMs. Scale bars, 100 μ m; **B:** Representative confocal images showing differentiation of tdTomato⁺ PDGFR α + CLCs and α MHC+ CMs into cardiomyocytes 15 d after the implantation. α SMA-expressing cells were negative for tdTomato or α -MHC-GFP signal as shown in the inset. Scale bars, 25 μ m; **C:** Percentages of α -actinin⁺ CMs, CD31 endothelial cells and α SMA⁺ smooth muscle cells of the implanted PDGFR α + CLCs and α MHC+ CMs. Each group, $n = 4$. Scale bars, 20 μ m. CLCs: Cardiac lineage-committed cells; CMs: Cardiomyocytes; MI: Myocardial infarction; ECs: Endothelial cells; SMCs: Smooth muscle cells.

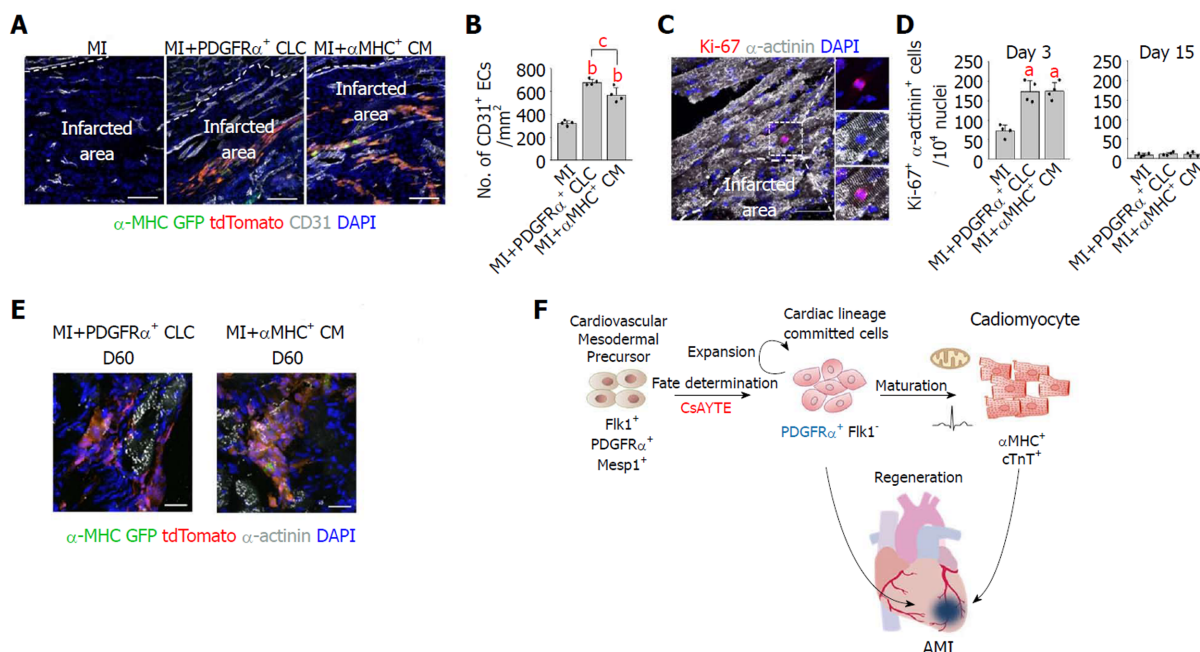


Figure 4 Proliferation and survival of implanted platelet-derived growth factor receptor- α cardiac lineage-committed cells in the infarcted heart. **A:** Representative confocal image of Ki-67⁺ host cardiomyocytes in the peri-infarcted region 3 d after cell injection. Dotted-lined rectangular region is magnified in right. Scale bar, 50 μ m; **B:** Quantifications of Ki-67⁺ α -actinin⁺ cardiomyocytes per 10⁴ nuclei in the peri-infarcted region 3 and 15 d after the implantation. Each group, $n = 4$. ^a $P < 0.05$ vs myocardial infarction (MI); **C:** Representative confocal images of revascularization within the infarcted areas 15 d after the implantation. Scale bars, 100 μ m; **D:** Quantifications of capillary density (No. of CD31⁺ ECs/mm²) within the infarcted areas. Each group, $n = 4$. ^b $P < 0.01$ vs MI; ^c $P < 0.01$ vs MI+ platelet-derived growth factor receptor- α (PDGFR α)⁺ cardiac lineage-committed cells (CLCs); **E:** Representative confocal images of tdTomato⁺ PDGFR α ⁺ CLCs and α MHC⁺ CMs 60 d after the implantation. Three independent experiments showed similar findings. Scale bars, 20 μ m; **F:** Schematic diagram illustrating the regenerative potential of PDGFR α ⁺ CLCs in the infarcted heart. CLCs: Cardiac lineage-committed cells; CMs: Cardiomyocytes; MI: Myocardial infarction.

ARTICLE HIGHLIGHTS

Research background

Pluripotent stem cell (PSC)-derived cardiomyocytes (CMs) have become one of the most attractive cellular resources for cell-based therapy to rescue damaged cardiac tissue.

Research motivation

The proliferative capacity of PSC-derived CMs is decreased after beating and terminal differentiation. Furthermore, there is no definite surface marker of differentiated PSC-derived CMs to facilitate purification.

Research objectives

We investigated the regenerative potential of mouse embryonic stem cell-derived PDGFR α ⁺ cardiac lineage-committed cells (CLCs) in a murine myocardial infarction (MI) model and compared their efficacy with differentiated CMs.

Research methods

We implanted platelet-derived growth factor receptor- α (PDGFR α)⁺ CLCs and differentiated α MHC⁺ CMs into a MI murine model and performed functional analysis using transthoracic echocardiography (TTE) and histologic analysis.

Research results

Compared with the untreated MI hearts, the anterior and septal regional wall motion and systolic functional parameters were notably and similarly improved in the MI hearts implanted with PDGFR α ⁺ CLCs and α MHC⁺ CMs based on TTE. In histologic analysis, the untreated MI hearts contained a thinner ventricular wall than did the controls, while the ventricular walls of MI hearts implanted with PDGFR α ⁺ CLCs and α MHC⁺ CMs were similarly thicker compared with that of the untreated MI hearts. Furthermore, implanted PDGFR α ⁺ CLCs aligned and integrated with host CMs and were mostly differentiated into α -actinin⁺ CMs, and they did not convert into CD31⁺ endothelial cells or α SMA⁺ mural cells.

Research conclusions

PDGFR α ⁺ CLCs from mouse ESCs exhibiting proliferative capacity showed a regenerative effect in infarcted myocardium. Therefore, mouse ESC-derived PDGFR α ⁺ CLCs may represent a potential cellular resource for cardiac regeneration.

Research perspectives

PDGFRα⁺ CLCs served as the potential donor population for cardiac regeneration, and our findings provide conceptual and technical advances in stem cell therapy for cardiac regeneration.

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