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Human Cardiac Progenitor Cells Engineered With Pim-I Kinase Enhance Myocardial Repair

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Objectives The study goal was to demonstrate the enhancement of human cardiac progenitor cell (hCPC) reparative and regenerative potential by genetic modification for the treatment of myocardial infarction.

Background Regenerative potential of stem cells to repair acute infarction is limited. Improved hCPC survival, proliferation, and differentiation into functional myocardium will increase efficacy and advance translational implementation of cardiac regeneration.

Methods hCPCs isolated from the myocardium of heart failure patients undergoing left ventricular assist device implantation were engineered to express green fluorescent protein (hCPCe) or Pim-1-GFP (hCPCeP). Functional tests of hCPC regenerative potential were performed with immunocompromised mice by using intramyocardial adoptive transfer injection after infarction. Myocardial structure and function were monitored by echocardiographic and hemodynamic assessment for 20 weeks after delivery. hCPCe and hCPCeP expressing luciferase were observed by using bioluminescence imaging to noninvasively track persistence.

Results hCPCeP exhibited augmentation of reparative potential relative to hCPCe control cells, as shown by significantly increased proliferation coupled with amelioration of infarction injury and increased hemodynamic performance at 20 weeks post-transplantation. Concurrent with enhanced cardiac structure and function, hCPCeP demonstrated increased cellular engraftment and differentiation with improved vasculature and reduced infarct size. Enhanced persistence of hCPCeP versus hCPCe was revealed by bioluminescence imaging at up to 8 weeks post-delivery.

Conclusions Genetic engineering of hCPCs with Pim-1 enhanced repair of damaged myocardium. Ex vivo gene delivery to modify stem cells has emerged as a viable option addressing current limitations in the field. This study demonstrates that efficacy of hCPCs from the failing myocardium can be safely and significantly enhanced through expression of Pim-1 kinase, setting the stage for use of engineered cells in pre-clinical settings. (J Am Coll Cardiol 2012;xx:xxx) © 2012 by the American College of Cardiology Foundation

The human heart harbors an adult stem cell population consistent with true characteristics of stemness such as self-renewal (1), clonogenicity (2), and multilineage differentiation potential (3). These "cardiac stem cells" populate the heart in highly conserved atrial and ventricular niches that regulate myocyte turnover (4). Recent evidence demonstrates the ability of resident human cardiac cells to differentiate into mechanically integrated cardiomyocytes (3,5) as well as vascular smooth muscle and endothelial cells, thereby supporting cardiac regeneration (6). Adoptive transfer of human cardiac stem cells results in modest repair due in part to lack of survival, proliferation, and commitment of the transplanted cells after myocardial infarction. Therapeutic stem cell performance is further complicated by the elderly target population for regenerative therapy, which possesses a stem cell pool adversely affected by age concomitant with up-regulation of senescence markers (4), shorter telomere length (3), and decreased metabolic activity (7).

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Abbreviations and Acronyms

BLI = bioluminescence imaging

Dex = dexamethasone

EF = ejection fraction

eGFP = enhanced green fluorescent protein

FS = fractional shortening

hCPCs = human cardiac progenitor cells

hCPCe = human cardiac progenitor cells overexpressing green fluorescent protein

hCPCeP = human cardiac progenitor cells overexpressing Pim-1

Luc = luciferase

vWF = von Willebrand factor

These detrimental insults collectively compromise the regenerative ability of stem cells in this aged population, limiting their use for autologous therapy.

Modification of human cardiac progenitor cells (hCPCs) to enhance proliferation, survival, and commitment increases effectiveness and buttresses use of stem cells as a viable therapeutic modality. Ex vivo genetic modification is an effective strategy to enhance stem cell function (8,9). Previously, our group has shown that Pim-1 kinase, a downstream effector of Akt, enhances cell survival (10) and metabolic activity (11), attenuates apoptosis (12), and maintains mitochondrial integrity (11,13). Mechanistically, apoptotic proteins such as Bad (14) and cell cycle proteins including p21 (15) have been identified as Pim-1 substrates. In the heart, Pim-1 is induced as a consequence of stress or pathological insult (10). Pim-1 also positively regulates neovasculogenesis (16), which forms an integral part of the myocardial repair response. Proof-of-principle studies performed with murine CPCs in a syngeneic system demonstrate that Pim-1 augments reparative processes after myocardial injury, with improved cellular survival, persistence, and differentiation of engrafted cells into cardiac lineages 32 weeks after transplantation (17). However, potentiation of hCPCs derived from patients who have heart failure presents a different challenge from the healthy young CPCs used in syngeneic murine studies. Utility of hCPCs as a viable therapeutic option would be further improved by interventional strategies designed to overcome inherent limitations in aged or pathologically challenged myocardial tissue.

Applicability of genetic modification to the clinical setting requires progression into an experimental model with hCPCs obtained from the target population of aged patients who would be candidates for regenerative therapy: individuals undergoing left ventricular assist device implantation as a bridge to transplant or destination therapy. In the present study, we demonstrate that hCPCs isolated from failing myocardium and modified with Pim-1 possess enhanced reparative potential relative to control hCPCs. Improvements mediated by hCPCs modified with Pim-1 were evident structurally and functionally, with durable human cellular persistence, engraftment, and acquisition of phenotypic characteristics consistent with differentiated myocardium. These results validate the utility of Pim-1 kinase as a molecular interventional approach to enhance hCPC-mediated regeneration, even when derived from a failing human heart.

Methods

See the Online Appendix for an explanation of the study methods.

Results

Pim-1 overexpression characterization in hCPCs. hCPCs are negative for hematopoietic markers CD34, CD45, CD2, CD16, and CD31 and are positive for c-kit (Online Figs. 1A and 1B). Human cardiac progenitor cells overexpressing green fluorescent protein (hCPCe) and human cardiac progenitor cells overexpressing Pim-1 (hCPCeP) were transduced with lentiviral vectors Lv-egfp and Lv-egfp+pim1 (Online Fig. 1C). Efficiency of modification after lentiviral transduction was 74.25% and 75.95% for hCPCe and hCPCeP, respectively, as measured by using flow cytometric analyses for enhanced green fluorescent protein (eGFP) (Online Fig. 1D). Expression of eGFP and Pim-1 in hCPCe and hCPCeP was confirmed by using immunoblot analysis (Online Fig. 1E). Karyotype analyses revealed normal chromosome content in either hCPCe or hCPCeP, indicating normal mitotic chromosomal segregation in the genetically engineered cells (Online Fig. 1F).

Enhanced proliferation, mitochondrial activity, and TRAP-activity in hCPCeP. Proliferation was increased in hCPCeP relative to hCPCs and hCPCe ($p < 0.001$) at day 3 as measured by using CyQUANT assay (Fig. 1A). Conversely, using the Pim-1 pharmacological blocker quercetagetin, proliferation was abrogated at day 1 ($p < 0.01$) and day 3 ($p < 0.001$), demonstrating involvement of Pim-1 in the proliferative response (Fig. 1B). hCPCeP also showed increased metabolic activity compared with hCPCs and hCPCe at day 3 ($p < 0.001$), as measured by using MTT assay (Fig. 1C). Similarly, relative telomerase reverse transcriptase activity measured by using a TRAP assay was significantly improved ($p < 0.05$) in hCPCeP compared with hCPCe (Fig. 1D). Increased levels of phospho-p21, a Pim-1 target substrate, confirm functional activity of expressed Pim-1 protein by using immunoblot analysis (Figs. 1E and 1F). Collectively, these results indicate that Pim-1 modification of hCPC confers phenotypic properties consistent with beneficial cellular signaling.

Increased cardiac commitment of hCPCeP after dexamethasone differentiation. Markers of cardiogenic lineage commitment, including MEF2C, von Willebrand factor (vWF), and GATA-6, were up-regulated in hCPCeP relative to hCPCe after dexamethasone (Dex) treatment, as confirmed by using quantitative real-time polymerase chain reaction analysis (Fig. 2A) and immunocytochemistry (Fig. 2B). MEF2C and GATA-6 signal were absent in hCPCe or hCPCeP before Dex treatment, with sparse reactivity for vWF in hCPCeP (Fig. 2B) and marked increases in immunolabeling for all 3 markers as well as morphological remodeling of hCPCe and hCPCeP after Dex exposure (Fig. 2C). Morphological remodeling (flattening) of cells treated with Dex was consistent with

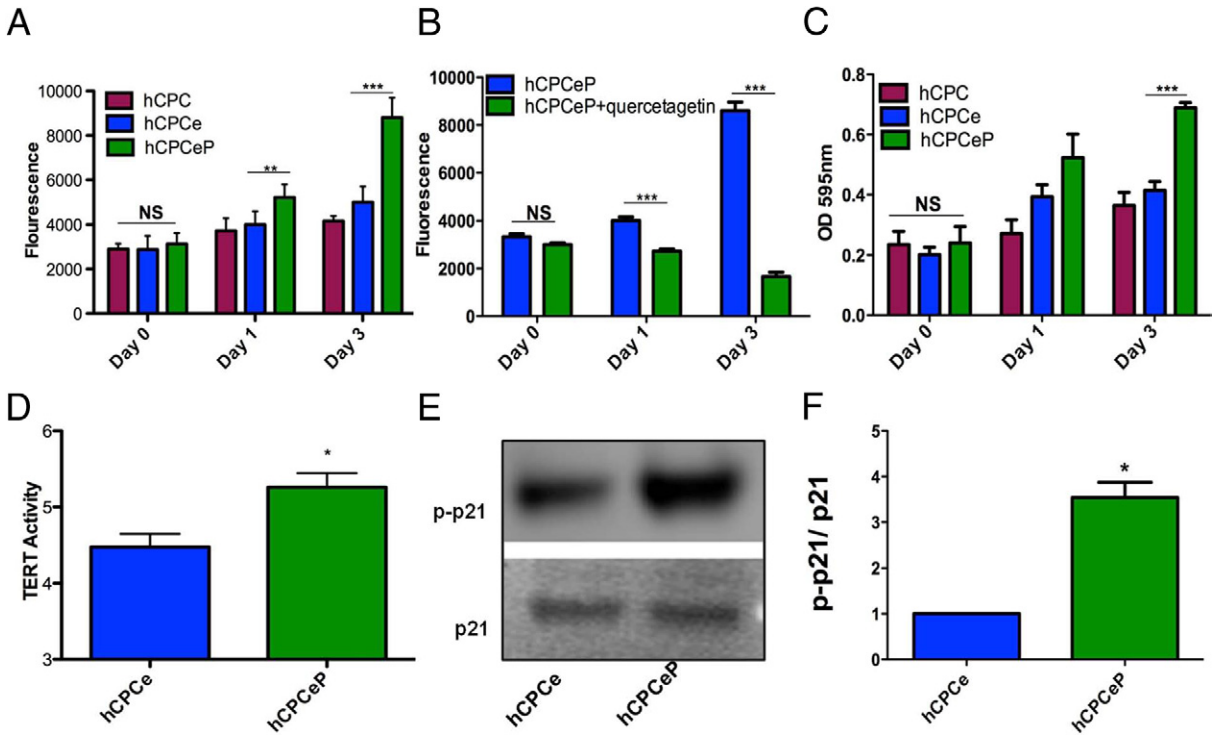

Figure 1 **Enhancement of Proliferation, Mitochondrial Activity, and Telomerase Activity in hCPCeP**

(A) CyQUANT assay: human cardiac progenitor cells overexpressing Pim-1 (hCPCeP) exhibited enhanced proliferation compared with human cardiac progenitor cells (hCPCs) and human cardiac progenitor cells overexpressing green fluorescent protein (hCPCe) for 3 days (n = 4). **(B)** CyQUANT assay: hCPCeP treated with 10 μM of quercetagetin show decreased proliferation relative to nontreated hCPCeP (n = 4). **(C)** Metabolic activity measured by using MTT reagent: hCPCeP demonstrated improved metabolic activity relative to hCPC and hCPCe (n = 4). **(D)** Telomerase reverse transcriptase (TERT) activity is significantly higher in hCPCeP relative to hCPCe (n = 3). **(E)** Immunoblot analysis for p21 and p-p21. **(F)** Quantitation of immunoblot (n = 3). *p < 0.01, **p < 0.001, ***p < 0.05.

previous findings (17–19). These results indicate augmentation of lineage commitment signals in hCPCeP relative to control hCPCe after rudimentary differentiation induction with Dex.

hCPCeP augment cardiac function and reduce infarct size. Delivery of hCPCeP, hCPCe, or vehicle alone by intramyocardial injection into SCID mice concurrent with myocardial infarction was performed to determine reparative potential. Loss of function in all groups indicated comparability of infarction damage as assessed at 1 week post-challenge by using echocardiography (Figs. 3A and 3B). Within 4 weeks after cell injection, myocardial function was significantly improved ($p < 0.001$) (Online Table 3) in hearts of mice receiving either hCPCe or hCPCeP compared with vehicle, as measured via echocardiographic assessment of ejection fraction (EF) or fractional shortening (FS) (Figs. 3A and 3B). Differences in myocardial function between hCPCe and hCPCeP 4 weeks after transplantation were not significant ($p > 0.05$) (Online Table 1). However, EF and FS performance improved in the hCPCeP group from 4 to 8 weeks post-delivery, in contrast with depressed contractility for hCPCe-treated mice that was not significantly different ($p > 0.05$) from the vehicle group (Figs. 3A and 3B). Myocardial contractile performance of hearts receiving hCPCeP increased by 1.81-fold in EF and 1.86-fold in FS compared with hCPCe 20 weeks after transplantation. Hemodynamic parameters were also significantly improved in hCPCeP-treated hearts compared with those treated with hCPCe ($p < 0.01$) or vehicle ($p < 0.001$) 20 weeks after transplantation. hCPCeP-treated hearts increased dP/dt_{max} and dP/dt_{min} (maximum and minimum rate of pressure change in the ventricle) by 1.29-fold and 1.23-fold, respectively (Fig. 3C) together with a 1.37-fold increase in left ventricular developed pressure (Fig. 3D) relative to hCPCe. Collectively, these results demonstrate the enhanced capacity of hCPCeP to preserve and/or restore myocardial function after infarction injury.

Enhancement of myocyte formation and neovascularization resulting from hCPCeP delivery. Improvement of hemodynamic performance in hearts receiving hCPCeP (Fig. 3) was accompanied by evidence of cardiogenic lineage commitment. Cardiomyocyte immunoreactivity with alpha-sarcomeric actin labeling in hearts receiving hCPCeP demonstrated cardiogenic commitment, together with a coincident eGFP signal indicative of derivation from hCPCeP. Human origin of cells in myocardial sections from mice was evident by immunolabeling for eGFP co-localized with human-specific mitochondrial marker or by detection of

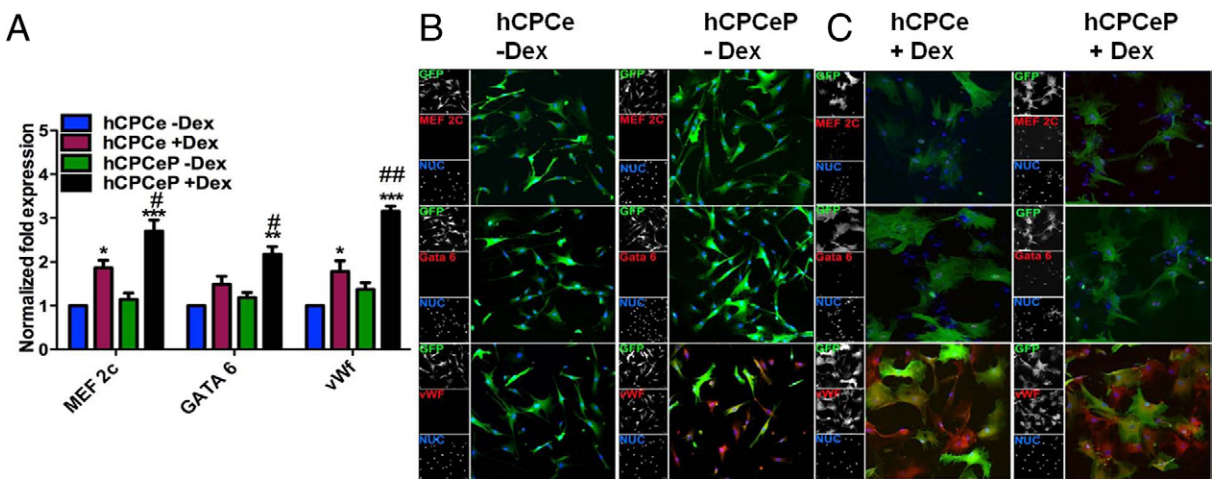

Figure 2 **Increases in Cardiac Commitment of hCPCeP After Dex Differentiation**

(A) Quantitative real-time polymerase chain reaction analysis for hCPCe and hCPCeP after dexamethasone (Dex) treatment for MEF2C, GATA-6, and von Willebrand factor (vWF) (n = 3). *p < 0.05, **p < 0.001, ***p < 0.01, hCPCeP-Dex versus hCPCeP+Dex; #p < 0.05, ##p < 0.01, hCPCe+Dex versus hCPCeP+Dex. **(B, C)** Immunostaining for MEF2C (cardiac), vWF (endothelial), and GATA-6 (smooth muscle) before and after Dex treatment for 7 days. GFP = green fluorescent protein; other abbreviations as in Figure 1.

characteristic repetitive *Alu* DNA sequence (Online Fig. 2). hCPCeP displayed a significant 2.32-fold increase ($p < 0.05$) in telomere length after adoptive transfer to infarcted hearts relative to hCPCe 20 weeks after transplantation, consistent with a youthful cellular phenotype (Figs. 4A to 4C). Infarction size was significantly smaller at 20 weeks in hCPCeP-transplanted mice compared with hCPCe-transplanted mice. Infarction damage involving 61.3% of the left ventricular free wall in hearts receiving hCPCeP compared favorably with 84.3% in hearts receiving hCPCe ($p < 0.05$). Infarct size was not significantly different in hearts receiving either vehicle or hCPCe (Figs. 4D and 4E). Presence of c-kit+/GFP+ cells derived from the adoptively transferred population increased 4.0-fold in hearts receiving hCPCeP relative to hCPCe 12 weeks after delivery. Total c-kit+ cell number was significantly higher by 1.75-fold ($p < 0.05$) in heart sections from hCPCeP relative to hearts receiving hCPCe at 12 weeks (Figs. 4F to 4I). New myocyte formation in these hearts was identified according to eGFP signal together with alpha-sarcomeric actin staining 12 weeks after transplantation (Fig. 5A). New vessel formation was evident by coincidence of eGFP immunolabeling with smooth muscle actin (SM22) to label vascular walls as well as vWF to label endothelial vessel lining (Fig. 5B–C). Myocardial sections from hearts receiving hCPCeP exhibited 28% GFP+/SM22+ cells versus 17% in hCPCe (Fig. 5D). Similarly, hCPCeP-treated hearts possessed 22% GFP+/vWF+ cells compared with 12% in hCPCe. Expression of Pim-1 was maintained for at least 12 weeks after delivery with increased immunolabeling for Pim-1 (Online Fig. 3). Collectively, these results suggest the enhanced ability of hCPCeP to survive and proliferate, significantly augmenting angiogenesis and myogenesis in the infarcted heart.

Persistence of hCPCeP after delivery confers long-term hemodynamic performance improvement revealed by non-invasive imaging. hCPC persistence in vivo was longitudinally assessed over an 8-week period by using bioluminescence imaging (BLI) of luciferase (Luc) signal in hearts receiving either hCPCe or hCPCeP transduced with Luc reporter construct (Fig. 6A) immediately after infarction injury. Both hCPCe-Luc or hCPCeP-Luc produced a robust BLI signal at day 2 in all recipient animals, indicative of successful cell delivery to the heart. The BLI signal remained detectable in the hCPCeP-Luc cohort throughout 56 days post-delivery (Figs. 6B and 6C), in stark contrast to loss of signal by 14 days after delivery in the hCPCe-Luc group. These results suggest the superior persistence of hCPCeP-Luc after delivery, particularly in the critical window of 2 to 4 weeks post-infarction.

Myocardial contractile performance impairment was initially comparable shortly after cardiomyopathic challenge in cohorts of hCPCe-Luc or hCPCeP-Luc, indicative of similar infarction injury according to echocardiographic assessment. Subsequently, mice receiving hCPCeP-Luc exhibited improvement in FS, left ventricular end-diastolic dimension, left ventricular end-systolic dimension, and anterior wall thickness at 8 weeks post-injury (Figs. 7A to 7D) ($p < 0.05$). Magnetic resonance imaging at 1 and 8 weeks post-infarction substantiated the echocardiographic results, revealing improved hemodynamic parameters at 8 weeks in mice receiving hCPCeP-Luc compared with hCPCe-Luc (Figs. 7E to 7H) with respect to left ventricular end-diastolic volume, left ventricular end-systolic volume, and

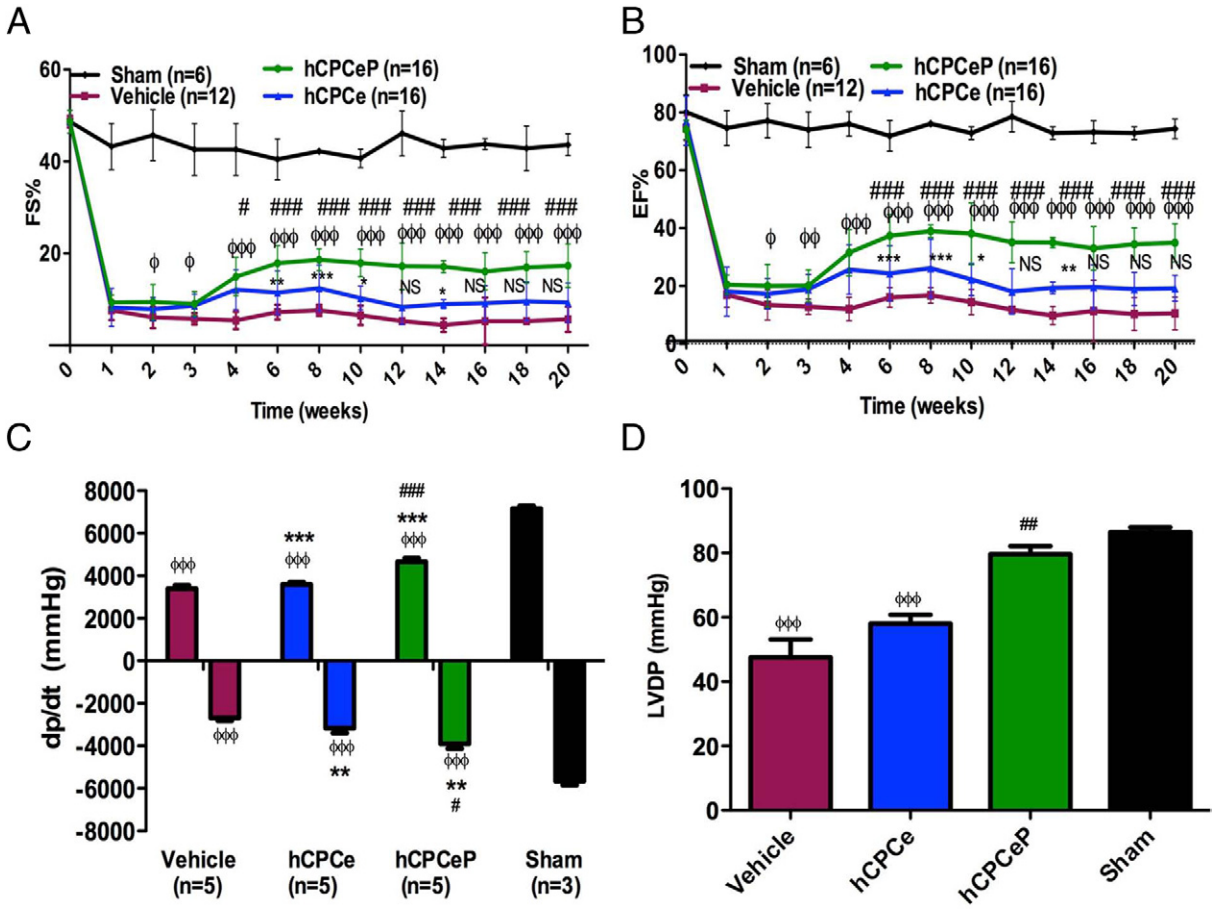

Figure 3 **Improvement of Cardiac Performance of Mice Treated With hCPCeP 20 Weeks After Transplantation in SCID Mice**

(A, B) Percentage of fractional shortening (FS) and ejection fraction (EF) measured by using echocardiography; sham: n = 6; vehicle: n = 12; hCPCe: n = 16; and hCPCeP: n = 16. **(C)** Hemodynamic assessment of rate of rise of left ventricular pressure (dP/dt) after cell transplantation (sham: n = 3; vehicle: n = 5; hCPCe: n = 5; and hCPCeP: n = 5). **(D)** Left ventricular developed pressure (LVDP). *p < 0.05, **p < 0.001, ***p < 0.01, for vehicle versus hCPCeP; ϕp < 0.05, $\phi\phi$p < 0.001, $\phi\phi\phi$p < 0.01, for hCPCe versus hCPCeP; #p < 0.01, ##p < 0.001, ###p < 0.05, for vehicle versus hCPCe. Abbreviations as in Figure 1.

EF ($p < 0.05$). P values for all parameters by magnetic resonance imaging or echocardiography are provided (Online Table 4). hCPCeP-Luc–treated hearts exhibited a 22.5% increase in anterior wall dimension with a 10% decrease in left ventricular end-diastolic dimension and a 20.6% decrease in left ventricular end-systolic dimension by echocardiography 8 weeks after transplantation relative to hCPCe-Luc.

Discussion

Discovery of hCPCs contributing to cardiomyogenesis within the heart and supporting myocardial repair has revolutionized the conceptual view of treatment for heart disease, as supported by the capacity of hCPCs to form functionally integrated cardiomyocytes and vasculature (20). However, survival and persistence of adoptively transferred hCPCs used for therapeutic purposes remain a major concern, particularly when the donor cell population used for autologous therapy is derived from pathologically stressed myocardium. Regenerative capabilities of adult hCPCs are likely to be impaired by age (21) and disease (22), limiting the reparative and regenerative potential of these autologously derived cells. Ex vivo modification or pre-conditioning has been shown to prime adoptively transferred cells for myocardial repair (23,24). Genetic modification to augment cellular survival and proliferation is a viable molecular interventional strategy, as previously published by our group, for syngeneic murine CPCs (17,25). The present study addresses a critical issue by demonstrating that Pim-1 modification augments regenerative and reparative potential of hCPC derived from heart failure patients, bringing this conceptual approach another step closer to therapeutic implementation.

hCPC isolated from heart failure patients amenable to modification with Pim-1 also display phenotypic characteristics consistent with enhanced survival, proliferation, and

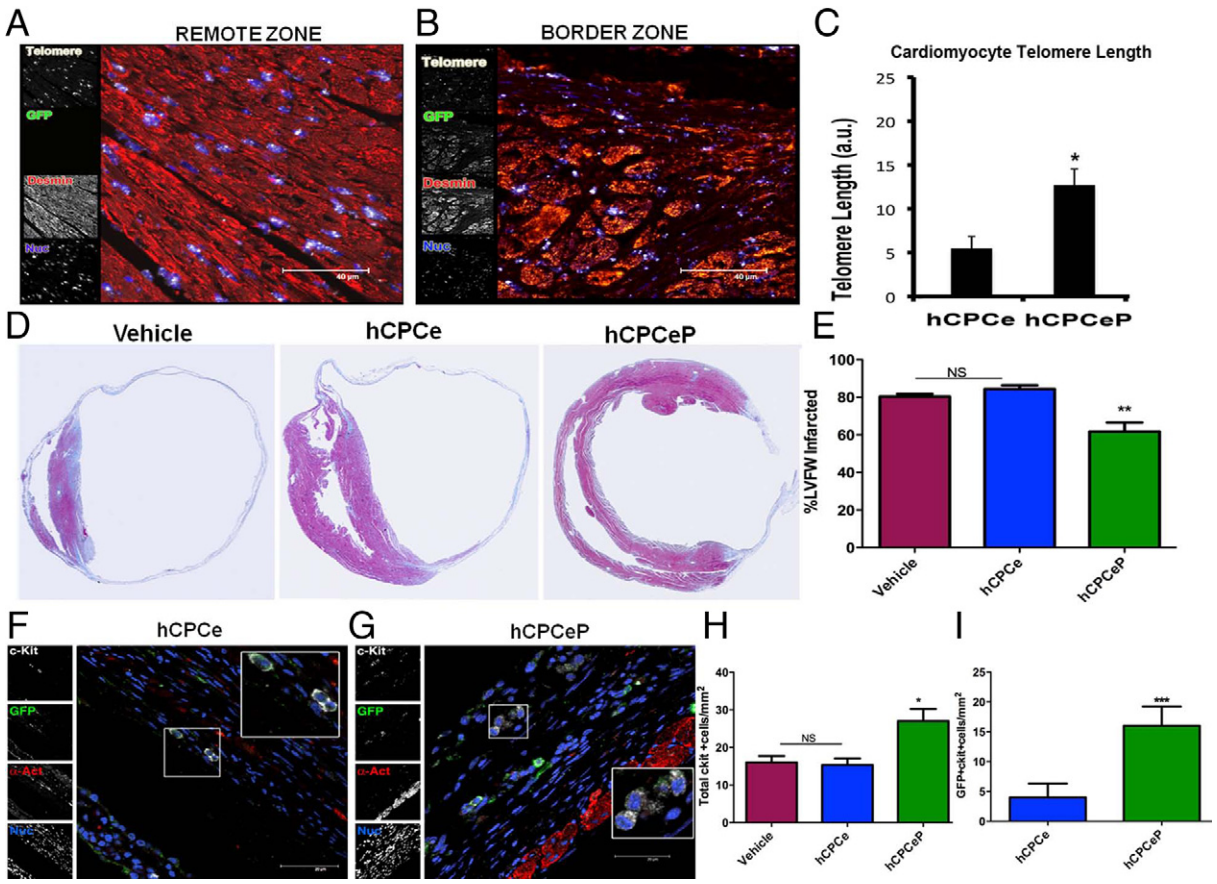

Figure 4 **hCPCeP Show Increases in Telomere Length, Enhancement of c-kit–Positive Cell Number, and Decreases in Fibrosis**

(A) Remote and **(B)** infarct zone telomere length in mice treated with hCPCeP, telomere **(white)**, GFP **(green)**, desmin **(red)**, and nuclei **(blue)**. **(C)** Quantitation of telomere length of hCPCe and hCPCeP ($p < 0.05$). **(D)** Masson's trichrome staining for vehicle-, hCPCe-, and hCPCeP-injected SCID mice. **(E)** Percentage of infarcted left ventricular free wall (LVFW) in vehicle, hCPCe, and hCPCeP ($n = 3$), $p < 0.01$. **(F, G)** Immunostaining for c-kit **(white)**, GFP **(green)**, alpha-sarcomeric actin **(red),** and nuclei 24 **(blue)** in hCPCe and hCPCeP, respectively. **(H)** Quantitation of total number of c-kit+ cells/mm^2 in vehicle, hCPCe, and hCPCeP. **(I)** Quantitation of GFP+ and c-kit+ cells/mm^2 in hCPCe- and hCPCeP-treated animals ($n = 3$). *p<0.05, **p<0.01, ***p<0.001. Abbreviations as in Figures 1 and 2.

reversal of senescent characteristics. hCPCeP exhibit high proliferation and metabolic activity in vitro (Figs. 1A through 1C). Telomere lengths of hCPCeP are also preserved, suggesting an important role of Pim-1 in maintaining telomere length (Fig. 1D) to antagonize cellular senescence; this topic is currently being investigated by our group. Pim-1 also increases phosphorylation of p21 (Figs. 1E and 1F), a cyclin-dependent kinase inhibitor, as well as stabilizing c-Myc and the nuclear mitotic apparatus (26). Increased survival of Pim-1–engineered cells is likely due to the ability of the kinase to promote proliferation and attenuate apoptotic signaling (10,27), moderating enhanced proliferation and persistence of the transplanted cells to augment the reparative process.

Potentiation of hCPCs from heart failure patients undergoing left ventricular assist device implantation reported in our study addresses the heretofore critical unanswered issue of whether aged hCPCs from pathologically damaged myocardium would retain the capacity to benefit from genetic engineering. Indeed, heart failure associated with aging has been proposed to be a "stem cell disease" characterized by impaired functional reserve of the endogenous stem cell pool due to exhaustion, senescence, depletion, or inability to cope with the environmental stressors (28). Recent clinical results using autologous hCPCs to restore myocardial performance in the SCIPIO (Cardiac Stem Cell Infusion in Patients With Ischemic Cardiomyopathy) trial found that the c-kit+ cell population is capable of mediating improvement in both EF as well as reduction in infarct size (29). With unequivocal evidence of clinical relevance for the treatment of heart failure using c-kit+ hCPC, the future of hCPC therapy will inevitably turn toward assessment of approaches to enhance the regenerative process.

Can Pim-1 be considered an appropriate molecular interventional strategy for enhancing cardiogenesis? Pim-1 induces proliferation of endothelial (16) and vascular smooth muscle (30) cells as well as promoting lineage commitment as evidenced by increased expression of cardiogenic transcripts in Pim-1–engineered CPCs (17)

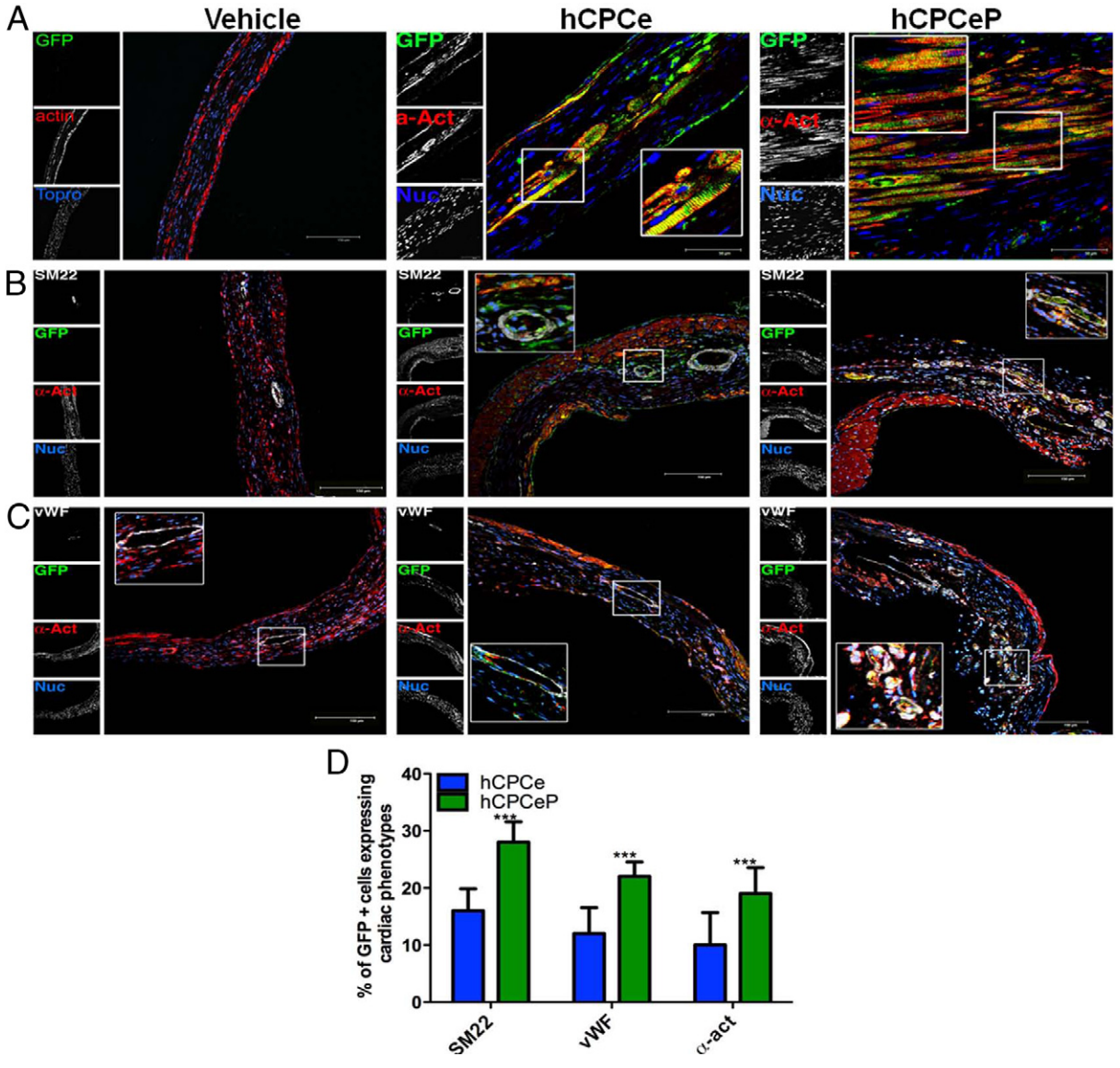

Figure 5 **hCPCeP Augment Myocardial Repair 12 Weeks After Transplantation**

Immunolabeling for **(A)** GFP **(green)**, alpha-sarcomeric actin **(red)**, and nuclei **(blue)**. **(B)** SM22 **(white)**, GFP **(green)**, alpha-sarcomeric actin **(red)**, and nuclei **(blue)**. **(C)** vW **(white)**, GFP **(green)**, alpha-sarcomeric actin **(red)**, and nuclei **(blue)**. **(D)** Quantitation of SM22, vWF and alpha-sarcomeric actin in hCPCe and hCPCeP ($p < 0.001$). **Boxes** indicate enlarged areas. Scale bars = 150 μm for all panels except row A, where hCPCe and hCPCeP widefield images = 50 μm and insets = 25 μm. Abbreviations as in Figures 1, 2, and 4.

(Fig. 2). hCPCeP express vWF transcript before and after differentiation in vitro and after transplantation into damaged myocardium. Moreover, clear evidence of myocytes derived from adoptively transferred hCPCeP was revealed after 20 weeks post-delivery by using immunohistochemistry (Fig. 5, Online Fig. 2). Persistence, expansion, and integration of the hCPCeP into myocardial tissue translate into progressive improvement in myocardial structure and function evident up to 20 weeks post-delivery relative to hCPCe (Figs. 3 and 6). The durability of repair, together with the superior improvement of functional parameters of myocardial hemodynamic performance, supports the use of Pim-1 as a plausible molecular strategy to enhance myocardial regeneration with modified hCPCs.

Despite pro-proliferative effects mediated by Pim-1, oncogenic transformation has never been observed in any of our human samples, and all engineered hCPCeP were amenable to differentiation in vitro that resulted in acquisition of post-mitotic characteristics (Fig. 2). In vivo studies show cardiogenic commitment of hCPCeP to all 3 essential cell lineages for reconstitution of myocardial tissue: cardiomyocytes, vasculature, and endothelium (Fig. 5). Further-

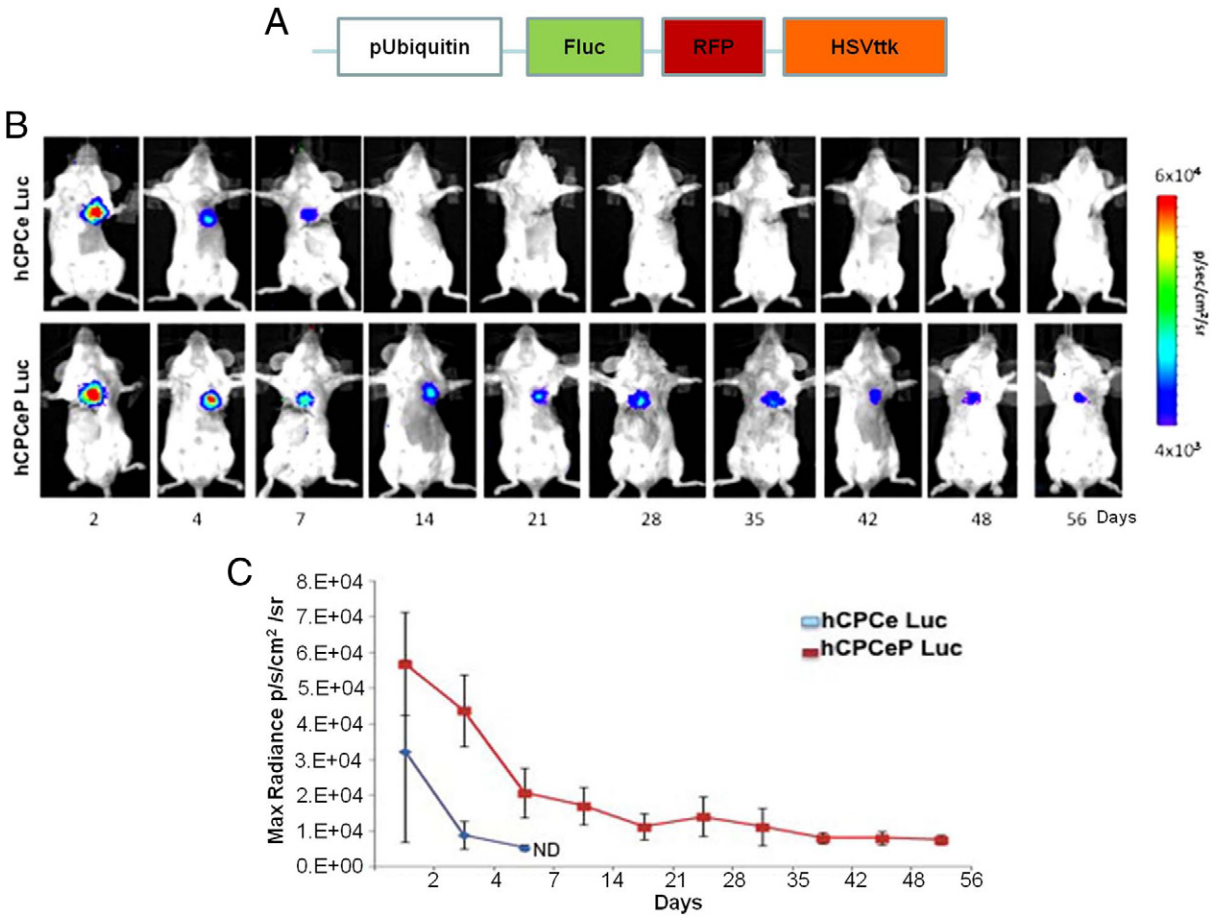

Figure 6 **Enhancement of hCPCeP Persistence**

(A) Firefly luciferase (Luc) reporter construct. **(B)** Bioluminescence imaging (BLI) imaging: pseudocolor images representing signal intensity in mice treated with hCPCe-Luc **(top)** and hCPCeP-Luc **(bottom)** after myocardial infarction. hCPCe-Luc signal was not detected 7 days after transplantation whereas hCPCeP-luc shows signal throughout the experimental cohort for 56 days. **(C)** Quantitation of pseudocolor images represented in maximum radiance (p/s/cm^2/sr). Abbreviations as in Figure 1.

more, karyotypic analyses show normal chromosome content in hCPCeP (Online Fig. 1F). Although oncogenic risk needs to be carefully evaluated when genetic engineering is proposed, it is important to consider that lentiviral vectors have also made their way into clinics as therapies (31), including for advanced forms of HIV infections (32), Parkinson's disease (33), and inherited disorders affecting hematopoietic cells (34). In addition, lentiviral vectors have integration sites away from transcriptional regulatory sites, making them a safe therapeutic option (35). These findings are in stark contrast to published literature showing chromosomal abnormalities in certain embryonic stem cells and induced pluripotent stem cells in which oncogenic transformation remains a significant barrier to therapeutic implementation (36,37).

Clinical trials using bone marrow–derived stem cells (TAC-HFT [Transendocardial Injection of Autologous Human Cells (bone marrow or mesenchymal) in Chronic Ischemic Left Ventricular Dysfunction and Heart Failure Secondary to Myocardial Infarction]) (38) and hCPCs (29) effectively demonstrate improved cardiac function after transplantation of stem cells. Narrow inclusion criteria for these clinical trials leave open the issue as to whether initially promising findings will be broadly applicable to the much greater segment of patients experiencing the debilitating consequences of aging and multiple concurrent cardiac problems. Nevertheless, despite severe deterioration of myocardium necessitating surgical intervention and mechanical assist device implantation in the 68-year-old source of our hCPC, Pim-1 expression effectively increased myocardial repair in immunosuppressed murine recipients, whereas hCPCe without Pim-1 expression were ineffective. Persistence of the BLI signal of hCPCeP until ~2 months after delivery (Fig. 6) reinforces the earlier findings with syngeneic CPCeP in mice (17) and supports the notion that hCPCeP become permanently integrated into the myocardium, unlike control hCPCe undetectable after 2 weeks post-delivery. Moreover, the notably enhanced signal from hCPCeP at 2 to 4 weeks after transfer coincides

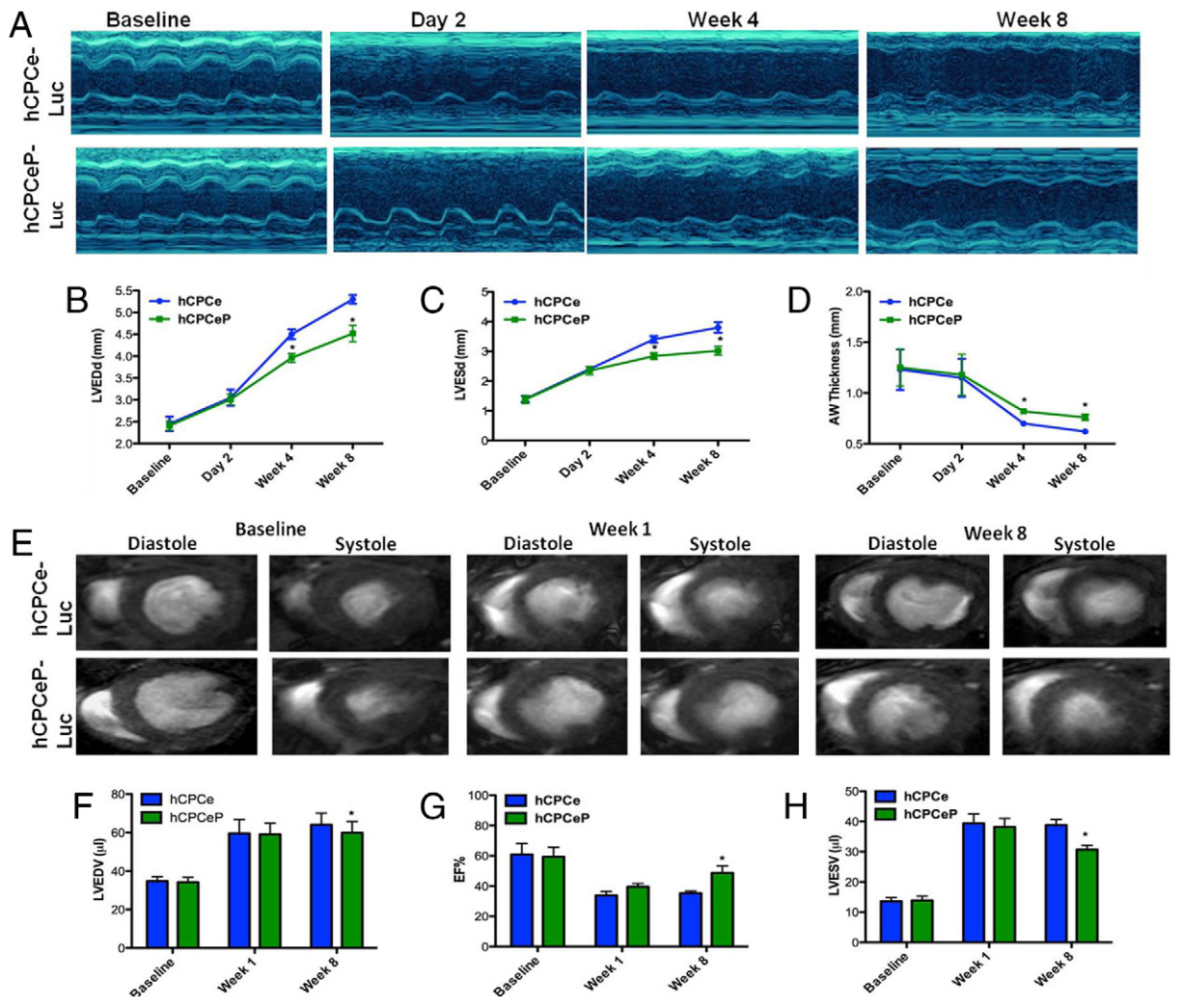

Figure 7 **Echocardiography and MRI of Mice 8 Weeks After Myocardial Infarction**

(A) Echocardiographic images from hCPCe-Luc and hCPCeP- Luc, **(B)** left ventricular end-diastolic dimension (LVEDd), **(C)** left ventricular end-systolic dimension (LVESd), and **(D)** anterior wall (AW) thickness. Magnetic resonance imaging (MRI): **(E)** MRI images, **(F)** left ventricular end-diastolic volume (LVEDV), **(G)** ejection fraction percentage (EF%), and (**H**) left ventricular end-systolic volume (LVESV). *$p < 0.05$ versus hCPCe. Abbreviations as in Figure 1.

with timing for recruitment of endogenous repair in the infarcted heart (25). Increased presence of total c-kit+ cells in the myocardium of hearts receiving hCPCeP (Figs. 4F to 4I) likely reflects augmentation of endogenous repair previously postulated to play a critical role in mediating myocardial repair (39). The ensuing progressive loss of BLI signal in hearts of recipient mice receiving hCPCeP over 2 months could be caused by ongoing molecular and cellular processes such as promoter silencing (29,40) or rejection of the allogenic human cells from remnants of non–T cell, non–B cell immunity in the NOD/SCID mice (41). It is reasonable to posit that persistence of hCPCeP could be further improved with autologous transfer as well as by using humanized expression vectors such as minicircles that can persist for months in nondividing cells without integrating into chromatin, thereby minimizing concerns of insertional mutagenesis (42). Ongoing studies are evaluating minicircle technology and other protocol modifications to further refine the safety and efficacy of Pim-1 genetic engineering to enhance myocardial regeneration.

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Key Words: heart repair ■ human cardiac progenitor cells ■ Pim-1 kinase.

APPENDIX

For an expanded Methods section, please see the online version of this article.