Repair of Acute Myocardial Infarction by Human Stemness Factors Induced Pluripotent Stem Cells

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Background—Nuclear reprogramming provides an emerging strategy to produce embryo-independent pluripotent stem cells from somatic tissue. Induced pluripotent stem cells (iPS) demonstrate aptitude for de novo cardiac differentiation, yet their potential for heart disease therapy has not been tested.

Methods and Results—In this study, fibroblasts transduced with human stemness factors OCT3/4, SOX2, KLF4, and c-MYC converted into an embryonic stem cell–like phenotype and demonstrated the ability to spontaneously assimilate into preimplantation host morula via diploid aggregation, unique to bona fide pluripotent cells. In utero, iPS-derived chimera executed differentiation programs to construct normal heart parenchyma patterning. Within infarcted hearts in the adult, intramyocardial delivery of iPS yielded progeny that properly engrafted without disrupting cytoarchitecture in immunocompetent recipients. In contrast to parental nonreparative fibroblasts, iPS treatment restored postischemic contractile performance, ventricular wall thickness, and electric stability while achieving in situ regeneration of cardiac, smooth muscle, and endothelial tissue.

Conclusions—Fibroblasts reprogrammed by human stemness factors thus acquire the potential to repair acute myocardial infarction, establishing iPS in the treatment of heart disease.

Key Words: pluripotent stem cells ■ regenerative medicine ■ transplantation

Regenerative medicine offers the potential of curative therapy to repair damaged tissues.1,2 Pluripotent stem cells derived from the inner cell mass of early-stage embryos have provided a prototype for multilineage repair. Ethical considerations along with practical limitations, however, have precluded adoption of embryonic stem cell platforms, driving advances in nuclear reprogramming to establish viable alternatives.3 In this regard, induced pluripotent stem cell (iPS) technology provides an emerging innovation that promises the unlimited potential of embryonic stem cells while circumventing the need for embryonic sources.4

Although induced pluripotency reliably generates an embryonic-like ground state from healthy and diseased sources,13,14 the therapeutic value of reprogramming remains largely unknown. To date, only 3 disease models have been treated with iPS-derived strategies,15–17 Interventions in sickle cell anemia, Parkinson’s disease, and hemophilia A have been limited to lineages prespecified in vitro.15–17 Despite recapitulating the cardiomyogenic phenotype from both murine and human somatic fibroblasts,18–21 multilineage repair of heart tissue with iPS-based intervention has yet to be documented.

We here demonstrate for the first time the efficacy of iPS to treat acute myocardial infarction. Murine fibroblasts were transduced with human stemness-related factors through an efficient vector system to generate valid iPS clones with inherent cardiogenic potential. Head-to-head comparison between parental fibroblasts and reprogrammed progeny established the acquired propensity for cardiac tissue regeneration in a setting of ischemic heart disease. iPS progeny engrafed in the context of immunocompetent allogeneic transplantation and rescued postischemic myocardial structure and function. This proof-of-principle study pioneers fibroblast-derived iPS in cardiac repair.

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Nuclear reprogramming designed to convert somatic tissues into stem cells capable of germline transmission has been achieved recently with ectopic expression of various pluripotent gene combinations.5–8 Delivery of stemness-related gene sets, exemplified by the Oct-3/4, Sox2, Klf4, and c-Myc quartet,5 reprograms adult somatic cells by promoting reacquisition of evolutionarily conserved embryonic gene networks.9 By reinstating pluripotency, iPS reset the biological age of a cell reflected in the relengthening of telomeres.10 The potential for reversing adult cell fates has been systematically exploited, producing iPS from diverse tissue origins across phylogenetic barriers.11,12

Received March 13, 2009; accepted June 1, 2009.
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Circulation is available at http://circ.ahajournals.org

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Molecular Cardiology

DOI: 10.1161/CIRCULATIONAHA.109.865154
Transduction
pSIN-CSGWD6NotI–derived transfer vectors were generated with human OCT3/4, SOX2, KLF4, and c-MYC cDNAs (Open Biosystems).22 The packaging plasmid, pCMV8.91, was engineered with H87Q mutation in the HIV-1 capsid region for increased transduction efficiency of purified infectious supernatants.22 Mouse embryonic fibroblasts, obtained from embryos at 14.5 days postcoitum (dpc), were expanded in maintenance medium containing Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, Calif) supplemented with 10% fetal calf serum, 1% L-glutamine (Invitrogen), and 1% penicillin/streptomycin and plated at 10^5 per 24 wells before transduction for 12 hours with infectious supernatants. Transduced fibroblasts were replated at confluence, and iPS was isolated after 2 weeks for clonal expansion. Cells were labeled with HIV vectors carrying LacZ (pLenti6/UbC/V5-GW/LacZ, Invitrogen) or luciferase (pSIN-Luc).23

Pluripotent Induction
RI-derived embryonic stem cells and iPS were expanded in embryonic stem cell media. Cells were fixed with 3% paraformaldehyde, permeabilized, and stained with anti–SSEA-1 antibody (MAB4301; dilution 1:50; Chemicon, Temecula, Calif) along with secondary antibody (1:1000; Invitrogen) and Alexa-488-labeled phalloidin (Molecular Probes). Immunofluorescence was analyzed with the use of epi-fluorescence microscope (LEICA). For field-emission scanning or JEOL 1200 EXII transmission electron microscopy, cells were permeabilized, and stained with anti–SSEA-1 antibody (MAB4301; dilution 1:50; Chemicon, Temecula, Calif) along with secondary antibody (1:1000; Invitrogen). Growth and differentiation potential were determined on substractaneous injection in anesthetized (2% to 3% isoflurane) athymic nude mice. Cryopreserved tissue was processed for hematoxylin-eosin procedures.25-26

Differentiation
iPS were differentiated into embryoid bodies with the use of the hanging-drop method.23 Expression of precardiac mesoderm and cardiac differentiation markers was detected by reverse transcription polymerase chain reaction. Total RNA was extracted with a combination of gDNA Eliminator and RNasey columns (Qiagen). cDNA was prepared from RNA samples with the use of Superscript III First Strand Synthesis System (Invitrogen). Mouse Gapdh (4352932E; Applied Biosystems, Foster City, Calif) was used as control. Analyzed genes included Gata4 (Mm00484689_m1), Myocd (Mm01340839_m1; Applied Biosystems), Tbx5 (Mm00455051_m1), and Mef2c (Mm01340839_m1; Applied Biosystems).

Diploid Aggregation
Contribution to embryonic development was assessed through diploid aggregation.23 Host embryos from CD-1 superovulated females were collected at 2.5 dpc. Fibroblasts, iPS, and R1-derived embryonic stem cells (α-MHC-lacZ and EF-lacZ provided by Drs Ravi Misra and John Lough, Medical College of Wisconsin) were partially digested with the use of trypsin 0.25%–EDTA (Invitrogen), and 8 to 15 cell clumps were placed with paired embryos denuded of zona pellucida. The aggregation complex was incubated for 24 hours (in 5% CO2/5% O2/90% N2) until blastocyst cavitation.24 Chimeric embryos were transplanted into anesthetized (2% to 3% isoflurane) pseudopregnant surrogate CD-1 mothers, harvested at 9.5 dpc, and analyzed for distribution of LacZ-labeled progenitors.23

iPS Therapy
Male 8- to 12-week-old C57BL/6 or athymic nude mice were anesthetized (1.5% to 2% isoflurane) and intubated (Mini Vent 845, Hugo Sacks Electronic), and the left coronary artery was ligated with a 9-0 suture under direct visualization after minimally invasive thoracotomy. Myocardial ischemia was confirmed by ECG, echocardiography, and color change of left ventricular wall. Fibroblasts or iPS (200,000 to 10 μL of differentiation medium) were transplanted with 4 injections of 2.5 μL within 30 minutes after ligation. Cryosections (7 μm thickness) were processed for hematoxylin-eosin, Masson’s trichrome, luciferase, and β-galactosidase staining.23,26 Sections were labeled with luciferase (1:5000; Sigma, St Louis, Mo) or β-galactosidase antibody (1:5000; Abcam) coupled with Alexa-568 secondary antibody (1:1000; Invitrogen) and colocalized with α-actinin (1:200; Sigma), smooth muscle actin (1:200 Abcam), CD31 (1:200; Abcam), or SSEA-1 (1:50; Chemicon) antibodies all paired with Alexa-488 antibody (1:1000; Invitrogen).

Live Cell Imaging and Heart Performance
Luciferase-transfected fibroblasts or iPS were cultured for multiple passages including a freeze/thaw cycle before expansion and transplantation. Cells were tracked with the IVIS 200 Bioluminescence Imaging System (Xenogen) after intraperitoneal injection of 150 mg/kg D-luciferin (Xenogen), and signals were analyzed with the Living Image Software (Xenogen). Ventricular performance was quantified by echocardiography (RMV-707B scan head, Vevo770, Visual Sonics). Ejection fraction (EF) (%) was calculated as [(LVVd–LVVs)/LVVd]×100, where LVVd is left ventricular end-diastolic volume (μL) and LVVs is left ventricular end-systolic volume (μL). Left ventricular fractional shortening (%) was calculated as [(LVVd–LVVs)/LVVd]×100, where LVVd is left ventricular end-diastolic dimension (mm) and LVVs is left ventricular end-systolic dimension (mm).23 Electric activity was monitored by ECG (MP150, Biopac). Data were collected and analyzed by blinded investigators.

Statistical Analysis
Results are presented as mean±SEM. Median is additionally reported when grouped data were compared with nonparametric Mann–Whitney U test. Comparison between groups over time was performed by 2-way repeated-measures ANOVA. Kaplan–Meier analysis was applied with log-rank testing. P<0.05 was predetermined as significant, and all values >0.001 were reported.

Results
Nuclear Reprogramming Resets Primitive Morphology and Unlocks Functional Pluripotency
Transduced with human stemness factors OCT3/4, KLF4, SOX2, and c-MYC, reprogrammed fibroblasts were isolated according to compact clusters of embryonic stem cell–like morphology distinct from monomorphic, single-cell layers of parental fibroblasts (Figure 1A). Reprogrammed cells displayed distinct subcellular architecture, reorganized from original fibroblasts to recapitulate salient features of undifferentiated embryonic stem cells with high nucleo/cytoplasmic ratio, predominance of nuclear euchromatin, and scant density of cytosolic organelles (Figure 1B). Reprogramming induced expression of the early embryonic SSEA-1 antigen, an initial marker of stemness absent in parental fibroblasts (Figure 1C). To determine functional pluripotency, the inherent capacity for embryonic integration was probed by diploid aggregation with a pair of denuded host embryos (Figure 1D, top). Whereas morula-derived blastomeres incorporated into an embryonic structure after 24 hours in microwells, fibroblasts aborted engraftment and failed to contribute to ex utero blastocyst development (Figure 1D, bottom left). In contrast, reprogrammed fibroblasts demonstrated spontaneous integration and contributed to preimplantation blastocyst formation (Figure 1D, bottom right). Noncoerced assimilation into early-stage embryos thereby established bona fide iPS clones, providing a high-stringency quality control measure for functional pluripotency.

Chimeric Embryos Authenticate iPS-Derived Patterning of Normal Cardiogenesis
As iPS differentiated within 5-day-old embryoid bodies (Figure 2A), upregulation of precardiac markers Mesp1,
Tbx5, Cxcr4, and Flk-1 indicated engagement beyond the original fibroblast lineage (not illustrated). Within 12 days, increased expression of canonical cardiac transcription factors Mef2c (P<0.049; n=3), Gata4 (P<0.049; n=3), and Myocardin (P<0.049; n=3) indicated the capacity for cardiac tissue maturation (Figure 2B). Beyond redirection of somatic cell fate in vitro, chimeric embryos were utilized to examine the ability of iPS clones to ensure tissue formation during embryonic development in utero. Preimplantation blastocysts containing lacZ-labeled iPS progenitors were transferred into surrogate uterus and tracked at early stages of organogenesis. iPS labeled with a constitutively active reporter construct mimicked the stochastic distribution of embryonic stem cells throughout the developing embryo at 9.5 dpc (Figure 2C). Labeled iPS progeny demonstrated robust contribution to the heart field, including cardiac inflow and outflow tracts as well as left and right ventricles of the embryonic heart parenchyma (Figure 2D and 2E). Thereby, qualified iPS clones demonstrated de novo organogenesis and patterning of cardiogenic tissue within a developing embryo.

**iPS Engraft Into Infarcted Immunocompetent Adult Hearts**

In contrast to fibroblasts unable to proliferate even after prolonged incubation, subcutaneous injection of iPS clones within an immunodeficient adult environment demonstrated aggressive growth (Figure 3A). Transplanted cells, initially labeled with retroviral reporter constructs and expanded

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**Figure 1.** iPS demonstrate pluripotent features. A, Flat fibroblasts reprogrammed with human stemness factors metamorphosed into rounded clusters shown by field-emission scanning electron microscopy. Bar=5 μm. B, In transmission electron microscopy, derived iPS demonstrated nuclear/cytoplasmic composition similar to embryonic stem cells (ES). C, Counterstained by nuclear DAPI, iPS expressed the pluripotent marker SSEA-1 (red), absent from fibroblasts (0 hour; left). Bar=50 μm. D, Fibroblasts or iPS clumps were placed along with two 8-cell host embryos for diploid aggregation (1 hour; top). Bar=50 μm. Within 24 hours, iPS spontaneously integrated to form an early-stage chimeric blastocyst (24 hours; bottom right), in contrast to fibroblasts that were excluded (24 hours; bottom left).

**Figure 2.** iPS recapitulate in utero cardiogenic propensity. A, LacZ-labeled iPS clones, detected by β-galactosidase (β-gal) staining, were maintained as undifferentiated colonies at day 0 before aggregation into embryoid bodies (EB). B, Gene expression profiles at day 0 (d0) compared with day 12 (d12) of differentiation demonstrated induction of cardiac transcription factors Mef2c (P<0.049; n=3), Gata4 (P<0.049; n=3), and Myocardin (P<0.049; n=3). C, Embryos provide a wild-type (WT) environment to determine tissue-specific differentiation (top left). Derived by diploid aggregations, embryonic stem cells (ES) stochastically contribute to tissue patterning with diffuse integration tracked with constitutively labeled EF-lacZ cell line (top right) and cardiac-specific integration identified by α-MHC-lacZ reporter (bottom left). iPS, labeled with ubiquitously expressing reporter with CMV promoter, identifies progeny throughout developing embryo (bottom right). D, Chimerism with lacZ-labeled iPS demonstrated robust contribution to developing hearts within 9.5 dpc embryos. Bar=100 μm. E, Heart parenchyma of 9.5 dpc chimeric embryo contained integrated iPS progeny expressing β-galactosidase. Bar=50 μm.
through multiple passages (>5) in vitro, were tracked with in vivo imaging with the use of emitted bioluminescence from iPS-derived progeny. On permanent occlusion of epicardial coronary vasculature and microsurgical transfer into the ischemic myocardium, iPS remained within injected hearts and produced gradual tumor outgrowth between 2 and 4 weeks (Figure 3B). Echocardiography confirmed a significant tumor burden, which compromised hemodynamics 4 weeks after transplant (Figure 3C). Autopsy in immunodeficient recipients (n=6) verified consistent teratoma formation with extension beyond the myocardial wall and tumor infiltration within the postinjured myocardium (Figure 3C). In contrast to tumorigenesis that compromised the safety within immunodeficient environments, subcutaneous transplantation of iPS into immunocompetent hosts demonstrated a persistent absence of tumor growth in all animals (n=6) even at 8 weeks of follow-up (Figure 3D). Furthermore, intramyocardial transplantation of 200 000 iPS per heart, a dose selected on the basis of tumor-free outcome with embryonic stem cell intervention26,27 produced stable engraftment without detectable tumor formation (n=6; Figure 3E). According to bioluminescence emitted from labeled progeny, differentiated iPS within ischemic immunocompetent hearts were detectable by 4 weeks after transplantation without metastatic dissemination after 4 weeks of engraftment (n=6; Figure 3E). In fact, immunostaining of hearts at 4 weeks demonstrated rare pockets of SSEA-1–positive progeny within the postischemic myocardium (Figure 3F). Immunocompetent recipients thus ensured controlled iPS engraftment (Figure 3G) with tissue integration that did not perturb electric homeostasis (n=6; Figure 3H). In this way, the immunocompetent adult host provided a permissive environment for differentiation, offering the opportunity to test the therapeutic potential of iPS clones.
iPS Therapy Restores Myocardial Performance Lost by Ischemic Injury

Within immunocompetent hosts, recovery of posts ischemic cardiac performance was compared in randomized cohorts transplanted with parental fibroblasts versus derived iPS. Monitored by echocardiography, irreversible occlusion of the epicardial coronary blood flow consistently impaired anterior wall motion, depressed global cardiac function, and halved EF from 82% to 38% within 1 day after infarction (n=12; Figure 4A). Whereas blinded transplantation with parental fibroblasts demonstrated persistent functional decline with EF dropping to 37±4% at 4 weeks (n=6), iPS intervention improved cardiac contractility to achieve an EF of 56±2% within the first 2 weeks of therapy and 50±5% by 4 weeks (n=6; P=0.002, iPS versus fibroblasts; Figure 4A). Functional benefit in response to iPS was verified by the improved fractional shortening, from 20±1% (median 18%; n=6) at 1 day after infarction to 31±3% (median 29%; n=6) after 4 weeks, in contrast to a lack of recovery in fibroblast-treated hearts (n=6; P=0.01; Figure 4B). Moreover, the regional septal wall thickness in systole was significantly rebuilt with iPS (1.31±0.11 mm; median 1.20 mm; n=5) but not with fibroblast (0.88±0.06 mm; median 0.90 mm; n=6) treatment (P=0.006; Figure 4C). Impaired cardiac contractility in the injured anterior wall resulted in akinetic regions with paradoxical motion in systole indicative of aneurysms in fibroblast-treated hearts, in contrast to coordinated concentric contractions in response to iPS treatment visualized by long-axis and short-axis imaging (Figure 4D and 4E). Thus, compared with nonreparative parental fibroblasts, iPS intervention improved functional performance after acute myocardial infarction.

iPS Therapy Halts Progression of Pathological Remodeling in Infarcted Hearts

Beyond functional deterioration, maladaptive remodeling with detrimental structural changes prognosticates poor outcome after ischemic injury. In this study, iPS-based intervention attenuated global LVDd. Preinfarction LVDd measured 3.2±0.1 mm (median 3.1 mm) but increased after infarction to 4.9±0.1 mm (median 4.9 mm) by 4 weeks of fibroblast treatment (n=6), a value significantly higher (P=0.007) than 4.2±0.2 mm (median 4.2 mm) with iPS treatment (n=6; Figure 5A). Furthermore, echocardiography demonstrated regional structural deficits with deleterious wall thinning and chamber dilation in fibroblast-treated hearts (n=6), rescued
by iPS intervention (n=6; Figure 5B). Pathological structural remodeling leads to electrophysiological consequences with prolongation of the QT interval, which increases risk of arrhythmia. Infarction increased QT interval from 28.9 ± 1.4 ms (median 28.1 ms) to 55.9 ± 1.3 ms (median 55.8 ms) in fibroblast-treated hearts (n=6), which was abrogated to 40.8 ± 1.5 ms (median 40.3 ms, n=6) with iPS treatment (P=0.004; Figure 5C). These real-time surrogates for tissue remodeling were confirmed on autopsy on inspection of gross specimen that demonstrated reduced heart size, lack of aneurysmal formation, and absence of severe wall thinning in iPS- compared with fibroblast-treated hearts (Figure 5D).

Collectively, the favorable remodeling at global, regional, and electric levels demonstrates the overall benefit of iPS therapy in the setting of myocardial infarction.

Multilineage Cardiac Tissue Regeneration After iPS Therapy

Histological analysis demarcated demuscularization and extensive scarring within left ventricles distal to coronary ligation in hearts treated with fibroblasts 4 weeks after transplantation (Figure 6A). In contrast, iPS treatment halted structural deterioration of infarcted tissue with antifibrotic benefit and remuscularization within the left ventricular free wall (Figure 6A). Surgical dissection and postmortem histopathological analysis verified absence of tumor infiltration or dysregulated cell expansion after iPS transplantation in the myocardium itself, as well as in the liver, lung, and spleen, organs with high metastatic risk (n >10 staggered sections throughout respective organs; Figure 6B). In postischemic myocardium, immunohistochemistry confirmed engraftment of iPS-derived progeny that expressed transgene markers luciferase (not illustrated) or β-galactosidase (Figure 6C through 6E). Colocalization of transgene expression with cardiac α-actinin was consistent within the damaged territory, as documented by microscopy of serial transverse sections (n >10 at 10- to 20-μm intervals) immediately adjacent to the site of coronary ligation (Figure 6C). Smooth muscle α-actin (Figure 6D) and endothelial CD31 (Figure 6E) were also detectable, albeit at lower frequency, consistent with multilineage cardiovascular differentiation of iPS. Thus, in contrast to ineffective parental fibroblasts, targeted delivery of iPS generated de novo cardiovascular tissue in postischemic adult myocardium.

Discussion

Nuclear reprogramming with iPS technology offers embryo-independent pluripotent progenitors. Although enabling patient-specific regenerative platforms, before this study iPS have been limited to 3 noncardiac disease conditions, namely, sickle cell anemia, Parkinson’s disease, and hemophilia A.15–17 The present study expands the therapeutic
indications of iPS by providing the first evidence for repair in the context of heart disease.

The ability to reprogram somatic tissue and reactivate atavistic functions of pluripotency has forged a new strategy for regenerative biology.\textsuperscript{31–34} Somatic cell nuclear transfer provided the initial empirical evidence for epigenetic conversion of non-stem cells to acquire pluripotency.\textsuperscript{35} More recently, nuclear reprogramming has been induced with the minimal requirement of ectopic gene expression of stemness factors.\textsuperscript{36,37} High-stringency testing for bona fide pluripotency has been restricted to low-throughput procedures, such as germline transmission and tetraploid aggregation.\textsuperscript{6,7} Non-coerced diploid aggregation, presented here, offers a rapid yet reliable surrogate marker for functional pluripotency with definitive readout feasible within 24 hours. Acquired pluripotency from somatic fibroblasts was herein verified by the inability of parental, nonreprogrammed counterparts to contribute to blastocyst development. As iPS technology continues to advance,\textsuperscript{36–38} diploid aggregation techniques offer thereby a high-throughput functional assay to screen for the pluripotent state of a clonal progenitor population.

Stem cell–based repair integrates multifactorial mechanisms with cardiac regeneration predicated on the ability of diseased myocardium to be replenished with healthy multilineage tissue.\textsuperscript{39–45} Rejuvenation can be achieved from endogenous stem cell pools that respond to injury, albeit at levels typically insufficient to compensate for severe cardiac damage.\textsuperscript{39} Exogenous stem cell types have been employed to augment therapeutic healing through paracrine-mediated mechanisms\textsuperscript{40} and/or cell-autonomous contribution to tissue reconstruction.\textsuperscript{41,42} In this way, embryonic stem cells have reproduced the complete cellular repertoire within ischemic heart\textsuperscript{43–45} and have demonstrated the ability to augment the physical force of cardiac contraction on transplantation.\textsuperscript{45} The present study introduces iPS therapy into a diseased myocardium as an alternative to produce cardiogenic tissue independently of an embryonic source. The iPS platform for cell-based regeneration relies on the acquired ground state of pluripotency to enable somatic tissue–derived cardiopiosis, here validated by in vitro differentiation with canonical cardiac transcription factor expression and in utero organogenesis with heart parenchyma patterning. Live-cell imaging documented in situ reconstruction of damaged tissues and engraftment of iPS-derived progeny within the heart, as transplanted cells were extensively processed to avoid carryover of lentiviral particles that could cross-contaminate native tissue and confound analysis of in vivo cell tracking.\textsuperscript{46,47} Intramyocardial transplantation of parental fibroblasts within acutely infarcted tissue was unproductive, despite previous indications that a multitude of cell types may improve cardiac function.\textsuperscript{48} In contrast, iPS responded to cardiac injury with controlled integration and chimeric tissue formation within allogeneic host. Cell integration translated into performance recovery as qualified iPS clones contributed to tissue reconstruction with synchronized cardiovascular, composed predominantly of cardiac lineage with accompanying smooth muscle and endothelium. Although multiple mechanisms likely contribute to the benefit associated with iPS-based therapy, converting fibroblasts, which are main contributors to postischemic scar, into reparative progenitors can thus now be considered a goal to engage and promote de novo tissue repair.

The heterogeneity inherent to the epigenetics and gene expression profiles within pluripotent cells, however, raises concern for pleiotropic outcomes.\textsuperscript{2,21,49} Both embryonic stem cells and iPS harbor the potential risk of producing teratoma. The propensity for uncontrolled growth of embryonic stem cells is nevertheless modulated by the immunity of nonnative environment, as here demonstrated for iPS within adult immunocompetent hosts. A properly matched milieu, such as that of the developing embryo, furthermore ensures precise differentiation and in utero growth.\textsuperscript{28} Diploid aggregation,
utilizing the chimeric environment, revealed the reliability of iPS clones to orchestrate tissue patterns without triggering tumorigenic outgrowth, in agreement with tumor-free organogenesis of iPS offspring.3,6,7 Fully differentiated cell types, such as native fibroblasts used here, are, in contrast, unable to contribute to primordial embryo or heart repair. Exemplified by the inability to integrate into the electric syncytium of failing myocardium, transplantation of non–stem cell progeny has been plagued by the rigidity associated with advanced cell fate.48,50 Accordingly, the risk/benefit of iPS-based interventions will critically depend on the state of lineage differentiation and the interface with host milieu.

In conclusion, this study expands the therapeutic potential of iPS treatment from noncardiac to cardiac disease. Created by ectopic expression of 4 human stemness-related factors, iPS demonstrated acquired cardiogenicity and ensured functional and structural repair of infarcted myocardium. Although individual iPS clones are likely to exhibit a spectrum of reparative potential, advances in nuclear reprogramming provide the tools to customize the reversibility of cell fate for “on-demand” cardiovascular regenerative medicine.

Acknowledgments

We thank Jonathan Nesbitt for surgical expertise and Lois Rowe for histological analysis.

Sources of Funding

This study was supported by the National Institutes of Health (R01HL083439, T32HL007111, R01HL085208, R56AI074736), American Heart Association, American Society for Clinical Pharmacology and Therapeutics, National Hemophilia Foundation, La Caixa Foundation Graduate Program, Marriott Individualized Medicine Program, Marriott Heart Disease Research Program, and Mayo Foundation.

Disclosures

None.

References


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**CLINICAL PERSPECTIVE**

Triggered nuclear reprogramming through ectopic transgene expression of stemness factors offers a revolutionary strategy for embryo-independent derivation of pluripotent stem cells from an ordinary adult source. Induced pluripotent stem cell (iPS) technology invalidates chronological age, reversing cell fate to reset an atavistic embryonic-like potential of somatic tissue. In this way, iPS have attained functions previously demonstrated only by natural embryonic stem cells to independently produce all tissue types and develop the complete organism within an embryonic environment. In the present study, fibroblasts redirected by a foursome of human stemness factors, OCT3/4, SOX2, KLF4, and c-MYC, acquired bona fide pluripotent features ensuring de novo tissue replacement in embryonic and adult host environments. Head-to-head comparison between parental fibroblasts and reprogrammed progeny established the therapeutic value for cardiac tissue regeneration in a setting of ischemic heart disease. Specifically, transplantation of iPS in the acutely infarcted myocardium yielded structural and functional repair to secure performance recovery as qualified clones contributed to in vivo tissue reconstruction with “on-demand” cardiovascularogenesis. Although the full clinical impact of iPS-based technology will be revealed when safeguards are fully validated, the proof-of-principle study here establishes the practical application of bioengineered stem cells to respond to the ischemic myocardium and heal the damaged areas with iPS-derived integrated functional tissues. Therefore, converting self-derived fibroblasts, which are main contributors to postischemic scar, into reparative progenitors can now be considered a goal of regenerative medicine to individualize treatment algorithms for multilineage cardiovascular repair.
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_Circulation_. 2009;120:408-416; originally published online July 20, 2009; doi: 10.1161/CIRCULATIONAHA.109.865154

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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