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Reporter-Based Isolation of Induced Pluripotent Stem Cell– and Embryonic Stem Cell–Derived Cardiac Progenitors Reveals Limited Gene Expression Variance

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Rationale: Induced pluripotent stem (iPS) cells can differentiate into multiple cell types, including cardiomyocytes and have tremendous potential for drug discovery and regenerative therapies. However, it is unknown how much variability exists between differentiated lineages from independent iPS cell lines and, specifically, how similar iPS cell–derived cardiomyocytes (iPS-CMs) are to embryonic stem (ES) cell–derived cardiomyocytes (ES-CMs).

Objective: We investigated how much variability exists between differentiated lineages from independent iPS cell lines and how similar iPS-CMs are to ES-CMs.

Methods and Results: We generated mouse iPS cells in which expression of NKX2-5, an early cardiac transcription factor, was marked by transgenic green fluorescent protein (GFP). Isolation of iPS- and ES-derived NKX2-5–GFP[+] cardiac progenitor pools, marked by identical reporters, revealed unexpectedly high similarity in genome-wide mRNA expression levels. Furthermore, the variability between cardiac progenitors derived from independent iPS lines was minimal. The NKX2-5–GFP^{+} iPS cells formed cardiomyocytes by numerous induction protocols and could survive upon transplantation into the infarcted mouse heart without formation of teratomas.

Conclusions: Despite the line-to-line variability of gene expression in the undifferentiated state of ES and iPS cells, the variance narrows significantly in lineage-specific iPS-derived cardiac progenitors, and these progenitor cells can be isolated and used for transplantation without generation of unwanted cell types. (*Circ Res*. 2010;107:340-347.)

Key Words: induced pluripotent stem cells ■ embryonic stem cells ■ cardiomyocytes ■ reporter ■ GFP ■ NKX2-5 ■ microarray

Induced pluripotent stem (iPS) cells may be a promising alternative to embryonic stem (ES) cells for both drug discovery and regenerative therapies, because they can differentiate into derivatives of all 3 germ layers.[1–4] However, because each iPS cell line is generated through nuclear reprogramming of a somatic cell, it is likely that the genome-wide epigenetic changes differ among individual cell lines. Indeed, numerous reports have examined the variation in gene expression among independent, undifferentiated iPS and ES cell lines.[1,3,5] However, the paucity of iPS cell lines with lineage-specific reporters has precluded determination of the degree to which such heterogeneity persists as cells adopt specific lineages and epigenetically silence much of the genome.

iPS cells can be induced to form cardiomyocytes in vitro, much like ES cells.[6,7] However, it remains unknown how much variability exists between cardiomyocytes derived from independent iPS cell lines or between iPS and ES cell–derived cardio-

myocytes (iPS-CMs and ES-CMs), particularly in terms of gene expression. Analysis of iPS-CMs has been hampered by the unavailability of reporter lines that would enable selection of cardiac progenitors. iPS cell lines with a cardiac-specific fluorescent reporter would enable more accurate quantification of cardiomyocyte yield by various differentiation methods, selection of cardiomyocytes for regenerative purposes, and isolation of cardiac progenitors to assess the line-to-line variability in gene expression among lineage-specific iPS cell derivatives. Furthermore, generation of iPS cell lines containing the same lineage-specific reporter as an ES cell line would facilitate isolation of comparable progenitor populations to determine the true degree of gene expression similarity between lineages derived from ES and iPS cells.

Here, we describe the generation of mouse iPS cells containing green fluorescent protein (GFP) regulated by a human NKX2-5 promoter–containing bacterial artificial

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chromosome (BAC).[8] NKX2-5 is expressed in early multipotent cardiac progenitors and differentiated cardiomyocytes.[8–12] Analyses of multiple independent NKX2-5–GFP iPS cell lines with ES cells containing the same reporter revealed unexpectedly high similarity in gene expression between sorted iPS and ES-derived cardiomyocyte progenitors and lower than expected differences in gene expression among NKX2-5–GFP$^+$ cells sorted from individual iPS cell lines.

Methods

NKX2-5 Reporter Mice

NKX2-5 reporter mice were generated by the Gladstone Transgenic Mouse Core facility by injection of the feeder-independent mouse ES cell line, E14, carrying the RP11-88L12/NKX2-5-Emerald GFP transgene (Mutant Mouse Regional Resource Centers [MMRRC] accession no. 30473) into 8 cell embryos using the same method as previously described for F0 analysis.[8] Mice with germline transmission were maintained as heterozygotes and genotyped from tail DNA by PCR (forward, gacgtgaccctgttcatcag; reverse, GTTTCTTGGG-GACGAAAG; 36-bp product) using the REDExtract-N-Amp PCR reaction (Sigma-Aldrich, XNAT-1KT) as recommended by the manufacturer. Cycling conditions were 94°C for 5 minutes; 35 cycles of 94°C for 1 minute, 56°C for 1 minute, 75°C for 1 minute; 72°C for 15 minutes. The NKX2-5 EmGFP mice were deposited with the Mutant Mouse Regional Resource Centers (MMRRC accession no. 030440).

Cell Culture, Pluripotency Induction, and Cardiomyocyte Differentiation

The tail tip was cut from an adult (8-week-old) male NKX2-5–GFP mouse,[8] cleaned with ethanol, washed in PBS, chopped into small pieces, and placed in collagenase IV/trypsin 0.25% solution at 37°C for 1 hour with 3 breaks for additional rounds of chopping. The resulting tissue was cultured in DMEM/F12 (+glutamine+Hepes+penicillin-streptomycin) with 10% FBS to isolate fibroblasts as described.[1] Induction of pluripotency by infection with 4 factors (Oct-4, Sox-2, Klf4, c-Myc) was performed essentially as described,[1] with one important modification: iPS cells were grown feeder-free in gelatinized culture plates at all stages (induction and maintenance). Our standard mES medium was used (GMEM supplemented with glutamine, sodium pyruvate, 0.1 mmol/L MEM nonessential amino acids, 10% [vol/vol] FBS [characterized, Hyclone], a 1:1000 dilution of β-mercaptoethanol stock solution [0.35% made from Sigma M7522], and 500 to 1000 U/mL leukocyte inhibitory factor [Chemicon ESG1107]). Valproic acid (2 mmol/L, Calbiochem) was added to this medium from day 2 to 9 after infection.[13]

To induce cardiomyocyte differentiation, hanging drops or suspension embryoid bodies (EBs) were made as described, including stimulation by Wnt3a where indicated.[10,14] Coaggregates with END-2 cells[15] were made as follows: mitotically inactivated END-2 cells were cultured under standard conditions,[16] dissociated and added to undifferentiated iPS cells in a 1:1 ratio, after which the standard protocol for suspension EBs was followed. To quantify beating, day 8 EBs were placed in 96-well plates at one EB per well and beating was scored after 3 days in a blinded fashion (n=8 plates derived from 3 batches of undifferentiated cells per line).

Calcium Imaging

NKX2-5–GFP$^+$ cardiomyocytes differentiated from iPS cells were dissociated and replated as single cells in regular differentiation medium. Cells were incubated with 50% Fluo4 Direct (Invitrogen) for 20 minutes before recording. Control and drug-treated cells were imaged with a Carl Zeiss Axio MiCAM02 and AxioVision version 4.7 software (Carl Zeiss) and configured with a linearly scaled stage and an XL S incubation unit (Carl Zeiss) to maintain physiological atmospheric conditions at 37°C and 5% CO2 with humidification.

Non-standard Abbreviations and Acronyms	
BAC	bacterial artificial chromosome
CM	cardiomyocyte
E	embryonic day
EB	embryoid body
ES	embryonic stem (cell)
FACS	fluorescence-activated cell sorting
GFP	green fluorescent protein
IPS	induced pluripotent stem (cell)
NOD-SCID	nonobese diabetic severe combined immunodeficient
qPCR	reverse transcriptase–quantitative PCR
SSEA-1	stage-specific embryonic antigen 1

Isoproterenol and carbachol were used at 1 μmol/L. Washing was performed with Hanks' BBS with 0.5% serum. Each experiment was repeated at least 3 times, and representative tracing images are displayed from cardiomyocytes after 45 days of differentiation.

Histology and Immunofluorescence

Immunocytochemistry was performed as described with cells on coverslips[17] or cryosections of EBs[18] (n ≥3 biological samples per cell line) using the following antibodies: Oct-4 (mouse, 1:100, Santa Cruz Biotechnology), SSEA-1 (stage-specific embryonic antigen 1) (mouse IgM, 1:50, Chemicon), α-actinin (mouse, 1:400, Sigma), smooth muscle actin (mouse, undiluted, DAKO), Nestin (mouse, 1:100, Chemicon), GFAP (glial fibrillary acidic protein) (rabbit, 1:100, DAKO), α-fetoprotein (mouse, 1:100, R&D), and GFP (rabbit, 1:500, Alexa-488–conjugated, Invitrogen). Secondary antibodies were Alexa-546–conjugated (Invitrogen).

Teratomas and hearts were processed for cryosectioning and staining as described.[19]

Quantitative PCR

RNA was extracted with TRIzol (Invitrogen). Reverse transcriptase–quantitative PCR (qPCR) was performed using the Superscript III first-strand synthesis system (Invitrogen) followed by use of TaqMan probes on the ABI 7900HT (TaqMan, Applied Biosystems) per the manufacturer's protocols with technical triplicates (n ≥3 biological samples except for initial pluripotency screening where n=2). Optimized primers from TaqMan Gene Expression Array were used. Expression levels were normalized to Gapdh expression.

Fluorescence-Activated Cell Sorting

For live cell fluorescence-activated cell sorting (FACS) to select pluripotent cells, dissociated cells were washed in PBS +10% FBS, blocked with CD16/32 antibody (eBioscience) for 10 minutes on ice, washed, incubated with first antibody SSEA-1 (Chemicon) 1:50 for 1 hour on ice, washed, incubated with second antibody (anti-mouse IgM+fluorophore PE or Alexa-488) 1:50 for 1 hour on ice, washed, and resuspended in 1% BSA/PBS, followed by FACS (FACSAria, BD) into 96-well plates at 1 cell per well to ensure clonal growth.

To quantify the number of GFP$^+$ cells after differentiation or select those cells for injection in the mouse heart, dissociated cells from EBs were washed in PBS+10% FBS and resuspended in 1% BSA/PBS and analyzed using the same sorter (n ≥3 per condition per cell line). The same gating and settings were used for every experiment.

iPS-Derived Chimeric Mice

Embryos were created by 8-cell-stage injection of NKX2-5-EmGFP$^+$ iPS cells as described.[20] E9.5 embryos were isolated and imaged without additional staining (n ≥8 per cell line).

Cell Injection in Adult Mice

For teratoma analysis, 500 000 iPS cells were injected subcutaneously and intramuscularly in 6-week-old male NOD-SCID (nonobese diabetic severe combined immunodeficient) mice (n=3 mice [6 injections] per cell line). The animals were monitored for tumor growth by inspection and palpation. Myocardial infarction was induced in 12-week old male NOD-SCID mice (Charles River) by ligation of the left anterior descending coronary artery under isoflurane anesthesia, as described previously.[19] Immediately after ligation, 400 000 iPS cells were injected in the infarcted myocardium through a 29-gauge needle (n=5 for unselected differentiated cells; n=12 for selected day 8 GFP$^+$ cells). All investigations involving experimental animals conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH, 1996) and were approved by the Institutional Animal Care and Use Committee (UCSF).

Gene Expression Microarray and Statistical Analysis

Mouse genome-wide gene expression analysis was performed using an Affymetrix Mouse Gene 1.0 ST Array. Day 8 Sorted GFP$^+$ differentiated iPS cells (iPS#3, iPS#25, and iPS#33), sorted GFP$^+$ differentiated ES cells (NKX2-5–GFP E14 and mCherry-NKX2-5–GFP E14), and undifferentiated ES cells (NKX2-5–GFP E14) were compared. RNA was extracted with the PicoPure RNA isolation kit (Molecular Devices). Linear models were fitted for each gene to estimate cell-type effects and associated significance using R (Bioconductor). Pairwise contrasts were set up to identify differentially expressed genes. Moderated t statistics and the associated probability values were calculated, as well as B statistics (log odds). Probability values were adjusted for multiple testing by (1) controlling for false-discovery rate using the Benjamini–Hochberg method and (2) controlling for family-wise error rate using the Bonferroni correction.

For all other experiments, error bars indicate standard error of the mean (SEM).

Results

To obtain iPS cells with a cardiomyocyte reporter label, tail tip fibroblasts were isolated from an NKX2-5–GFP transgenic mouse containing GFP in a BAC containing the human NKX2-5 locus.[8] Mouse ES cells containing the same NKX2-5–GFP BAC were previously reported.[8] Fibroblasts from the NKX2-5–GFP reporter mice were infected with a combination of 4 retroviruses encoding factors known to induce pluripotency: Oct-4, Sox-2, Klf4, and c-Myc.[2] Three-dimensional colonies were observed from the fifth day after viral infection. By day 8, colonies were large enough to be selected and expanded in a feeder-free culture system (Figure 1A). The cells derived from these colonies expressed SSEA-1 and Oct-4 protein as shown by immunostaining (Figure 1B and 1C). To select the best reprogrammed lines, qPCR for the pluripotency genes Nanog and Oct-4 was performed, revealing mRNA levels comparable to those of ES cells in both low (p4) and high (p20 to p25) passage samples (Figure 1D).

We also tested whether the cells were able to grow clonally by selecting SSEA-1-expressing cells by FACS (Figure 1E and 1F) and culturing in 96-well plates at 1 cell per well. Indeed, new colonies were obtained from single SSEA-1$^+$ cells that could be passaged and expanded while maintaining expression of pluripotency markers. This sorting strategy allowed for purification of potentially heterogenous colonies of iPS cells at the single-cell level. All studies were done with clonally derived cells. After subcutaneous and intramuscular injection into NOD-SCID mice, undifferentiated iPS cells of each selected line formed teratomas containing derivatives of all germ lines (Figure 1G through 1Q).

We next evaluated whether the NKX2-5–GFP iPS cells could be differentiated in vitro, with a special interest in cardiomyocyte differentiation. iPS cells formed beating EBs with high efficiency when induced by the hanging drop system. Spontaneous aggregation in suspension in low-attachment plates is much less labor-intensive and also produced some beating EBs, but with lower efficiency. However, when coaggregates in suspension were made with the endoderm cell line END-2,[15] a high percentage of beating EBs were isolated (Figure 1R). As expected, the beating foci within these EBs were green because of the NKX2-5–GFP reporter (Online Videos I and II, available at http://circres.ahajournals.org). The iPS cell–derived EBs contained derivatives of all 3 germ layers (Figure 1S through 1W), including α-actinin–positive cardiomyocytes with typical sarcomeric patterns (Figure 1V). Thus, we concluded that we had derived several independent NKX2-5–GFP iPS cell lines from an adult mouse and that the lines were able to retain their pluripotency under feeder-free culture conditions and responded to guided differentiation in vitro.

We quantified the percentage of GFP$^+$ cells in EBs, representing the NKX2-5–expressing population of cardiac progenitor cells and cardiomyocytes at day 8, obtained by several differentiation methods (Figure 2A). Consistent with the count of beating EBs, the hanging drop system yielded the highest percentage of GFP$^+$ cells (Figure 2B). END-2 coaggregates were not included in this analysis, because the END-2 cells present in the mixture of differentiated cells would confound the result by altering the denominator when calculating percent cardiac progenitors. The expression of a panel of cardiac-enriched genes was higher in sorted GFP$^+$ cells compared to sorted GFP$^-$ cells relative to undifferentiated ES or iPS cells (Figure 2C through 2F). In addition, the cardiomyocytes derived from the iPS cells after 45 days of differentiation had spontaneous calcium flux and responded to β-adrenergic agonist isoproterenol and muscarinic receptor agonist carbachol stimulation (Figure 2G). While the NKX2-5–GFP iPS cell lines we generated could efficiently differentiate into cardiomyocytes in vitro, we investigated their potential in vivo in embryos also. We took advantage of the recent demonstration that injection of ES cells into 8-cell embryos can result in generation of 95% to 100% chimeras, effectively generating embryos composed almost entirely of the injected ES cells.[20] Injection of NKX2-5–GFP iPS cells into 8-cell embryos revealed that the cells could give rise to viable embryos with hearts almost entirely populated by NKX2-5–GFP iPS-derived cardiomyocytes, illustrated by the green fluorescent hearts (Figure 2H).

To determine the similarity of iPS- and ES cell–derived cardiomyocytes at the gene expression level, we took advantage of an NKX2-5–GFP ES cell line generated with exactly the same BAC reporter transgene used to create the NKX2-5–GFP mouse line used for iPS generation.[8] Thus, NKX2-5–GFP$^+$ cells isolated from the iPS and ES cell lines should in principle mark very similar populations. Microarray analysis of mRNA from sorted NKX2-5–GFP$^+$ cells after 8 days of differentiation revealed that only 195 of the 28 853 transcripts represented were significantly different between ES and iPS

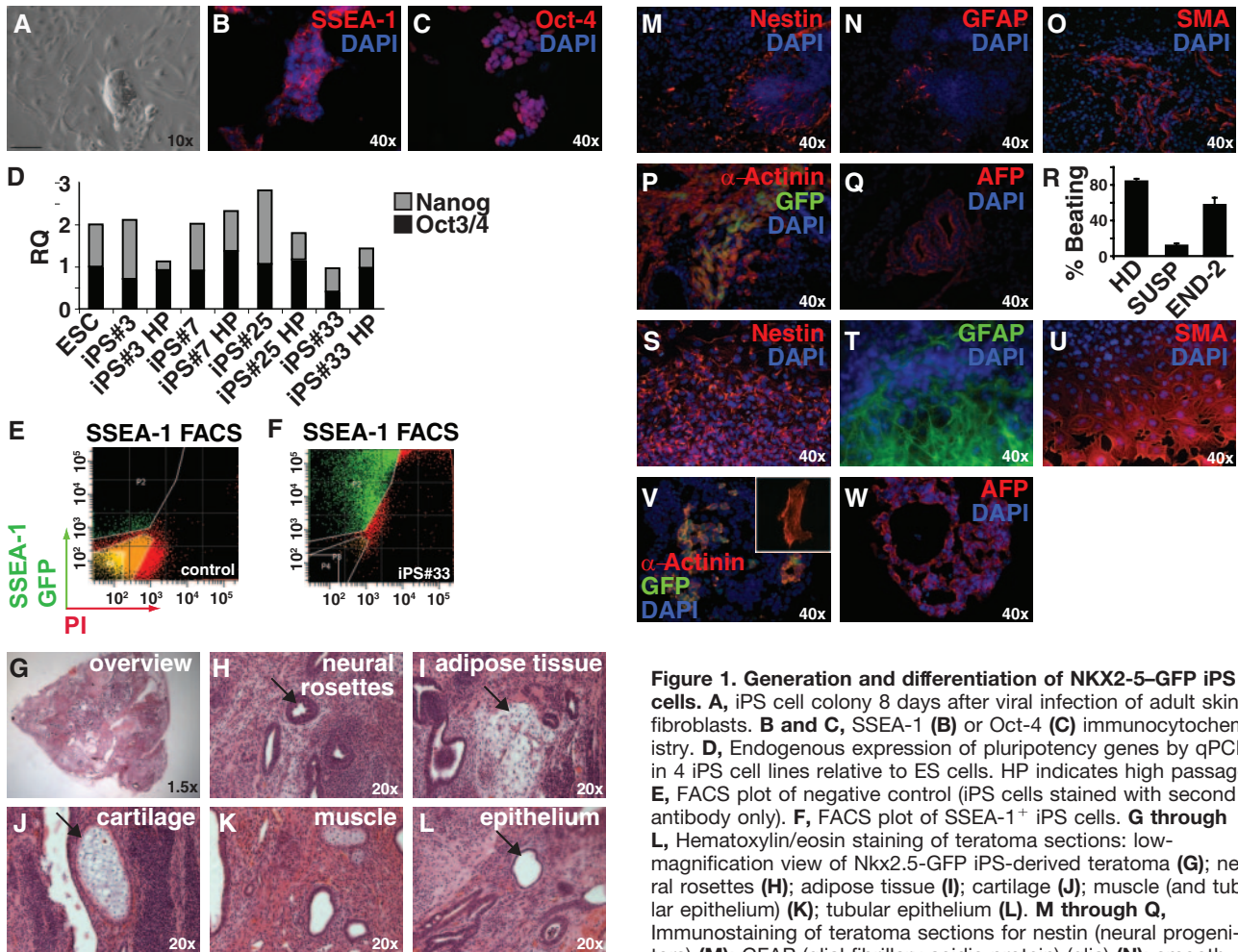**Figure 1. Generation and differentiation of NKX2-5–GFP iPS cells. A,** iPS cell colony 8 days after viral infection of adult skin fibroblasts. **B and C,** SSEA-1 **(B)** or Oct-4 **(C)** immunocytochemistry. **D,** Endogenous expression of pluripotency genes by qPCR in 4 iPS cell lines relative to ES cells. HP indicates high passage. **E,** FACS plot of negative control (iPS cells stained with second antibody only). **F,** FACS plot of SSEA-1+ iPS cells. **G through L,** Hematoxylin/eosin staining of teratoma sections: low-magnification view of Nkx2.5-GFP iPS-derived teratoma **(G)**; neural rosettes **(H)**; adipose tissue **(I)**; cartilage **(J)**; muscle (and tubular epithelium) **(K)**; tubular epithelium **(L)**. **M through Q,** Immunostaining of teratoma sections for nestin (neural progenitors) **(M)**; GFAP (glial fibrillary acidic protein) (glia) **(N)**; smooth muscle actin (SMA) **(O)**; α-actinin **(red)**, GFP **(green) (P)**; α-fetoprotein (AFP) (endoderm) **(Q)**. **R,** Percentage of beating EBs with different induction methods: hanging drop (HD), suspension (SUSP), or END-2 coculture. **S through W,** Derivatives of all 3 germ layers in EBs in vitro demonstrated by immunostaining with indicated markers. **S through U,** Suspension EBs **(V and W)** co-culture with END-2 cells immunostained with indicated markers.

cell–derived cardiac progenitors, even when applying the least stringent statistical method for defining differential expression (FDR; adjusted probability value to control for false discovery rate using Benjamini–Hochberg method, with cutoff probability value set at $P<0.1$). Of those, only 38 annotated genes were altered greater than 2-fold, revealing a surprisingly high degree of similarity in the differentiated progeny of ES and iPS cells (Figure 3; Online Tables I and II).

Concerns exist regarding the potential variability between iPS cell lines compared to the variability between ES lines, but the degree of variability of gene expression in lineage-specific cells derived from ES and iPS cells has not been interrogated for any cell type. This issue is particularly important for interpreting studies with multiple disease-specific iPS lines. Using the microarray data sets of NKX2-5–GFP-positive ES and iPS cells, we found that the variation between independent iPS cell–derived cardiac progenitors was no larger than that between cardiac progenitors derived from separate ES lines (Figure 3B and 3C). The correlation between cell lines was extremely high ($R=0.99$) (Figure 3B), suggesting that not only were the ES and iPS-derived cardiac progenitors more similar than

expected, but the line-to-line variation of the lineage-specific iPS cells was also very low.

To assess the potential of the NKX2-5–GFP iPS cells in regenerative applications, day 8 EBs were dissociated and prepared for injection into acutely infarcted hearts of NOD-SCID mice. Although this approach resulted in large patches of new GFP+ myocardium being formed (Figure 4A), clusters of SSEA-1– and Oct-4–expressing cells were also observed (Figure 4B and 4C), indicating that progenitor selection was necessary to avoid tumor formation. We thus sorted for GFP+ cells by flow cytometry to isolate the cardiac progenitor pool before intramyocardial transplantation. Although the grafts from NKX2-5–GFP+ cells were smaller than those observed after total cell injection, they did not form teratomas and were negative for markers of pluripotency (Figure 4D). These findings suggest that the selected NKX2-5–GFP+ cells may be safe for regenerative approaches, at least with respect to tumor formation.

Discussion

Here, we demonstrate the feeder-free generation and maintenance of clonally derived NKX2-5–GFP iPS cell lines that
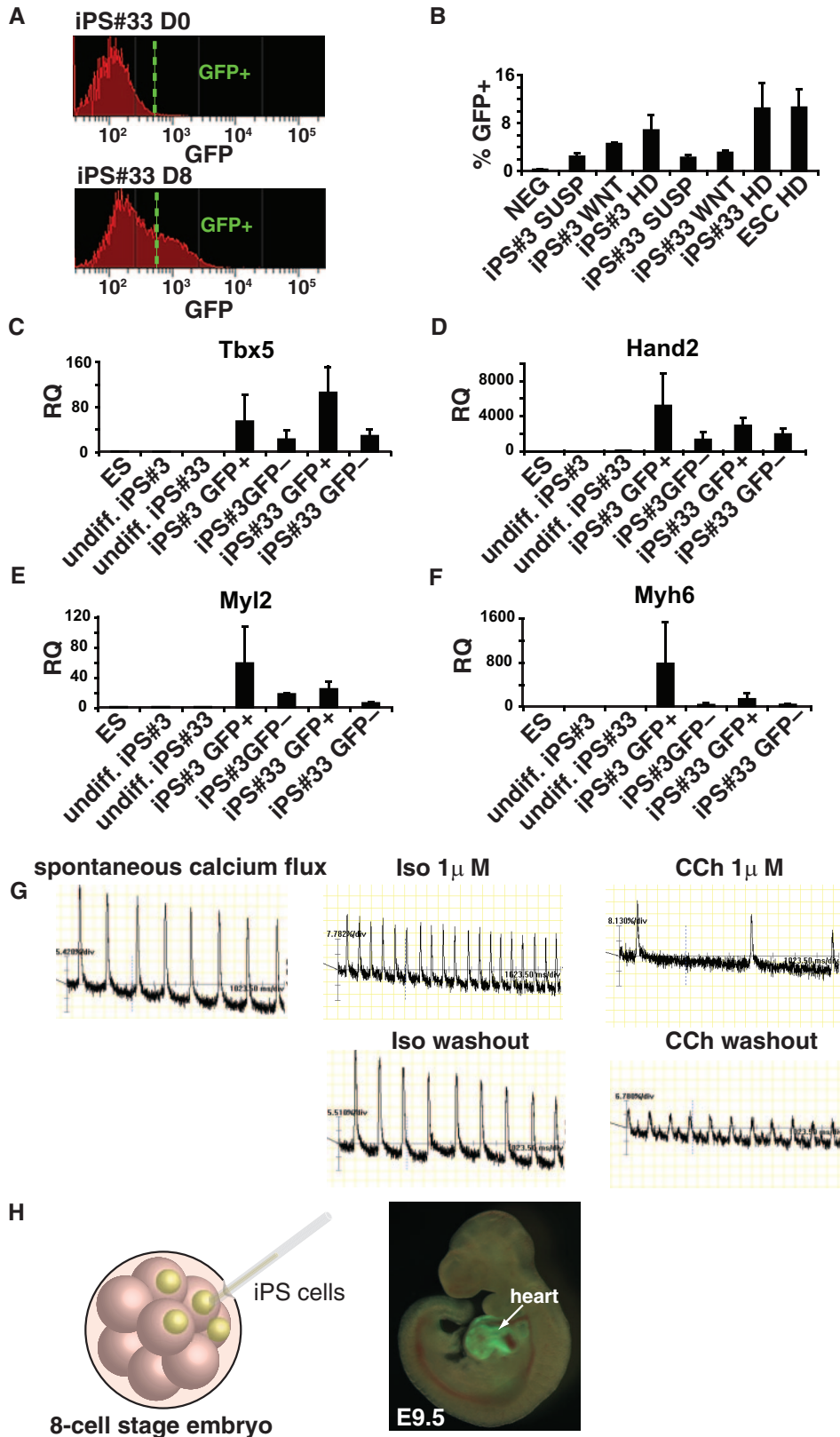
Figure 2. Cardiac differentiation and quantification from NKX2-5–GFP iPS cells. A, Example of FACS to select GFP+ cells from negative control cells at day 0 (D0) or day 8 (D8) NKX2-5–GFP iPS cells differentiated by hanging drop (HD) EBs. **B,** Quantification of GFP+ cells in day 8 EBs by FACS using HD, suspension (SUSP), or suspension+stimulation with Wnt3a (WNT) methods for differentiation. **C through F,** Relative expression of Tbx5, Hand2, Mlc2, and Myh6 in sorted differentiated iPS cells compared with undifferentiated iPS cells by qPCR. **G,** Calcium imaging traces of iPS cell–derived cardiomyocytes after 45 days of differentiation at baseline and in response to isoproteronol (Iso) or carbachol (CCh) treatment. **H, Left,** Schematic diagram showing iPS cells injected into a 8-cell-stage embryo. **H, Right,** E9.5 mouse embryo derived from injection of undifferentiated NKX2-5–GFP iPS cells into a wild-type 8-cell embryo, indicating GFP cells in the heart **(arrow)**.
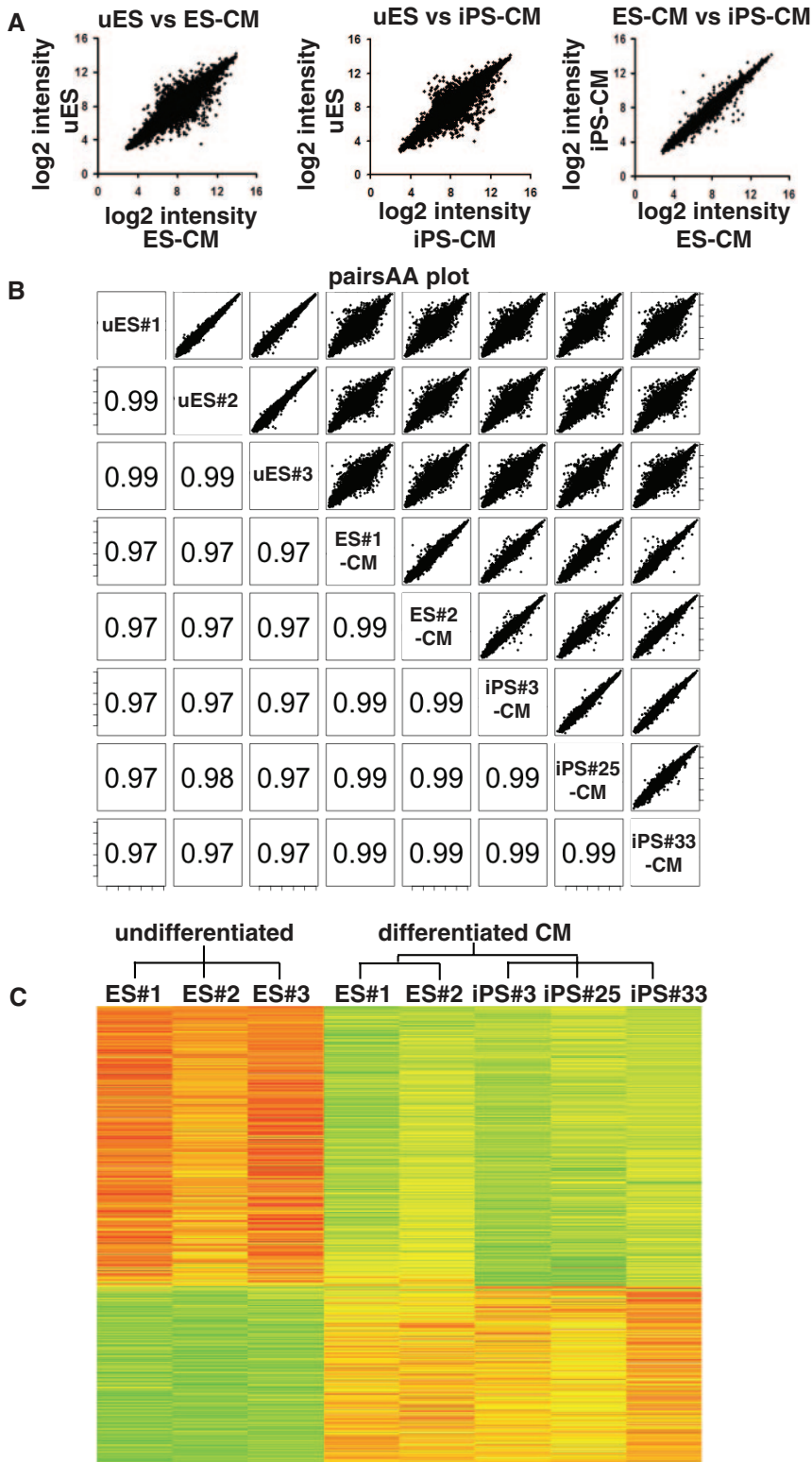
Figure 3. Gene expression microarray analyses comparing sorted GFP⁺ iPS or ES-derived cardiomyocyte progenitors (iPS-CMs or ES-CMs) and undifferentiated ES cells (uES). A, Log2 intensity plots showing the averaged variation between undifferentiated ES cells, sorted GFP⁺ differentiated ES cells, and sorted GFP⁺ differentiated iPS cells after 8 days of differentiation. **B,** Concordance between different cell lines of undifferentiated ES cells (uES#1, uES#2, uES#3), sorted GFP⁺ differentiated ES cells (ES#1-CM, ES#2-CM), and sorted GFP⁺ differentiated iPS cells (iPS#3-CM, iPS#25-CM, iPS#33-CM). **C,** Heat maps of microarray comparing (from left to right) undifferentiated ES cells, sorted GFP⁺ differentiated ES cells, and sorted GFP⁺ differentiated iPS cells.

allowed identification, selection, and analyses of iPS-derived cardiac progenitors. Our finding that ES and iPS cell–derived NKX2-5–GFP⁺ cardiac progenitors are highly similar at the gene expression level represents the first comparison of ES and iPS derivatives of any cell type and was facilitated by the generation of pluripotent lines containing the same reporter gene. Despite the differences reported in gene expression

between ES and iPS cells, it appears that as genomic loci are silenced or activated during lineage commitment, these differences rapidly narrow. The few genes that were differentially expressed do not appear to signify functional differences between the cells (Online Table II), although we did note increased expression of some markers of less differentiated cells in ES-derived cells (eg, Dppa2, Rex1) with a
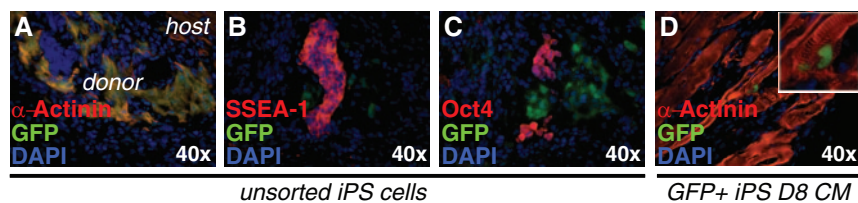
Figure 4. NKX2–5–GFP iPS cell–derived cardiac progenitors survive in the infarcted mouse heart after transplantation. A through C, Histology section of hearts 2 weeks after transplantation of unsorted dissociated NKX2-5–GFP cells from day 8 EBs into the infarcted mouse heart immunostained with α-actinin **(red)** and GFP **(green) (A)**; SSEA-1 **(red)** and GFP **(green) (B)**; Oct-4 **(red)** and GFP **(green) (C)**. **D,** Immunostaining of heart sections 2 weeks after transplantation of sorted NKX2-5–GFP⁺ dissociated cells from day 8 EBs into the infarcted mouse heart, α-actinin **(red)**, GFP **(green)**. DAPI indicates nuclei **(blue)**. Inset indicates higher magnification of GFP⁺ cell.

reciprocal increase in some markers of differentiated lineages in iPS-derived cells (eg, Lhx1, Cer1, Otx2). Given the small number of altered genes, there is no statistical significance to these categories, but it will be interesting to determine whether the iPS cells differentiate at a slightly accelerated pace.

The limited variability of gene expression between cardiac progenitors isolated from independent iPS cell lines also suggests that the broader heterogeneity of genome-wide reprogramming in the pluripotent state becomes less consequential as cells adopt specific lineages. This finding is promising for the study of disease-specific iPS cell lines and the use of iPS cells for drug toxicity, because the signal-to-noise ratio in the gene expression of lineage-specific iPS-derived cells may be significantly less than expected.

Finally, quantification of cardiomyocytes by FACS facilitated the evaluation of multiple cardiomyogenic differentiation protocols in iPS cells, revealing that iPS cells quantitatively had similar cardiomyogenic potential compared to ES cells.[10,14,15] Furthermore, the ability of iPS cells to contribute to the embryonic heart in vivo and for transplanted iPS cell–derived cardiac progenitors to form new myocardium in the infarcted mouse heart provide an important foundation for the future use of iPS cell–derived cardiac progenitors in cardiac regenerative approaches. It will be important to evaluate the functional maturation and contribution of such progenitors in vivo as technology improves to retain introduced cells within the host myocardium.

Disclosures

D.S. is a member of the Scientific Advisory Board of iPierian Inc and RegeneRx Pharmaceuticals. B.R.C. is a consultant for iPierian Inc.

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Novelty and Significance

What Is Known?

- Adult skin fibroblasts can be transformed into induced pluripotent stem (iPS) cells.
- iPS cells are similar to embryonic stem (ES) cells but have differences in gene expression and have significant line-to-line variability in gene expression.
- iPS cells can differentiate into cardiac progenitor cells (CPCs).

What New Information Does This Article Contribute?

- CPCs derived from individual induced pluripotent stem cells lines show no more variability than cardiac progenitors derived from separate lines of ES cells.
- CPCs derived from induced pluripotent stem cells display a gene expression profile that is highly similar to that of ES cell–derived cardiomyocytes.
- CPCs derived from iPS cells can survive after intramyocardial transplantation without tumor formation.

The iPS cells could provide an alternative to ES cells as a source of CPCs or cardiomyocytes for drug discovery and eventually regenerative therapies. However, it has been postulated that there may be significant variability between CPCs derived from independent iPS cell lines or between iPS cell– and ES cell–derived CPCs. Reporter lines that would enable selection of CPCs are required for analysis of iPS cell–derived CPCs but were unavailable up until now. We generated mouse iPS cell lines containing the same lineage-specific reporter (NKX2-5–GFP) as ES cell lines. With this unique tool, we were able to isolate comparable progenitor populations derived from each cell type. We found unexpectedly high similarity in genome-wide mRNA expression levels. Furthermore, the variability between CPCs derived from independent iPS lines was minimal. The NKX2-5–GFP$^+$ iPS cells formed cardiomyocytes by numerous induction protocols and could survive on transplantation into the infarcted mouse heart without formation of teratomas. Our findings suggest that genetic variance is limited in lineage-specific iPS-derived CPCs and that these progenitor cells can be isolated and used for transplantation without the generation of unwanted cell types. This is an important step forward toward application of iPS cells for in vitro drug testing and cardiac repair.