JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2014 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.
VOL. 64, NO. 5, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.04.056

Effect of Human Donor Cell Source on Differentiation and Function of Cardiac Induced Pluripotent Stem Cells

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ABSTRACT

BACKGROUND Human-induced pluripotent stem cells (iPSCs) are a potentially unlimited source for generation of cardiomyocytes (iPSC-CMs). However, current protocols for iPSC-CM derivation face several challenges, including variability in somatic cell sources and inconsistencies in cardiac differentiation efficiency.

OBJECTIVES This study aimed to assess the effect of epigenetic memory on differentiation and function of iPSC-CMs generated from somatic cell sources of cardiac versus noncardiac origins.

METHODS Cardiac progenitor cells (CPCs) and skin fibroblasts from the same donors were reprogrammed into iPSCs and differentiated into iPSC-CMs via embryoid body and monolayer-based differentiation protocols.

RESULTS Differentiation efficiency was found to be higher in CPC-derived iPSC-CMs (CPC-iPSC-CMs) than in fibroblast-derived iPSC-CMs (Fib-iPSC-CMs). Gene expression analysis during cardiac differentiation demonstrated up-regulation of cardiac transcription factors in CPC-iPSC-CMs, including *NKX2-5*, *MESP1*, *ISL1*, *HAND2*, *MYOCD*, *MEF2C*, and *GATA4*. Epigenetic assessment revealed higher methylation in the promoter region of *NKX2-5* in Fib-iPSC-CMs compared with CPC-iPSC-CMs. Epigenetic differences were found to dissipate with increased cell passaging, and a battery of in vitro assays revealed no significant differences in their morphological and electrophysiological properties at early passage. Finally, cell delivery into a small animal myocardial infarction model indicated that CPC-iPSC-CMs and Fib-iPSC-CMs possess comparable therapeutic capabilities in improving functional recovery in vivo.

CONCLUSIONS This is the first study to compare differentiation of iPSC-CMs from human CPCs versus human fibroblasts from the same donors. The authors demonstrate that although epigenetic memory improves differentiation efficiency of cardiac versus noncardiac somatic cell sources in vitro, it does not contribute to improved functional outcome in vivo. (J Am Coll Cardiol 2014;64:436–48) © 2014 by the American College of Cardiology Foundation.

Coronary heart disease is the leading cause of death in developed countries, accounting for approximately 550,000 deaths annually in the United States alone (1). As the only option for end-stage treatment, heart transplantation excludes many patients who are not suitable candidates because of preexisting comorbidities and is furthermore limited by the low number of available organ donors. In recent years, cell-based

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Manuscript received December 23, 2013; revised manuscript received March 31, 2014, accepted April 30, 2014.

therapies have emerged as a promising alternative (2). Human-induced pluripotent stem cells (iPSCs), in particular, are an attractive donor cell source in view of their capacity for unlimited self-renewal and pluripotency (3). In addition, because iPSCs can be derived from various somatic tissue sources (4), their use largely circumvents ethical and immunologic concerns associated with embryonic stem cell (ESC)-based therapies.

SEE PAGE 449

However, several studies have demonstrated that iPSCs are not as similar to ESCs as initially believed (5). Newer published data suggests that epigenetic memory has the potential to influence the differentiation potential and functional maturity of iPSC-derived cell types (6).

In this study, the authors aimed to assess the contributions of epigenetic memory to the differentiation potential, function, and maturity of human iPSCs derived from cardiac and noncardiac sources. To do so, the study generated human iPSCs from cardiac progenitor cells (CPC-iPSCs) and dermal fibroblasts (Fib-iPSCs) from the same donors and differentiated these cells to iPSC-CMs for in vitro and in vivo characterization. Because human CPCs have been shown to give rise to multiple lineages of cells found in the heart (7–9), the authors also compared the ability of CPC-iPSCs and Fib-iPSCs to differentiate into other cell types found in the heart.

METHODS

A detailed Methods section is available in the Online Appendix. Stanford University (Stanford, California) Human Subjects Research Institutional Review Board approved all the protocols in this study. iPSCs were generated using lentiviral transduction, as previously described (4). A modified protocol from Yang et al. (10) was followed to differentiate iPSC-CMs by 3-dimensional (3D) embryoid body (EB) formation. As an alternate method of cardiac differentiation, a 2-dimensional (2D) monolayer protocol from Lian et al. (11) was also used. Following differentiation, iPSC-CMs were cultured in vitro using SCT Cardiac Maintenance Media (Stem Cell Theranostics, Menlo Park, California). A detailed description is included in the Methods section in the Online Appendix.

STATISTICAL ANALYSIS. Normality distribution was studied with the Kolmogorov-Smirnov test ($p < 0.05$). Statistically significant differences were determined using the Mann-Whitney test or Wilcoxon signed rank test, with alpha set to 0.05 for samples not displaying a normal distribution, and with Student *t* test or paired Student *t* test, with alpha set to 0.05 for samples with a normal distribution. Unless specified, data are expressed as mean ± SEM. All statistical analysis was carried out using Prism5 (GraphPad Software, La Jolla, California).

RESULTS

Skin fibroblasts and CPCs were isolated from 2 matched human fetal donors (**Fig. 1A**). To perform CPC isolation, heart tissue was digested to a single cell suspension and labeled with Sca-1 antibody for magnetic cell sorting (12). Following a brief period of cell expansion, fetal CPCs and fibroblasts were characterized for gene expression. Fetal CPCs were found to express genes associated with cardiac lineage, such as *KDR*, *NKX2-5*, *TBX18*, *WT1 MEF2C*, and *GATA4*, whereas fibroblasts expressed only *TBX18* (Online Fig. 1A). Neither the CPCs nor fibroblasts were found to express genes associated with pluripotency such as *NANOG* and *OCT4*, both of which were highly expressed in iPSCs (Online Fig. 1B). CPCs and fibroblasts were subsequently reprogrammed through lentiviral infection using *OCT4*, *SOX2*, *KLF4*, and *c-MYC* (3). After approximately 3 weeks, colonies positive for alkaline phosphatase (**Fig. 1B**) with ESC-like morphology were mechanically isolated and expanded on Matrigel-coated dishes. No differences in reprogramming efficiency were observed between the 2 cell types. Both CPC-iPSCs and Fib-iPSCs exhibited identical morphologies and presence of pluripotency markers such as Tra-1-60, and Oct4 (**Fig. 1B**). Teratoma formation assays using CPC-iPSCs and Fib-iPSCs produced derivatives from all 3 germ layers (**Fig. 1C**). Paired CPC-iPSCs and Fib-iPSCs also were generated from an adult 65-year old donor as an additional control. Reprogramming was conducted in a manner identical to that used in fetal donor sources. Adult CPC-iPSCs and Fib-iPSCs similarly exhibited ESC-like morphologies and markers of pluripotency (Online Fig. 2).

Following iPSC characterization and a brief period of cell expansion, CPC-iPSCs and Fib-iPSCs were differentiated into iPSC-CMs via 3D EB formation (Online Fig. 3A) (10). At day 15 following induction of cardiac differentiation, spontaneously beating EBs were observed under brightfield microscopy. Beating EBs were dissociated into single cells and characterized by confocal microscopy for immunostaining against cardiac-specific markers, such as cardiac troponin T (cTnT) and sarcomeric alpha-actinin (**Fig. 2A**, Online Fig. 3B). A fluorescence-activated cell sorting (FACS) analysis of dissociated EBs confirmed a significantly higher percent of cTnT-positive cells in CPC-iPSC-CMs than in Fib-iPSC-CMs

ABBREVIATIONS AND ACRONYMS

CM = cardiomyocyte
CPC = cardiac progenitor cell
cTnT = cardiac troponin T
EB = embryoid body
EC = endothelial cell
FACS = fluorescence-activated cell sorting
Fib = fibroblast
iPSC = induced pluripotent stem cell
MEA = multielectrode array
MI = myocardial infarction
Sca = stem cell antigen
SMC = smooth muscle cell