Get With the (Re)Program
Cardiovascular Potential of Skin-Derived Induced Pluripotent Stem Cells
Nathaniel L. Tulloch, BS; Lil Pabon, PhD; Charles E. Murry, MD, PhD

The reprogramming of adult cells to a pluripotent state resembling embryonic stem (ES) cells is one of the most exciting advances in stem cell biology in the last decade. These induced pluripotent stem (iPS) cells offer the potential for autologous regenerative therapies, new models to understand disease, and systems for drug discovery. Little is known, however, about the ability of iPS cells to generate cell types of relevance to the cardiovascular system. Two articles1,2 in this issue of Circulation indicate that cardiomyocytes, smooth muscle cells, and multiple types of endothelium can be derived from mouse iPS cells, encouraging efforts toward developing patient-matched cells for cardiovascular disorders.

Articles pp 498 and 507

Creating Pluripotent Non-ES Cells
The cloning of Dolly the sheep3 involved reprogramming of an udder cell to a totipotent state by fusion with an enucleated sheep oocyte. Although this breakthrough demonstrated that all mammalian tissues could arise from a single differentiated nucleus, the factors required remained enigmatic. The mechanistic breakthrough came when Takahashi and Yamanaka4 overexpressed a battery of candidate genes in mouse dermal fibroblasts and then grew the cells under conditions favoring expansion of ES cells. They found that ES-like cells emerged, and they systematically winnowed the required list down to 4 transcription factors: Oct4, Sox2, c-myc, and Klf4 (Figure 1). Oct4 and Sox2 are part of the core transcriptional network required for pluripotency. c-myc is a protooncogene required for cell cycle progression. Klf4 is a cell cycle regulator that may control self-renewal of ES cells and block apoptotic pathways induced by c-myc. Recently, reprogramming of human dermal fibroblasts was achieved with the same 4 factors5,6 or with a slightly different cocktail of Oct4, Sox2, Nanog, and Lin28.7 Fortuitously, retrovirally expressed genes typically are silenced in pluripotent cells, meaning that, once established, pluripotency is maintained by endogenous genes subject to normal regulation after differentiation.

Mouse iPS cells generate teratomas (tumors comprising ectoderm, mesoderm, and endoderm derivatives) when injected into adult mice and form chimeric mice after blastocyst injection.4,5,7 The chimeras transmitted the iPS genome to offspring, indicating germline transmission. These properties indicate that mouse iPS cells are as potent as ES cells. Human iPS cells similarly generate teratomas after transplantation, and differentiation to neural progenitors and cardiomyocytes8 suggests that human iPS cells may be as potent as their mouse counterparts.

Although iPS cells can generate all mammalian cell types, many intriguing questions remain. For instance, are the yields for generating the cells of interest from iPS cells comparable to those of ES cells? Can different cardiovascular cell subtypes be generated? Are the cells generated from iPS and ES cells functionally equivalent? Do the different iPS lines have distinct differentiation properties? The 2 iPS cell articles1,2 presented in this issue characterize more comprehensively the cardiovascular differentiation capabilities of independently derived iPS lines with respect to ES cells.

Generation of iPS-Derived Cardiovascular Cells
Both of these groups used iPS cells generated with the original 4-factor cocktail including c-myc. Although Mauritz et al1 used a single line of iPS cells selected by the Oct4 promoter, Narazaki et al2 used 3 independent iPS lines selected by the Nanog promoter (Figure 1). The differentiation protocols also differed between the 2 groups. Mauritz’s group induced differentiation by forming 3-dimensional aggregates (embryoid bodies [EBs]) and treating the cells with serum.1 Narazaki et al differentiated in monolayers using serum and selected by fluorescence-activated cell sorting for mesodermal cells expressing the vascular endothelial growth factor (VEGF) receptor Flk1.2 They then obtained endothelial and mural cells by adding VEGF plus serum and cardiomyocytes by coculture with OP9 cells.

The results of the 2 studies disagree slightly regarding several aspects of differentiation. Mauritz et al report delayed iPS cell-cardiomyocyte differentiation compared with ES cells measured both by percentage of spontaneously contracting EBs and by expression of cardiomyocyte-specific mRNA for troponin (3- to 5-fold lower in iPS EBs).1 In this regard, it is important to note that their line of ES cells showed unusually efficient differentiation into cardiomyocytes (100% of EBs beating by day 8), whereas cardiogenesis in iPS cells was comparable to that in published reports with other ES cell lines.8 Along these lines, Narazaki and colleagues found that...
cardiac differentiation of iPSCs was within the range of the different lines of ES cells they studied in terms of both beating activity and cardiac gene expression. Given that cardiac differentiation efficiency and yield vary significantly between ES cell lines, it seems reasonable to conclude that cardiogenesis from mouse iPSCs is in the same ballpark as mouse ES cells. It would be interesting to systematically evaluate various iPSC cell lines to assess their variability in differentiation.

Both groups found that iPSC–derived cardiomyocytes are structurally and functionally similar to ES cell–derived cardiomyocytes. iPSC cell–derived cardiomyocytes exhibit regular sarcomeric organization and express myocyte markers, including troponin T, connexin43, sarcomeric α-actinin, and titin. Furthermore, both groups showed that these cells beat spontaneously at frequencies similar to cardiomyocytes derived from ES cells. Mauritz et al performed additional characterization of the functional properties of iPSC-derived cardiomyocytes. They found synchronous calcium transients among groups of cells, indicating electric connection through gap junctions, and calcium release after caffeine treatment suggested similar stores of calcium in the sarcoplasmic reticulum for both cell types. Furthermore, beating rates in iPSC cell-cardiomyocytes increased with isoproterenol and decreased with carbachol, indicating functional β-adrenergic and muscarinic signaling pathways.

Narazaki and colleagues also demonstrated iPSC cell differentiation into several other mesodermal derivatives. They showed that Flk1+ cells from iPSCs can be induced along vascular lineages with VEGF, expressing either endothelial markers CD31 and VE-cadherin or smooth muscle markers α-actin, SM22α, and calponin. These cells formed tubular structures in 3-dimensional gels of type I collagen. Although the endothelium induced by VEGF exhibited a venous phenotype, they further showed that differentiation with VEGF and a cAMP analog could induce expression of arterial markers such as CXCR4 and ephrinB2. This group also demonstrated that iPSC–derived Flk1+ progenitors differentiate in coculture with the OP9 stromal line into cells expressing lymphatic markers LYVE-1 and prox1 or the hematopoietic marker CD45. These results suggest that iPSCs are comparable to ES cells in their vascular, lymphatic, and hematopoietic potential.

These studies are complemented by a study from Schenke-Layland et al., whose article was published electronically after these 2 manuscripts were accepted. Schenke-Layland et al compared mouse ES and iPSCs for their ability to differentiate into cardiovascular cells. Using EB differentiation or isolated Flk1+ cells grown on collagen IV or with OP9 cells, they found that induction of cardiomyocytes, smooth muscle cells, and endothelial cells occurred at comparable efficiency in ES and iPSCs.

Limitations and Improvements

The reprogramming of adult cells to ES cell–like pluripotent states provides far-reaching possibilities. However, multiple issues have to be addressed before this technology can be used in patients. Perhaps most pressing is the need to develop techniques to generate iPSCs without integrating viruses, thereby lowering the risk of malignant transformation. Furthermore, silencing of virally encoded genes in iPSCs is not always complete. Myc-expressing tumors develop in 15% to 20% of iPSC–derived chimeric mice and their offspring. Along these lines, Narasaki and colleagues found incomplete silencing of Klf4 and c-myc as late as 2 months after differentiation. Recent progress indicates that c-myc is not essential for iPSC cell generation and that iPSCs can be selected on the basis of morphology rather than the use of virally encoded antibiotic resistance genes. This eliminates 2 of the 5 transgenes from the original system, and the absence of c-myc markedly reduced tumor incidence in iPSC cell–derived mice. Work is underway in multiple laboratories to identify alternative reprogramming techniques such as cell–permeant protein reagents, nonintegrating viruses, and
small molecules. In the meantime, it is essential that investigators using iPS cells screen for expression of the virally encoded reprogramming factors.

It is important to assess how fully pluripotent cells differentiate because residual undifferentiated pluripotent cells could form teratomas. In this regard, Mauritz and colleagues showed incomplete downregulation of endogenous pluripotency markers Oct4 and Nanog in iPS cell EBs. The sustained expression of these transcripts might be intrinsic to the cell lines used in this study. Alternatively, the heterogeneous nature of EB differentiation might result in slower differentiation (although silencing of Oct4 is commonly seen in EB differentiation systems). In contrast, the guided approach used by Narazaki et al resulted in a progenitor population in which neither Nanog nor Oct4 was significantly expressed after 4 days of differentiation. Thus, iPS cells intended for therapeutic purposes should be screened for their ability to appropriately downregulate endogenous pluripotency factors. We think that directing differentiation toward the desired cell type maximizes yield and reduces the chances of tumor formation. In this regard, use of undefined components such as serum and cell cocultures to guide differentiation can be a roadblock to therapy. Optimized methods with fully defined components are needed for eventual use in patients.

Future Applications: Cell Therapy, Disease Models, and Drug Screens

The feasibility of ES cell–derived therapies has already been demonstrated in animal models. Engraftment of human ES cell–derived cardiomyocytes in the infarcted hearts of immunocompromised rats or mice attenuates ventricular dilation and improves contractile function. Additionally, human ES cells have been differentiated into pancreatic islet cells, oligodendrocyte precursors, and dopamine neurons and have been shown to benefit function in models of diabetes, spinal cord injury, and Parkinson disease, respectively.

The potential for iPS cell–derived therapies was recently demonstrated in murine sickle cell anemia. iPS cells were generated from humanized sickle cell mouse fibroblasts with the original 4-factor reprogramming cocktail. Next, c-myc was removed by a cre-lox system to prevent its reactivation, and the sickle β-hemoglobin allele was repaired by homologous recombination. Finally, the modified iPS cells were differentiated into hematopoietic cells and transplanted into irradiated sickle cell mice to correct the disease. After the limitations mentioned earlier have been addressed, it is fairly straightforward to see how this process could be translated into a human therapy (Figure 2A). Although many human applications would not require the step of repairing a genetic defect, with our current understanding, the process still would be complicated, time consuming, and expensive. (Note that this is true to a lesser extent for most autologous cell therapies requiring in vitro expansion.) Thus, therapies with patient-specific iPS cells may not be widely available for quite some time and may never be available in an acute setting. By analogy to bone marrow banking, it may be more feasible to bank iPS lines that match the population’s HLA diversity, offering cell therapies for off-the-shelf use (Figure 2B).

Beyond cell replacement therapy, iPS cells may prove useful for understanding disease pathogenesis and treatment. For example, it should be possible to obtain iPS cells from patients with familial cardiomyopathy and to generate cardiomyocytes that perfectly match the patient’s genetics. The diseased cardiomyocytes could be studied to better understand the pathophysiology, thereby improving genotype-phenotype correlations. In addition, the ability to grow large numbers of patient-specific human cardiomyocytes may facilitate high-throughput screening of small molecules for drug development. Similar strategies could be performed for other genetically based diseases, eg, neurodegenerative diseases, storage diseases, and metabolic disorders.
Closing Comments

Despite some variability from line to line, these 2 studies demonstrate that murine iPS cells are able to differentiate into cardiomyocytes and other vascular cells as easily as murine ES cells. Furthermore, ES and iPS cell–derived cardiomyocytes have similar functional properties. Although there are limitations in current reprogramming techniques, iPS cells provide exciting possibilities for clinically applicable cellular therapies, disease research, and drug discovery.

Acknowledgments

Human and rodent depiction in Figures 1 and 2 were adapted from Servier Medical Art collection (www.servier.com).

Disclosures

None.

References


Key Words: Editorials cardiovascular diseases myocytes reprogramming stem cells
Get With the (Re)Program: Cardiovascular Potential of Skin-Derived Induced Pluripotent Stem Cells
Nathaniel L. Tulloch, Lil Pabon and Charles E. Murry

Circulation. 2008;118:472-475
doi: 10.1161/CIRCULATIONAHA.108.791442
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/118/5/472