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On the Road to iPS Cell Cardiovascular Applications

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The ability to generate induced pluripotent stem (iPS) cells from somatic cells by the overexpression of a limited number of stem cell related genes has generated great excitement and interest in the biomedical research community including cardiovascular researchers. The pioneering study by Yamanaka and colleagues showing that overexpression of *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc* could reprogram mouse fibroblasts to a pluripotent state similar to that of embryonic stem (ES) cells opened major new avenues of research.[1] This epigenetic reprogramming was rapidly extrapolated to the human system using either the same combination of reprogramming factors or a slightly different combination of transgenes (*OCT4*, *NANOG*, *SOX2*, *LIN28*).[2-4] Like embryonic stem (ES) cells, iPS cells can be used for basic developmental biology research and also as a cell source to generate theoretically unlimited quantities of desired cell types such as cardiomyocytes. Such differentiated cells types can be used in a wide range of basic research studies and potentially in clinical applications, which not only include cellular therapies but also drug discovery and safety testing. One appealing aspect of human iPS cells compared to human ES cells is that they can be more readily generated without specialized expertise and access to human embryos, which also avoids the ethical challenges associated with human embryo research. Potentially the most powerful advantage of iPS cells over ES cells is that they can be generated from any patient to produce genetically identical pluripotent cells that can create human disease models or generate patient-specific cells for therapy. Already a number of iPS cell human disease models have been generated,[5, 6] and proof-of-principle iPS cellular therapies have been pioneered in mouse models.[7-9]

Despite the speed at which the iPS cell field is racing forward, we are just at the beginning of a long road. Many major questions remain regarding iPS cells. Will they prove to be equivalent to ES cells in their properties? In other words, will iPS cells be equally as pluripotent as ES cells and readily generate all cell lineages represented in the three embryonic germ layers? Will different iPS cell lines show distinct differentiation profiles which may be an advantage or disadvantage for a given application? For example, will certain iPS cell lines be less able to differentiate into cardiovascular relevant cell types compared to other lines? Another critical question for therapeutic applications is whether reprogrammed iPS cell lines are prone to tumorigenesis. Does the starting cell source matter? How does the technique of reprogramming impact iPS cell behavior? In this issue of Circulation Research, Martinez-Fernandez and colleagues address some of these questions by carefully examining the cardiogenic potential

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of mouse iPS cells generated from murine fibroblasts using three reprogramming factors (*OCT4*, *KLF4*, and *SOX2*).[10]

Most of the detailed understanding of the developmental potential of iPS cells comes from studies using mouse iPS cell lines generated with integrating retroviral vectors encoding four reprogramming factors (*Oct3/4*, *Sox2*, *Klf4*, and *c-Myc*), because this technology has been available the longest, for three full years. Some of these four factor iPS cells have been demonstrated to be competent to integrate into blastocyst stage embryos and generate chimeric mice which can exhibit germline transmission.[11-13] This is strong evidence of pluripotency, but initial efforts at the most rigorous developmental test of pluripotency, what is called tetraploid complementation, failed.[12] In tetraploid complementation, an embryo is made tetraploid by fusing cells at the two cell stage. The resulting tetraploid blastocyst can develop the cells that become placenta but cannot develop the embryo proper. Stem cells transplanted at the early blastocyst stage of the tetraploid embryo will generate a viable mouse strictly from the donor cells, if the cells are truly pluripotent. Very recently, two studies have demonstrated using tetraploid complementation that four factor iPS cell lines can support development of full term viable mice.[14, 15] Thus, some four factor iPS cell lines seem to be fully capable of developing all cell types present in the adult mouse, but the hint of caution is that in the limited data available, not all tested iPS cell lines proved successful in tetraploid complementation.[15] This could be because of experimental limitations and inadequate testing or fundamental differences in the iPS cell lines. Likewise, in vitro studies of cardiac differentiation of four factor mouse iPS cell lines have produced mixed results, with some lines showing comparable cardiogenesis to ES cells and others showing delayed cardiogenesis.[16, 17] Thus with the oldest of iPS cell technologies, some iPS cell lines seem to meet even the most stringent criteria for pluripotency, but it is unlikely that all 4 factor iPS cell lines will meet this standard.

It was quickly recognized that chimeric animals generated from 4 factor iPS cells were at increased risk for tumorigenesis due to reactivation of the *c-Myc* transgene,[11] which clearly represents a safety concern for translation of this technology to humans. However, *c-Myc* was soon found not to be essential for generation of iPS cells which could be generated using three virally encoded factors *Oct3/4*, *Sox2*, *and Klf4* (3F iPS cells).[18, 19] These 3F iPS cells expressed the standard markers of pluripotency in culture and could generate chimeric offspring following blastocyst injection, providing the first developmental assay supporting pluripotency and, importantly, they did not display any evident increase in risk for tumorigenesis.[18] However, at this stage, it remains to be determined whether these 3F iPS cells are fully pluripotent like ES cells and apparently like some four factor iPS cell lines.

To address this question with a focus on cardiac differentiation, in this issue of Circulation Research.[10] Martinez-Fernandez and colleagues present an elegant study of 3F mouse iPS cells focused on their ability to undergo cardiogenesis. These authors began their studies by transducing mouse embryonic fibroblasts using lentiviral delivered human transgenes for *SOX2*, *OCT4*, and *KLF4* to produce 3F iPS cells. The first test of the cardiogenic potential of the 3F iPS cells was performed by subjecting them to in vitro differentiation in embryoid bodies (EBs). The anticipated temporal change in expression of genes indicative of cardiac differentiation was observed, with an upregulation of cardiac transcription factors followed by myofilament proteins and presence of contracting cardiomyocytes in the EBs. The cardiomyocytes exhibited organized myofibrils and were electrically coupled based on the presence of gap junction and synchronized multicellular contraction patterns. Furthermore, they demonstrated spontaneous cardiac-like action potentials and Ca^{2+} transients. Initial voltage clamp experiments showed that the iPS cell-derived cardiomyocytes exhibited some of the anticipated ionic currents expected in cardiomyocytes.

Although this in vitro characterization convincingly demonstrates the ability of the 3F iPS cells to generate functional cardiomyocytes, it is only a beginning study of these cardiomyocytes. How do these iPS cell cardiomyocytes compare to ES cell cardiomyocytes under the authors' same experimental conditions? For example, is there evidence (from the action potentials or other markers) for different types of cardiomyocytes developing, e.g. atrial, ventricular and nodal-like? Likewise, the authors see primarily Ca^{2+} inward currents without evidence for Na^{+} current in these cardiomyocytes despite using a ramp protocol that should allow measurement of Na^{+} current. Mouse ES cell-derived cardiomyocytes in contrast express voltage-dependent Na^{+} channels and inward Na^{+} currents at comparable stages of maturation.[20] In addition, the authors demonstrate Ca^{2+} transients, but do these transient exhibit properties typical of cardiac muscle such as robust intracellular Ca^{2+} stores with Ca^{2+}-induced Ca^{2+} release?

Despite these unresolved questions, the authors provide evidence that these 3F iPS cells can robustly undergo cardiogenesis based on their embryonic developmental studies. The authors take a slightly different route by aggregating the 3F iPS cells to morula stage embryos using a technique called non-coerced diploid aggregation. If the iPS cells are fully pluripotent, they would be anticipated to contribute to the generation of a chimeric blastocyst which develops into a chimeric mouse. Since the iPS cells include a lacZ transgene, they can be readily tracked, and the study demonstrates a mosaic distribution of lacZ positive iPS cell progeny in the developing mouse embryos. The iPS cells were found to be clearly present in the heart at all stages of development studied, and live-born chimeric mice exhibited normal cardiac function. Although these observations do not quite reach the pluripotency-defining bar of tetraploid complementation, at least for the focused analysis on cardiac development, it seems likely that the 3F iPS cells generated by the Martinez-Fernandez can undergo normal cardiac development. Furthermore, the authors did not observe tumors in the chimeric offspring from the 3F iPS cells, but the number of mice studied was relatively small. Overall, the study of Martinez-Fernandez provides the most detailed analysis of cardiogenesis of 3F iPS cells to date, and the news is good thus far in that these cells are readily able differentiate to form cardiac muscle.

However, we have not reached our desired destination with current 3F iPS cells because reprogramming without c-Myc reduces but does not eliminate the risk of tumorigenesis. A recent safety study of iPS cell lines examining their potential to generate teratomas from differentiated progeny revealed that secondary neurospheres differentiated from iPS cells could form teratomas when transplanted into mouse brains regardless of the presence or absence of *c-Myc* transgene.[21] Another clear risk for oncogenic transformation of iPS cells is related to the uncontrolled integration of the transgenes into the genomes of the reprogrammed cells. Unfortunately, the potential for insertional mutagenesis to activate proto-oncogenes has been demonstrated to be a real risk in the case of some gene therapy protocols. Hence integrating lentiviral 3F iPS cells are only one more stop on the road of perfecting iPS cell technology.

The approaches to generate iPS cells are changing at a remarkable pace. Small molecules have been used to increase the efficiency of generating iPS cell lines and have enabled the use of only two transgenes to generate iPS cell lines.[22, 23] Mouse iPS cells have now been generated using adenovirus or plasmid-mediated transfections, which avoid the potential problems associated with viral integration of transgenes.[24, 25] Generation of mouse iPS cells using only recombinant proteins without transgenes has also been described.[26] Furthermore, techniques using nonintegrating transgenes have succeeded in generating iPS cells from human cells.[27] This exponential growth in technologies to produce iPS cells has, however, outpaced the ability to thoroughly investigate the properties of the resulting iPS cells. Careful studies like those of Martinez-Fernandez are needed for next generation iPS cells.

The groundbreaking studies done with mouse iPS cells have greatly advanced the field, but ultimately, for clinical applications, human iPS cells need the closest scrutiny. But how can we demonstrate that human iPS cells are pluripotent without access to embryological studies? In vitro studies will certainly play an important role in characterizing lineage potential development.[28] What will be the best way to assess tumor risk in the human iPS cells? What human iPS cell technologies will provide cells optimal for repair of the injured myocardium? We are on the road to progress, but this remains a largely unmapped route that requires us to proceed expeditiously but cautiously.

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References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663–676. [PubMed: 16904174]
2. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131(5):861–872. [PubMed: 18035408]
3. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318(5858):1917–1920. [PubMed: 18029452]
4. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008;451(7175): 141–146. [PubMed: 18157115]
5. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ. Disease-specific induced pluripotent stem cells. Cell 2008;134(5):877–886. [PubMed: 18691744]
6. Ebert AD, Yu J, Rose FF Jr, Mattis VB, Lorson CL, Thomson JA, Svendsen CN. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 2009;457(7227):277–280. [PubMed: 19098894]
7. Nelson TJ, Martinez-Fernandez A, Yamada S, Perez-Terzic C, Ikeda Y, Terzic A. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. Circulation 2009;120 (5):408–416. [PubMed: 19620500]
8. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 2007;318(5858):1920–1923. [PubMed: 18063756]
9. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, Broccoli V, Constantine-Paton M, Isacson O, Jaenisch R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A 2008;105 (15):5856–5861. [PubMed: 18391196]
10. Martinez-Fernandez A, Nelson TJ, Yamada S, Reyes S, Alekseev AE, Perez-Terzic C, Ikeda Y, Terzic A. iPS Programmed Without c-MYC Yield Proficient Cardiogenesis for Functional Heart Chimerism. Circ Res. 2009
11. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448(7151):313–317. [PubMed: 17554338]
12. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 2007;448(7151): 318–324. [PubMed: 17554336]
13. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic

remodeling and widespread tissue contribution. Cell Stem Cell 2007;1(1):55–70. [PubMed: 18371336]

14. Kang L, Wang J, Zhang Y, Kou Z, Gao S. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell 2009;5(2):135–138. [PubMed: 19631602]
15. Zhao XY, Li W, Lv Z, Liu L, Tong M, Hai T, Hao J, Guo CL, Ma QW, Wang L, Zeng F, Zhou Q. iPS cells produce viable mice through tetraploid complementation. Nature. 2009
16. Mauritz C, Schwanke K, Reppel M, Neef S, Katsirntaki K, Maier LS, Nguemo F, Menke S, Haustein M, Hescheler J, Hasenfuss G, Martin U. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. Circulation 2008;118(5):507–517. [PubMed: 18625890]
17. Narazaki G, Uosaki H, Teranishi M, Okita K, Kim B, Matsuoka S, Yamanaka S, Yamashita JK. Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. Circulation 2008;118(5):498–506. [PubMed: 18625891]
18. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 2008;26(1):101–106. [PubMed: 18059259]
19. Wernig M, Meissner A, Cassady JP, Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. Cell Stem Cell 2008;2(1):10–12. [PubMed: 18371415]
20. Maltsev VA, Wobus AM, Rohwedel J, Bader M, Hescheler J. Cardiomyocytes differentiated in vitro from embryonic stem cells developmentally express cardiac-specific genes and ionic currents. Circ Res 1994;75:233–244. [PubMed: 8033337]
21. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 2009;27(8):743–745. [PubMed: 19590502]
22. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol 2008;26(11):1269–1275. [PubMed: 18849973]
23. Shi Y, Desponts C, Do JT, Hahm HS, Scholer HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 2008;3(5):568–574. [PubMed: 18983970]
24. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. Science 2008;322(5903):945–949. [PubMed: 18818365]
25. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322(5903):949–953. [PubMed: 18845712]
26. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Scholer HR, Duan L, Ding S. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009;4(5):381–384. [PubMed: 19398399]
27. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324(5928):797–801. [PubMed: 19325077]
28. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 2009;104(4):e30–41. [PubMed: 19213953]