10. THE PROMISE OF INDUCED PLURIPOTENT STEM CELLS (iPSCs)

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In 2006, researchers at Kyoto University in Japan identified conditions that would allow specialized adult cells to be genetically "reprogrammed" to assume a stem cell-like state. These adult cells, called induced pluripotent stem cells (iPSCs), were reprogrammed to an embryonic stem cell-like state by introducing genes important for maintaining the essential properties of embryonic stem cells (ESCs). Since this initial discovery, researchers have rapidly improved the techniques to generate iPSCs, creating a powerful new way to "de-differentiate" cells whose developmental fates had been previously assumed to be determined.

Although much additional research is needed, investigators are beginning to focus on the potential utility of iPSCs as a tool for drug development, modeling of disease, and transplantation medicine. The idea that a patient's tissues could provide him/her a copious, immune-matched supply of pluripotent cells has captured the imagination of researchers and clinicians worldwide. Furthermore, ethical issues associated with the production of ESCs do not apply to iPSCs, which offer a non-controversial strategy to generate patient-specific stem cell lines. As an introduction to this exciting new field of stem cell research, this chapter will review the characteristics of iPSCs, the technical challenges that must be overcome before this strategy can be deployed, and the cells' potential applications to regenerative medicine.

"REPROGRAMMING" CELLS: ACHIEVING PLURIPOTENCY

As noted in other chapters, stem cells represent a precious commodity. Although present in embryonic and adult tissues, practical considerations such as obtaining embryonic tissues and isolating relatively rare cell types have limited the large-scale production of populations of pure stem cells (see the Chapter, "Alternate Methods for Preparing Pluripotent Stem Cells" for details). As such, the logistical challenges of isolating, culturing, purifying, and differentiating stem cell lines that are extracted from tissues have led researchers to explore options for "creating" pluripotent cells using existing non-pluripotent cells. Coaxing abundant, readily available differentiated cells to pluripotency would in principle eliminate the search for rare cells while providing the opportunity to culture clinically useful quantities of stem-like cells.

One strategy to accomplish this goal is nuclear reprogramming, a technique that involves experimentally inducing a stable change in the nucleus of a mature cell that can then be maintained and replicated as the cell divides through mitosis. These changes are most frequently associated with the reacquisition of a pluripotent state, thereby endowing the cell with developmental potential. The strategy has historically been carried out using techniques such as somatic cell nuclear transfer (SCNT),[1,2] altered nuclear transfer (ANT),[3,4] and methods to fuse somatic cells with ESCs [5,6] (see "Alternate Methods for Preparing Pluripotent Stem Cells" for details of these approaches). From a clinical perspective, these methods feature several drawbacks, such as the creation of an embryo or the development of hybrid cells that are not viable to treat disease. However, in 2006, these efforts informed the development of nuclear reprogramming *in vitro,* the breakthrough method that creates iPSCs.

This approach involves taking mature "somatic" cells from an adult and introducing the genes that encode critical transcription factor proteins, which themselves regulate the function of other genes important for early steps in embryonic development (See Fig. 10.1). In the initial 2006 study, it was reported that only four transcription factors *(Oct4, Sox2, Klf4,* and *c-Myc)* were required to reprogram mouse fibroblasts (cells found in the skin and other connective tissue) to an embryonic stem cell–like state by forcing them to express genes important for maintaining the defining

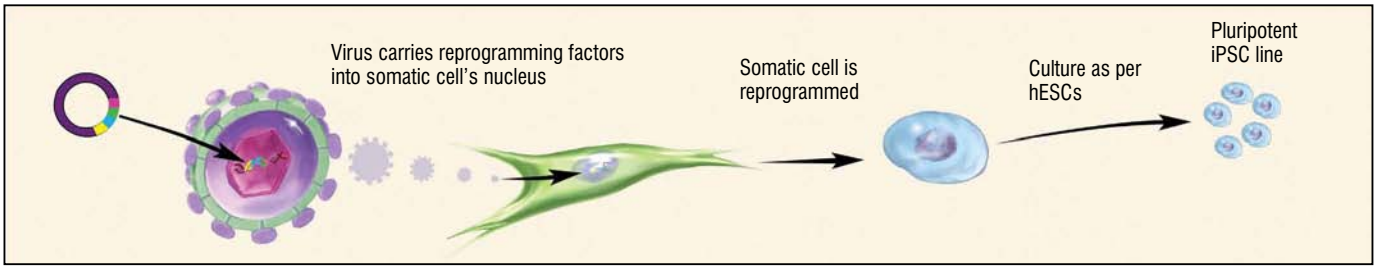

Figure 10.1. Generating Induced Pluripotent Stem Cells (iPSCs)

properties of ESCs.[7] These factors were chosen because they were known to be involved in the maintenance of pluripotency, which is the capability to generate all other cell types of the body. The newly-created iPSCs were found to be highly similar to ESCs and could be established after several weeks in culture.[7,8] In 2007, two different research groups reached a new milestone by deriving iPSCs from human cells, using either the original four genes[9] or a different combination containing *Oct4, Sox2, Nanog,* and *Lin28*.[10] Since then, researchers have reported generating iPSCs from somatic tissues of the monkey[11] and rat.[12,13]

However, these original methods of reprogramming are inefficient, yielding iPSCs in less than 1% of the starting adult cells.[14,15] The type of adult cell used also affects efficiency; fibroblasts require more time for factor expression and have lower efficiency of reprogramming than do human keratinocytes, mouse liver and stomach cells, or mouse neural stem cells.[14–19]

Several approaches have been investigated to improve reprogramming efficiency and decrease potentially detrimental side effects of the reprogramming process. Since the retroviruses used to deliver the four transcription factors in the earliest studies can potentially cause mutagenesis (see below), researchers have investigated whether all four factors are absolutely necessary. In particular, the gene *c-Myc* is known to promote tumor growth in some cases, which would negatively affect iPSC usefulness in transplantation therapies. To this end, researchers tested a three-factor approach that uses the orphan nuclear receptor *Esrrb* with *Oct4* and *Sox2,* and were able to convert mouse embryonic fibroblasts to iPSCs.[20] This achievement corroborates other reports that *c-Myc* is dispensable for direct reprogramming of mouse fibroblasts.[21] Subsequent studies have further reduced the number of genes required for reprogramming,[22–26] and researchers continue to identify chemicals that can either substitute for or enhance the efficiency of transcription factors in this process.[27] These breakthroughs continue to inform and to simplify the reprogramming process, thereby advancing the field toward the generation of patient-specific stem cells for clinical application. However, as the next section will discuss, the method by which transcription factors are delivered to the somatic cells is critical to their potential use in the clinic.

CURRENT CHALLENGES IN iPSC RESEARCH

Reprogramming poses several challenges for researchers who hope to apply it to regenerative medicine. To deliver the desired transcription factors, the DNA that encodes their production must be introduced and integrated into the genome of the somatic cells. Early efforts to generate iPSCs accomplished this goal using retroviral vectors. A retrovirus is an RNA virus that uses an enzyme, reverse transcriptase, to replicate in a host cell and subsequently produce DNA from its RNA genome. This DNA incorporates into the host's genome, allowing the virus to replicate as part of the host cell's DNA. However, the forced expression of these genes cannot be controlled fully, leading to unpredictable effects.[28] While other types of integrating viruses, such as lentiviruses, can increase the efficiency of reprogramming,[16] the expression of viral transgenes remains a critical clinical issue. Given the dual needs of reducing the drawbacks of viral integration and maximizing reprogramming efficiency, researchers are exploring a number of strategies to reprogram cells in the absence of integrating viral vectors[27–30] or to use potentially more efficient integrative approaches.[31,32]

Before reprogramming can be considered for use as a clinical tool, the efficiency of the process must improve substantially. Although researchers have

begun to identify the myriad molecular pathways that are implicated in reprogramming somatic cells,[15] much more basic research will be required to identify the full spectrum of events that enable this process. Simply adding transcription factors to a population of differentiated cells does not guarantee reprogramming – the low efficiency of reprogramming *in vitro* suggests that additional rare events are necessary to generate iPSCs, and the efficiency of reprogramming decreases even further with fibroblasts that have been cultured for long time periods.[33] Furthermore, the differentiation stage of the starting cell appears to impact directly the reprogramming efficiency; mouse hematopoietic stem and progenitor cells give rise to iPSCs up to 300 times more efficiently than do their terminally-differentiated B- and T-cell counterparts.[34] As this field continues to develop, researchers are exploring the reprogramming of stem or adult progenitor cells from mice[24,25,34,35] and humans[23,26] as one strategy to increase efficiency compared to that observed with mature cells.

As these discussions suggest, clinical application of iPSCs will require safe and highly efficient generation of stem cells. As scientists increase their understanding of the molecular mechanisms that underlie reprogramming, they will be able to identify the cell types and conditions that most effectively enable the process and use this information to design tools for widespread use. Clinical application of these cells will require methods to reprogram cells while minimizing DNA alterations. To this end, researchers have found ways to introduce combinations of factors in a single viral "cassette" into a known genetic location.[36] Evolving tools such as these will enable researchers to induce programming more safely, thereby informing basic iPSC research and moving this technology closer to clinical application.

ARE iPSCs TRULY EQUIVALENT TO ESCs?

ESCs and iPSCs are created using different strategies and conditions, leading researchers to ask whether the cell types are truly equivalent. To assess this issue, investigators have begun extensive comparisons to determine pluripotency, gene expression, and function of differentiated cell derivatives. Ultimately, the two cell types exhibit some differences, yet they are remarkably similar in many key aspects that could impact their application to regenerative medicine. Future experiments will determine the clinical significance (if any) of the observed differences between the cell types.

Other than their derivation from adult tissues, iPSCs meet the defining criteria for ESCs. Mouse and human iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cell types from all three primitive embryonic layers, and displaying the capacity to contribute to many different tissues when injected into mouse embryos at a very early stage of development. Initially, it was unclear that iPSCs were truly pluripotent, as early iPSC lines contributed to mouse embryonic development but failed to produce live-born progeny as do ESCs. In late 2009, however, several research groups reported mouse iPSC lines that are capable of producing live births,[37,38] noting that the cells maintain a pluripotent potential that is "very close to" that of ESCs.[38] Therefore, iPSCs appear to be truly pluripotent, although they are less efficient than ESCs with respect to differentiating into all cell types.[38] In addition, the two cell types appear to have similar defense mechanisms to thwart the production of DNA-damaging reactive oxygen species, thereby conferring the cells with comparable capabilities to maintain genomic integrity.[39]

Undifferentiated iPSCs appear molecularly indistinguishable from ESCs. However, comparative genomic analyses reveal differences between the two cell types. For example, hundreds of genes are differentially expressed in ESCs and iPSCs,[40] and there appear to be subtle but detectable differences in epigenetic methylation between the two cell types.[41,42] Genomic differences are to be expected; it has been reported that gene-expression profiles of iPSCs and ESCs from the same species differ no more than observed variability among individual ESC lines.[43] It should be noted that the functional implications of these findings are presently unknown, and observed differences may ultimately prove functionally inconsequential.[44]

Recently, some of the researchers who first generated human iPSCs compared the ability of iPSCs and human ESCs to differentiate into neural cells (e.g., neurons and glia).[45] Their results demonstrated that both cell types follow the same steps and time course during differentiation. However, although human ESCs differentiate into neural cells with a similar efficiency regardless of the cell line used, iPSC-derived neural cells demonstrate lower efficiency and greater variability when differentiating into neural cells. These observations occurred regardless of which of several iPSC-generation protocols were used to reprogram

the original cell to the pluripotent state. Experimental evidence suggests that individual iPSC lines may be "epigenetically unique" and predisposed to generate cells of a particular lineage. However, the authors believe that improvements to the culturing techniques may be able to overcome the variability and inefficiency described in this report.

These findings underpin the importance of understanding the inherent variability among discrete cell populations, whether they are iPSCs or ESCs. Characterizing the variability among iPSC lines will be crucial to apply the cells clinically. Indeed, the factors that make each iPSC line unique may also delay the cells' widespread use, as differences among the cell lines will affect comparisons and potentially influence their clinical behavior. For example, successfully modeling disease requires being able to identify the cellular differences between patients and controls that lead to dysfunction. These differences must be framed in the context of the biologic variability inherent in a given patient population. If iPSC lines are to be used to model disease or screen candidate drugs, then variability among lines must be minimized and characterized fully so that researchers can understand how their observed results match to the biology of the disease being studied. As such, standardized assays and methods will become increasingly important for the clinical application of iPSCs, and controls must be developed that account for variability among the iPSCs and their derivatives.

Additionally, researchers must understand the factors that initiate reprogramming towards pluripotency in different cell types. A recent report has identified one factor that initiates reprogramming in human fibroblasts,[46] setting the groundwork for developing predictive models to identify those cells that will become iPSCs. An iPSC may carry a genetic "memory" of the cell type that it once was, and this "memory" will likely influence its ability to be reprogrammed. Understanding how this memory varies among different cell types and tissues will be necessary to reprogram successfully.

POTENTIAL MEDICAL APPLICATIONS OF IPSCs

iPSCs have the potential to become multipurpose research and clinical tools to understand and model diseases, develop and screen candidate drugs, and deliver cell-replacement therapy to support regenerative medicine. This section will explore the possibilities and the challenges that accompany these medical applications, with the caveat that some uses are more immediate than others. For example, researchers currently use stem cells to test/screen drugs or as study material to identify molecules or genes implicated in regeneration. Conducting experiments or testing candidate drugs on human cells grown in culture enables researchers to understand fundamental principles and relationships that will ultimately inform the use of stem cells as a source of tissue for transplantation. Therefore, using iPSCs in cell-replacement therapies is a future application of these cells, albeit one that has tremendous clinical potential. The following discussion will highlight recent efforts toward this goal while recognizing the challenges that must be overcome for these cells to reach the clinic.

Reprogramming technology offers the potential to treat many diseases, including Alzheimer's disease, Parkinson's disease, cardiovascular disease, diabetes, and amyotrophic lateral sclerosis (ALS; also known as Lou Gehrig's disease). In theory, easily-accessible cell types (such as skin fibroblasts) could be biopsied from a patient and reprogrammed, effectively recapitulating the patient's disease in a culture dish. Such cells could then serve as the basis for autologous cell replacement therapy. Because the source cells originate within the patient, immune rejection of the differentiated derivatives would be minimized. As a result, the need for immunosuppressive drugs to accompany the cell transplant would be lessened and perhaps eliminated altogether. In addition, the reprogrammed cells could be directed to produce the cell types that are compromised or destroyed by the disease in question. A recent experiment has demonstrated the proof of principle in this regard,[47] as iPSCs derived from a patient with ALS were directed to differentiate into motor neurons, which are the cells that are destroyed in the disease.

Although much additional basic research will be required before iPSCs can be applied in the clinic, these cells represent multi-purpose tools for medical research. Using the techniques described in this article, researchers are now generating myriad disease-specific iPSCs. For example, dermal fibroblasts and bone marrow-derived mesenchymal cells have been used to establish iPSCs from patients with a variety of diseases, including ALS, adenosine deaminase deficiency-related

severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease type III, Duchenne and Becker muscular dystrophies, Parkinson's disease, Huntington's disease, type 1 diabetes mellitus, Down syndrome/trisomy 21, and spinal muscular atrophy.[47–49] iPSCs created from patients diagnosed with a specific genetically-inherited disease can then be used to model disease pathology. For example, iPSCs created from skin fibroblasts taken from a child with spinal muscular atrophy were used to generate motor neurons that showed selective deficits compared to those derived from the child's unaffected mother.[48] As iPSCs illuminate the development of normal and disease-specific pathologic tissues, it is expected that discoveries made using these cells will inform future drug development or other therapeutic interventions.

One particularly appealing aspect of iPSCs is that, in theory, they can be directed to differentiate into a specified lineage that will support treatment or tissue regeneration. Thus, somatic cells from a patient with cardiovascular disease could be used to generate iPSCs that could then be directed to give rise to functional adult cardiac muscle cells (cardiomyocytes) that replace diseased heart tissue, and so forth. Yet while iPSCs have great potential as sources of adult mature cells, much remains to be learned about the processes by which these cells differentiate. For example, iPSCs created from human[50] and murine fibroblasts[51–53] can give rise to functional cardiomyocytes that display hallmark cardiac action potentials. However, the maturation process into cardiomyocytes is impaired when iPSCs are used – cardiac development of iPSCs is delayed compared to that seen with cardiomyocytes derived from ESCs or fetal tissue. Furthermore, variation exists in the expression of genetic markers in the iPSC-derived cardiac cells as compared to that seen in ESC-derived cardiomyocytes. Therefore, iPSC-derived cardiomyocytes demonstrate normal commitment but impaired maturation, and it is unclear whether observed defects are due to technical (e.g., incomplete reprogramming of iPSCs) or biological barriers (e.g., functional impairment due to genetic factors). Thus, before these cells can be used for therapy, it will be critical to distinguish between iPSC-specific and disease-specific phenotypes.

However, it must be noted that this emerging field is continually evolving; additional basic iPSC research will be required in parallel with the development of disease models. Although the reprogramming technology that creates iPSCs is currently imperfect, these cells will likely impact future therapy, and "imperfect" cells can illuminate many areas related to regenerative medicine. However, iPSC-derived cells that will be used for therapy will require extensive characterization relative to what is sufficient to support disease modeling studies. To this end, researchers have begun to use imaging techniques to observe cells that are undergoing reprogramming to distinguish true iPSCs from partially-reprogrammed cells.[54] The potential for tumor formation must also be addressed fully before any iPSC derivatives can be considered for applied cell therapy. Furthermore, in proposed autologous therapy applications, somatic DNA mutations (e.g., non-inherited mutations that have accumulated during the person's lifetime) retained in the iPSCs and their derivatives could potentially impact downstream cellular function or promote tumor formation (an issue that may possibly be circumvented by creating iPSCs from a "youthful" cell source such as umbilical cord blood).[55] Whether these issues will prove consequential when weighed against the cells' therapeutic potential remains to be determined. While the promise of iPSCs is great, the current levels of understanding of the cells' biology, variability, and utility must also increase greatly before iPSCs become standard tools for regenerative medicine.

CONCLUSION

Since their discovery four years ago, induced pluripotent stem cells have captured the imagination of researchers and clinicians seeking to develop patient-specific therapies. Reprogramming adult tissues to embryonic-like states has countless prospective applications to regenerative medicine, drug development, and basic research on stem cells and developmental processes. To this point, a PubMed search conducted in April 2010 using the term "induced pluripotent stem cells" (which was coined in 2006) returned more than 1400 publications, indicating a highly active and rapidly-developing research field.

However, many technical and basic science issues remain before the promise offered by iPSC technology can be realized fully. For putative regenerative medicine applications, patient safety is the foremost consideration. Standardized methods must be developed to characterize iPSCs and their derivatives. Furthermore, reprogramming has demonstrated a proof-of-principle, yet the process is currently too inefficient for routine clinical application. Thus,

unraveling the molecular mechanisms that govern reprogramming is a critical first step toward standardizing protocols. A grasp on the molecular underpinnings of the process will shed light on the differences between iPSCs and ESCs (and determine whether these differences are clinically significant). Moreover, as researchers delve more deeply into this field, the effects of donor cell populations can be compared to support a given application; i.e., do muscle-derived iPSCs produce more muscle than skin-derived cells? Based on the exciting developments in this area to date, induced pluripotent stem cells will likely support future therapeutic interventions, either directly or as research tools to establish novel models for degenerative disease that will inform drug development. While much remains to be learned in the field of iPSC research, the development of reprogramming techniques represents a breakthrough that will ultimately open many new avenues of research and therapy.

REFERENCES

1. French AJ, Adams CA, Anderson LS, Kitchen JR, Hughes MR, Wood SH. Development of human cloned blastocysts following somatic cell nuclear transfer (SCNT) with adult fibroblasts. *Stem Cells.* 2008;26:485-483.
2. Byrne JA, Pedersen DA, Clepper LL, et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. *Nature.* 2007;450:497-502.
3. Hurlbut WB. Ethics and embryonic stem cell research: altered nuclear transfer as a way forward. *BioDrugs.* 2007;21:79-83.
4. Meissner A, Jaenisch R. Generation of nuclear transfer-derived pluripotent ES cells from cloned Cdx2-deficient blastocysts. *Nature.* 2006;439:212-215.
5. Cantz T, Bleidissel M, Stehling M, Schöler HR. In vitro differentiation of reprogrammed murine somatic cells into hepatic precursor cells. *Biol Chem.* 2008;389:889-896.
6. Cowan CA, Atienza J, Melton DA, Eggan K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science.* 2005;309:1369-1373.
7. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126:663-676.
8. Maherali N, Sridharan R, Xie W, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell.* 2007;1:55-70.
9. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;131:1-12.
10. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007;318:1917-1920.
11. Liu H, Zhu F, Yong J, et al. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell.* 2008;3:587-590.
12. Liao J, Cui C, Chen S, et al. Generation of induced pluripotent stem cell lines from adult rat cells. *Cell Stem Cell.* 2009;4:11-15.
13. Li W, Wei W, Zhu S, et al. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. *Cell Stem Cell.* 2009;4:16-19.
14. Brambrink T, Foreman R, Welstead GG, et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell.* 2008;2:151-159.
15. Stadtfeld M, Maherali N, Breault DT, Hochedlinger K. Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell.* 2008;2:230-240.
16. Maherali N, Ahfeldt T, Rigamonti A, Utikal J, Cowan C, Hochedlinger K. A high-efficiency system for the generation and study of human induced pluripotent stem cells. *Cell Stem Cell.* 2008;3:340-345.
17. Aasen T, Raya A, Barrero MJ, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol.* 2008;26:1276-1284.
18. Schöler HR. Commentary: Highlight on stem cell research. *Biol Chem.* 2008;389:789.
19. Aoi T, Yae K, Nakagawa M, et al. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science.* 2008;321:699-702.
20. Feng B, Jiang J, Kraus P, et al. Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb. *Nat Cell Biol.* 2009;11:197-203.
21. Wernig M, Meissner A, Cassady JP, Jaenisch R. *c-Myc* is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell.* 2008;2:10-12.
22. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only *Oct4* and *Sox2. Nat Biotechnol.* 2008;26:1269-1275.
23. Hester ME, Song S, Miranda CJ, Eagle A, Schwartz PH, Kaspar BK. Two factor reprogramming of human neural stem cells into pluripotency. *PLoS One.* 2009;4:e7044.
24. Kim JB, Zaehres H, Wu G, et al. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature.* 2008;454:646-650.
25. Kim JB, Sebastiano V, Wu G, et al. Oct4-induced pluripotency in adult neural stem cells. *Cell.* 2009;136:411-419.
26. Kim JB, Greber B, Arauzo Bravo MJ, et al. Direct reprogramming of human neural stem cells by *Oct4. Nature.* 2009;461:649-653.

27. Feng B, Ng JH, Heng JC, Ng HH. Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. *Cell Stem Cell.* 2009;4:301-312.

28. Stadtfeld M, Hochedlinger K. Without a trace? PiggyBac-ing to pluripotency. *Nat Methods.* 2009;6:329-330.

29. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science.* 2008;322:945-949.

30. Page RL, Ambady S, Holmes WF, et al. Induction of stem cell gene expression in adult human fibroblasts without transgenes. *Cloning Stem Cells.* 2009;11:1-10.

31. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science.* 2008;322:949-953.

32. Yusa K, Rad R, Takeda J, Bradley A. Generation of transgene-free induced pluripotent mouse stem cells by the *piggyBac* transposon. *Nat Methods.* 2009;6:363-369.

33. Utikal J, Polo JM, Stadtfeld M, et al. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature.* 2009;460:1145-1148.

34. Eminli S, Foudi A, Stadtfeld M, et al. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet.* 2009;41:968-976.

35. Ko K, Tapia N, Wu G, et al. Induction of pluripotency in adult unipotent germline stem cells. *Cell Stem Cell.* 2009;5:87-96.

36. Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells.* 2009;27:543-549.

37. 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;5:135-138.

38. Zhao XY, Li W, Lv Z, et al. iPS cells produce viable mice through tetraploid complementation. *Nature.* 2009;461:86-90.

39. Armstrong L, Tilgner K, Saretzki G, et al. Human induced pluripotent stem cell lines show similar stress defence mechanisms and mitochondrial regulation to human embryonic stem cells. *Stem Cells.* 2010;Jan 13 [Epub ahead of print].

40. Chin MH, Mason MJ, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell.* 2009;5:111-123.

41. Deng J, Shoemaker R, Xie B, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. *Nat Biotechnol.* 2009;27:353-360.

42. Doi A, Park I-H, Wen B, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet.* 2009;41:1350-1353.

43. Mikkelsen TS, Hanna J, Zhang X, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature.* 2008;454:794.

44. Colman A, Dressen O. Induced pluripotent stem cells and the stability of the differentiated state. E*MBO Reports.* 2009;10:714-721.

45. Hu BY, Weick JP, Yu J, et al. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci USA.* 2010;107:4335-4340.

46. Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature.* 2010;463:1042-1048.

47. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science.* 2008;321:1218-1221.

48. Ebert AD, Yu J, Rose FF Jr, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature.* 2009;457:277-280.

49. Park I-H, Arora N, Huo H, et al. Disease-specific induced pluripotent stem (iPS) cells. *Cell.* 2008;134:877-886.

50. Zhang J, Wilson GF, Soerens AG, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res.* 2009;104:e30-e41.

51. Mauritz C, Schwanke K, Reppel M, et al. Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation.* 2008;118:507-517.

52. Kuzmenkin A, Liang H, Xu G, et al. Functional characterization of cardiomyocytes derived from murine induced pluripotent stem cells in vitro. *FASEB J.* 2009;23:4168-4180.

53. Pfannkuche K, Liang H, Hannes T, et al. Cardiac myocytes derived from murine reprogrammed fibroblasts: intact hormonal regulation, cardiac ion channel expression and development of contractility. Cell Physiol Biochem. 2009;24:73-86.

54. Chan EM, Ratanasirintrawoot S, Park I-H, et al. Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells. Nat Biotechnol. 2009;27:1033-1037.

55. Haase A, Olmer R, Schwanke K, et al. Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell. 2009;5:434-441.