iPS cells for transplantation

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Abstract

Purpose of review

The induced pluripotent stem (iPS) cells from patient's somatic cells could be a useful source for drug discovery and cell transplantation therapies. However, there are still several problems to be solved in terms of safety concerns. We herein summarize the current knowledge about iPS cells and the obstacles that must be overcome before the cells can be used for medical applications.

Recent findings

Recent progress has enabled us to generate integration-free iPS cells from non-invasive tissues. However, several studies have also uncovered differences in iPS cells and ES cells in terms of gene expression, epigenetic modification, and differentiation potentials. Tissue origin affects the quality of iPS cells. However, in a rodent disease model, the transplantation of differentiated cells derived from iPS cells ameliorated their symptoms. Methods for gene correction and direct cell fate switching have also been reported. The successful generation of HLA-typed iPS cells has been described. These findings should therefore facilitate the further application of iPS cells.

Summary

Human iPS cells are able to provide functional neuronal cells, blood cells, and retinal cells, which would be a useful source for transplantation. Although recent reports have shown that it may be possible to overcome several difficulties associated with iPS cells, further improvements are needed, not only regarding safety aspects, but also in quality, before they can be medically applied.

Introduction

Reprogramming of somatic cells into pluripotent stem cells has been reported by introducing a combination of several transcription factors (Oct3/4, Sox2, Klf4, and c-Myc)(1). The induced pluripotent stem (iPS) cells from patient's somatic cells could provide a useful source of cells for drug discovery and cell transplantation therapies. However, most human iPS cells have been made using viral vectors, such as retroviruses and lentiviruses, which integrate the reprogramming factors into the host genomes and may increase the risk of tumor formation (2). To overcome the safety concerns about iPS cell generation, several non-integration methods have been reported, such as transient expression of the reprogramming factors using adenoviral vectors or plasmids, and direct delivery of reprogramming proteins and RNAs (3-6). These approaches could decrease the risk of genomic alteration, however, recent studies raised other obstacles in the way of future medical applications.

Human iPS cells are able to generate neuronal cells, blood cells, and retinal cells (7-9). However, there have been some reports that human iPS cells show attenuated differentiation potential into neuronal or hematopoietic lineages in comparison to ES cells (10, 11). These studies indicate the limited applications of human iPS cells and suggest the need for improvement of reprogramming, not only in

terms of safety, but also in quality. We herein summarize the current knowledge and problems associated with the development of iPS cells for medical applications.

Development of an efficient non-integrated method and derivation of iPS cells from non-invasive tissues

The first iPS cells were established by delivery of transgenes, Oct3/4, Sox2, Klf4, and c-Myc, by MMLV (Moloney Murine Leukemia Virus)-based retroviral vectors (1). Retroviruses can robustly infect fibroblasts and introduce their RNA genome into the host genome by reverse transcriptase. The resulting iPS cells had several genomic integrations of the transgenes. This enables effective reprogramming of somatic cells, however, the integrated sequences alter the genomic organization of iPS cells and can induce some abnormalities (12). One of the reprogramming factors, c-Myc, is a well-known proto-oncogene, and its reactivation could give rise to transgene derived-tumor formation (2). Because of these concerns, several improvements in the transduction methods have been studied for making non-integrated iPS cells. Generation of non-integrated iPS cells was first reported in mice using an adenoviral vector and by using a plasmid vector (3, 4), and then it was demonstrated that it was possible using human cells and viral vectors (adenovirus), DNA vectors (PiggyBac vector, episomal

plasmid, and minicircle vector), and direct protein delivery (13-18). Although these methods can avoid genomic alterations, most of them are highly inefficient and largely impractical. Recent progress was made by the employment of the non-transmissible Sendai virus vector for reprogramming (19). The Sendai virus is a minus-strand RNA virus and does not have the risk of integrating into the host genome. Fusaki et al. mixed 4 different Sendai viral vectors encoding different reprogramming factors, and transfected them into human fibroblasts. The viral vectors could constitutively replicate their RNA genome in the cytoplasm of the infected cells, to give a high reprogramming efficiency of up to 1%. The established iPS cells still contained viral RNA, however, the RNA were diluted and disappeared during cell growth. This elimination can be enhanced by using temperature-sensitive mutants and by antibody-mediated negative selection utilizing viral antigens expressed on the cell surface.

Direct delivery of synthetic mRNA encoding reprogramming factors also induced iPS cell generation at high efficiency (6). To suppress the activation of the cellular antiviral response, mRNAs were synthesized with modified residues of 5-methylcytidine triphosphate and pseudouridine triphosphate. In addition, a 5’ guanine cap was incorporated by inclusion of a synthetic cap analog to increase the RNA half-life in the cytoplasm. After purification, the RNAs were mixed and transfected into

human fibroblasts. This method provided rapid and large-scale generation of iPS cells. The estimated efficiency was beyond 4% when using a 5 factor cocktail (Oct3/4, Sox2, Klf4, c-Myc, and LIN28) and low oxygen conditions. The efficiency greatly surpassed that of all other reported protocols. The RNA-driven iPS cells showed pluripotency in their differentiation into cell types of all three germ layers. The method can also be applied to facilitate cell differentiation. Synthetic RNA encoding a myogenic factor, MYOD, enhanced the terminal differentiation of iPS cells into myotubes.

Many additional reprogramming factors have been reported that seem to affect both the induction efficiency and characteristics of iPS cells. For example, Hanna and colleagues found that some reprogramming factors, such as Lin28 and shRNA for p53, mainly regulate the reprogramming efficiency through the control of cell proliferation (20). In contrast, Nanog seems to enhance the efficiency of reprogramming by affecting the reprogramming process itself. Tbx3 was reported to improve the quality of mouse iPS cells, as it facilitated the germline transmission efficiency (21). Modification of cell signaling (by Wnt, inhibition of TGFbeta, and inhibition of MEK), or treatment with chemicals/natural compounds (valproic acid, 5´-azacytidine, thiazovivin, and vitamin C) have also been employed to develop better iPS cells (22-26). One company in the US has applied for a patent based on a process that generates iPS cells by using small

molecules. Although more detailed analyses are needed, one of these approaches could potentially be ideal for the future generation of iPS cells.

The origin of the iPS cells seems to be important, especially for humans. It is necessary to consider clinically available sources with respect to minimizing the invasiveness of collection and making them more easily accessible. Initially, iPS cells were established from fibroblasts (27, 28). Thereafter, numerous tissues sources have been investigated, and iPS cells can now be established from various tissues including keratinocytes, mesenchymal cells in fat, oral mucosa, molar teeth, peripheral blood, and cord blood (29-34). The generation of iPS cells from hematopoietic cells is noteworthy, because there are many established blood banks which already determine the HLA types of their blood. Seki et al. reported efficient generation of iPS cells from peripheral blood using Sendai viral vectors (35).

Genetic correction

iPS cells established from individual patients can provide a promising cell source for cell therapy, as they do not elicit immune rejection. However, when the patient suffers from a genetic disease, the cells need genetic correction. In general, human pluripotent stem cells are more difficult to modify compared to mouse ES cells. However, recent

studies have demonstrated effective gene modification of human pluripotent cells. Hockemeyer et al. employed a zinc-finger nuclease (ZFN) for genome editing (36). They designed a ZFN that recognized adjacent genomic sequences of the correction locus, by fusing the FokI nuclease domain on a DNA recognition domain composed of engineered C2H2 zinc-finger motifs. After transduction into iPS cells, the chimeric proteins bind at flanking genomic regions, dimerize with the nuclease domains, and cut the genomic DNA. Then a donor DNA, which is homologous to the target, is incorporated by homology-directed repair. This approach has several limitations, however, it can supply a powerful tool for gene correction.

Another method for efficient genetic correction is to use human iPS cells which show mouse ES cell-like characteristics. Conventional mouse and human ES cells share many characteristics, such as unlimited proliferation and differentiation potential into cells of all three germ layers, however, the cells have a significant number of distinct features including morphology, gene expression pattern, and cytokine dependence. Therefore, mouse and human pluripotent cells are termed “naïve” and “primed” pluripotent cells, respectively. Naïve mouse ES cells form compacted dome-shaped colonies in culture. They are largely dependent on external LIF signaling and have a high proliferation rate. On the other hand, human-type primed ES cells grow as flat

colonies, and are predominantly dependent on bFGF signaling, and have slow growth. Although both types of stem cells can differentiate into cell types from all three germ layers, the mouse naïve stem cells seem to have higher pluripotency, which enables them to contribute to chimera formation and germ cell lineage. Recently, the generation of human naïve ES and iPS cells has been reported in the presence of LIF and chemical supplementation (37, 38). In contrast to primed ES cells, the human naïve ES cells showed similar properties as mouse naïve ES cells with regard to their morphology, gene expression profile, and signaling pathway dependence. Interestingly, the human naïve ES cells also allowed efficient gene targeting by conventional homologous recombination methods, similar to the mouse naïve ES cells, which could provide a powerful method for gene repair. However, the culture conditions for naïve human pluripotent cells still needs to be optimized, as they easily revert to their primed state. Further studies will be necessary to enable stable handling of naïve human ES cells.

HLA banking

It is estimated that it would take at least a few years to establish clinical grade cells from a patient's own somatic cells. Each of the derived cell lines should be assessed for their medical stability, safety and efficacy. Thus, while ES cells might represent a novel

treatment approach for currently incurable diseases, it is necessary to consider the applications of iPS cells from an economical view point. Complete tailor-made iPS cell therapy would cost too much for most people. From this point of view, a banking system for iPS cells is proposed. To decrease immune rejection, iPS with various HLA haplotypes should be collected, similar to the way whole blood is currently banked. Based on the experiences with organ transplantation, it appears that the most important HLA molecules to match are the HLA class I molecules, HLA-A and HLA-B, and the class II molecule, HLA-DR. HLA matching of these loci can reduce the incidence of acute rejection, and has improved transplant survival. Estimations of the necessary stem cell bank size have been calculated for the Japanese and the UK populations (39, 40). If 170 lines of iPS cells were randomly established, 80% of such patients would be able to find a donor line with a single mismatch at one of the three HLA loci, HLA-A, -B, and –DR, with a better match found within the Japanese population. A bank size of 150 lines could therefore provide an acceptable match for 84.9% of the UK population. Importantly, when iPS cells are established from HLA homozygous cells, a smaller bank size of only 50 lines is expected to provide a three-locus match for 90.7% of the Japanese population (41). To identify at least one homozygote for each of the 50 different HLA haplotypes, it would be necessary to examine an HLA-type database of

24,000 individuals. This would be possible if the iPS bank cooperated with other banks, like cord blood banks and bone marrow banks. Generation of iPS cells from HLA-typed cells has already reported, thus supporting the feasibility of such a project. Tamaoki et al. focused on dental pulp cells, as they are easily obtained from extracted teeth, and such cells are generally considered to be medical waste, and can be expanded under simple culture conditions (42). Determination of their HLA types revealed that 2 out of 107 lines of dental pulp cells were homozygous for HLA-A, B, and DR loci.

Epigenetic memory of the source tissue

The origin of iPS cells affects their characteristics, including their safety and their differentiation efficiency. Miura et al. differentiated several mouse iPS cell lines into neural precursor cells in vitro and evaluated their safety by transplantation into immunodeficient mice (43). The iPS cells they used were derived from various tissues, such as embryonic fibroblasts, adult tail fibroblasts, liver, and stomach. Although iPS cells from embryonic fibroblasts and liver tissue efficiently differentiated into neural lineage cells, iPS cells from tail fibroblasts remained significantly undifferentiated after being treated using the same differentiation protocol, which resulted in teratoma formation after transplantation. Interestingly, the tumorigenicity was largely associated

with their origin, but not with inclusion of the c-Myc transgene. When a less tumorigenic iPS cell line was selected, the differentiated neural precursor cells could ameliorate the symptoms of spinal cord injury in a mouse model (44). Kim et al. also reported the effects of cell origin on the differentiation potential of iPS cells (45). In their study, iPS cells that originated from blood cells could easily differentiate into the hematopoietic linage, whereas iPS cells from a neuronal or fibroblast lineage showed limited differentiation potential. As treatment with inhibitors of DNA methyltransferase (5'-azacytidine) and histone deacetylase (trichostatin A) improved the hematopoietic differentiation capacity of iPS cells of neural origin, the epigenetic status appears to be important for the memory of iPS cells. In fact, several differentially methylated DNA regions (DMR) have been reported (46-48). Studies of the relationship between DMR and differentiation potential are therefore needed to determine the characteristics of iPS cells before inducing their differentiation. Some scientists believe that human naïve ES/iPS cells have less diversity than primed cells, as they are much closer to the actual pluripotent state and would therefore acquire full reprogramming more easily.

Direct conversion

The generation of iPS cells has clearly shown that forced expression of a few master

regulators can modify cell fate. With this in mind, several studies have been reported showing the direct conversion from one somatic cell type into another cell type without the use of stem cells. The generation of neuron-like cells from mouse fibroblasts was accomplished by transduction of several transcription factors including Ascl1, Brn2, and Myt1l (49). The induced neuronal cells expressed multiple neuron-specific proteins, generated action potentials, and formed functional synapses. Ieda et al. demonstrated derivation of cardiomyocyte-like cells from cardiac or dermal fibroblasts by transduction of three developmental transcription factors, Gata4, Mef2c, and Tbx5 (50). The cells termed "induced cardiomyocyte-like cells" expressed cardiac-specific markers, had a global gene expression profile similar to cardiomyocytes, and contracted spontaneously. The authors also transplanted fibroblasts into immunosuppressed mouse hearts after gene transduction and noted the formation of sarcomeric structures within 2 weeks. This approach represents one possible way to achieve transplantation. On the other hand, Zhou et al. reported direct *in vivo* induction of pancreatic exocrine cells to beta cells by transduction of three transcription factors, Ngn3, Pdx1, and Mafa (51). Adenoviral vectors encoding these genes were used for delivery to the pancreas of immunodeficient mice. The converted cells were capable of secreting insulin, and ameliorated hyperglycemia in streptozotocin-treated diabetic mice. As was observed for

iPS cells, these converted cells would have some epigenetic memory of their origin. Further studies are required, especially investigations regarding safety, before these approaches can be concluded to provide an alternative method for generating specific differentiated cells for use in medical treatment.

Conclusion

Artificial stem cells, iPS cells, are established by the modification of the intracellular and extracellular environments. The iPS cells seem to have pluripotency, as mouse and rat iPS cells can produce chimeric animals. A tetraploid complementation experiment revealed that mouse iPS cells have the ability to develop into a full-term mouse autonomously (52, 53). These data indicate that differentiated cells derived from iPS cells can at least partially replace the biological functions of various cells types. Several therapeutic attempts have been reported in rodent models, such as treatment of humanized sickle cell anemia, Parkinson's disease, and spinal cord injury (44, 54, 55). Although many problems still remain to be resolved, iPS cells may be applicable for medical treatment in the relatively near future. Studies of disease pathogenesis and drug discovery have already been launched, and the results thereof could provide important relief to countless people throughout the world who are currently suffering from

incurable diseases or injuries.

References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76.
2. Okita K, Ichisaka T, Yamanaka S. Generation of germ-line competent induced pluripotent stem cells. Nature. 2007 Jul 19;448:313-7.
3. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced Pluripotent Stem Cells Generated Without Viral Integration. Science. 2008 Sep 25;322(5903):945-9.
4. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of Mouse Induced Pluripotent Stem Cells Without Viral Vectors. Science. 2008 Oct 9;322(5903):949-53.
5. Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, et al. Generation of Induced Pluripotent Stem Cells Using Recombinant Proteins. Cell Stem Cell. 2009;in press, doi:10.1016/j.stem.2009.04.005.
6. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly Efficient Reprogramming to Pluripotency and Directed Differentiation of Human Cells with Synthetic Modified mRNA. Cell Stem Cell. 2010.

* The new virus- and integration-free method is remarkable because the synthetic mRNA can enhance not only reprogramming, but also directed differentiation.

7. Hirami Y, Osakada F, Takahashi K, Okita K, Yamanaka S, Ikeda H, et al. Generation of retinal cells from mouse and human induced pluripotent stem cells.
. Neurosci Lett 2009;Epub ahead of print.
8. Grigoriadis AE, Kennedy M, Bozec A, Brunton F, Stenbeck G, Park IH, et al. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells. Blood. 2010 Jan 11.
9. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, et al. Induced Pluripotent Stem Cells Generated from Patients with ALS Can Be Differentiated into Motor Neurons. Science. 2008 Jul 31;321:1218-21.
10. Hu BY, Weick JP, Yu J, Ma LX, Zhang XQ, Thomson JA, et al. Neural

differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci U S A. 2010 Feb 16.

11. Feng Q, Lu SJ, Klimanskaya I, Gomes I, Kim D, Chung Y, et al. Hemangioblastic Derivatives from Human Induced Pluripotent Stem Cells Exhibit Limited Expansion and Early Senescence. Stem Cells. 2010 Feb 12.

12. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science. 2003 Oct 17;302(5644):415-9.

13. Zhou W, Freed CR. Adenoviral Gene Delivery Can Reprogram Human Fibroblasts to Induced Pluripotent Stem Cells. Stem Cells. 2009 Aug 20.

14. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature. 2009 Mar 1.

15. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hamalainen R, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009 Mar 1.

16. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin, II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science. 2009 May 8;324(5928):797-801.

17. Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, et al. A nonviral minicircle vector for deriving human iPS cells. Nat Methods. 2010 Feb 7.

18. Kim DH, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, et al. Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins. Cell Stem Cell. 2009;Advanced online publication.

19. FUSAKI N, BAN H, NISHIYAMA A, SAEKI K, HASEGAWA M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome
Proc Jpn Acad, Ser B. 2009;85:15.

20. Hanna J, Saha K, Pando B, van Zon J, Lengner CJ, Creyghton MP, et al. Direct cell reprogramming is a stochastic process amenable to acceleration. Nature. 2009 Dec 3;462(7273):595-601.

21. Han J, Yuan P, Yang H, Zhang J, Soh BS, Li P, et al. Tbx3 improves the germ-line competency of induced pluripotent stem cells. Nature. 2010 Feb 7.

22. Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T, et al. Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell. 2009 Jan 9;4(1):16-9.

23. Marson A, Foreman R, Chevalier B, Bilodeau S, Kahn M, Young RA, et al. Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell. 2008 Aug 7;3(2):132-5.

24. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol. 2008 Oct 12;26(11):1269-75.

25. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, et al. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol. 2008 Jul;26(7):795-7.

26. Esteban MA, Wang T, Qin B, Yang J, Qin D, Cai J, et al. Vitamin C Enhances the Generation of Mouse and Human Induced Pluripotent Stem Cells. Cell Stem Cell. 2009 Dec 24.

27. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007 Nov 30;131(5):861-72.

28. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007 Dec 21;318(5858):1917-20.

29. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol. 2008 Nov;26(11):1276-84.

30. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, et al. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc Natl Acad Sci U S A. 2009 Sep 15;106(37):15720-5.

31. Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT. iPS cells reprogrammed from mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev. 2009 Oct 1.

32. Loh YH, Agarwal S, Park IH, Urbach A, Huo H, Heffner GC, et al. Generation of induced pluripotent stem cells from human blood.
. Blood. 2009;Advanced online publication.

33. Haase A, Olmer R, Schwanke K, Wunderlich S, Merkert S, Hess C, et al. Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell. 2009 Oct 2;5(4):434-41.

34. Giorgetti A, Montserrat N, Aasen T, Gonzalez F, Rodriguez-Piza I, Vassena R, et al. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. Cell Stem Cell. 2009 Oct 2;5(4):353-7.

35. Seki T, Yuasa S, Oda M, Egashira T, Yae K, Kusumoto D, et al. Generation of

induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell. 2010 Jul 2;7(1):11-4.

* They reported iPS cell generation from peripheral T cells by a non-integration method.

36. Hockemeyer D, Soldner F, Beard C, Gao Q, Mitalipova M, Dekelver RC, et al. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. Nat Biotechnol. 2009 Aug 13.

37. Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, et al. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. Proc Natl Acad Sci U S A. 2010 May 18;107(20):9222-7.

38. Buecker C, Chen HH, Polo JM, Daheron L, Bu L, Barakat TS, et al. A murine ESC-like state facilitates transgenesis and homologous recombination in human pluripotent stem cells. Cell Stem Cell. Jun 4;6(6):535-46.

39. Nakajima F, Tokunaga K, Nakatsuji N. HLA Matching Estimations in a Hypothetical Bank of Human Embryonic Stem Cell Lines in the Japanese Population for Use in Cell Transplantation Therapy. Stem Cells. 2006 Dec 21.

40. Taylor CJ, Bolton EM, Pocock S, Sharples LD, Pedersen RA, Bradley JA. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. Lancet. 2005 Dec 10;366(9502):2019-25.

41. Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and iPS cells. Nat Biotechnol. 2008 Jul;26(7):739-40.

42. Tamaoki N, Takahashi K, Tanaka T, Ichisaka T, Aoki H, Takeda-Kawaguchi T, et al. Dental Pulp Cells for Induced Pluripotent Stem Cell Banking. J Dent Res. 2010 Jun 16.

43. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, et al. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol. 2009 Jul 9.

44. Tsuji O, Miura K, Okada Y, Fujiyoshi K, Mukaino M, Nagoshi N, et al. Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci U S A. 2010 Jul 13;107(28):12704-9.

45. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, et al. Epigenetic memory in induced pluripotent stem cells. Nature. 2010 Jul 19.

46. Deng J, Shoemaker R, Xie B, Gore A, LeProust EM, Antosiewicz-Bourget J, et al. Targeted bisulfite sequencing reveals changes in DNA methylation associated with nuclear reprogramming. Nat Biotechnol. 2009;Advanced online publication.

47. Ball MP, Li JB, Gao Y, Lee JH, LeProust EM, Park IH, et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. Nat Biotechnol. 2009 Apr;27(4):361-8.

48. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, 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 Dec;41(12):1350-3.

49. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. Nature. 2010 Jan 27.

50. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell. 2010 Aug 6;142(3):375-86.

51. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature. 2008 Aug 27;455(7213):627-32.

52. 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 Jul 22.

53. Zhao XY, Li W, Lv Z, Liu L, Tong M, Hai T, et al. iPS cells produce viable mice through tetraploid complementation. Nature. 2009 Jul 23.

54. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, et al. Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin. Science. 2007 Dec 21;318(5858):1920-3.

55. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, et al. 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 Apr 15;105(15):5856-61.