Journal of Clinical Gerontology & Geriatrics 2 (2011) 1–6

Contents lists available at ScienceDirect

Journal of Clinical Gerontology & Geriatrics

journal homepage: www.e-jcgg.com

Review article

Induced pluripotent stem cells and regenerative medicine

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ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form
10 December 2010
Accepted 13 December 2010

Keywords:
Stem cells
Embryonic stem cell
Induced pluripotent stem cell
Regenerative medicine

ABSTRACT

Stem cells, a special subset of cells derived from embryo or adult tissues, are known to present the characteristics of self-renewal, multiple lineages of differentiation, high plastic capability, and long-term maintenance. Recent reports have further suggested that neural stem cells (NSCs) derived from the adult hippocampal and subventricular regions possess the utilizing potential to develop the transplantation strategies and to screen the candidate agents for neurogenesis, neuroprotection, and neuroplasticity in neurodegenerative diseases. In this article, we review the roles of NSCs and other stem cells in neuroprotective and neurorestorative therapies for neurological and psychiatric diseases. We show the evidences that NSCs play the key roles involved in the pathogenesis of several neurodegenerative disorders, including depression, stroke, and Parkinson's disease. Moreover, the potential and possible utilities of induced pluripotent stem cells, reprogramming from adult fibroblasts with ectopic expression of four embryonic genes, are also reviewed and further discussed. An understanding of the biophysiology of stem cells could help us elucidate the pathogenicity and develop new treatments for neurodegenerative disorders. In contrast to cell transplantation therapies, the application of stem cells can further provide a platform for drug discovery and small molecular testing, including Chinese herbal medicines. In addition, the high-throughput stem cell-based systems can be used to elucidate the mechanisms of neuroprotective candidates in translation medical research for neurodegenerative diseases.

1. Introduction

Stem cells are classified into three types according to their abilities to differentiate. The first type is totipotent stem cells, which can be implanted in the uterus of a living animal and give rise to a full organism. The second type is pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. They can give rise to every cell of an organism except extraembryonic tissues, such as placenta. This limitation restricts pluripotent stem cells from developing into a full organism. The third type is multipotent stem cells. They are adult stem cells, which only generate specific lineages of cells.[1] ES cells are pluripotent stem cells derived from the inner cell mass of mammalian blastocysts. They have remarkable abilities to proliferate indefinitely under appropriate *in vitro* culture system and to differentiate into any cell types of all three germ layers.[2,3] Since isolation of human ES in 1998, ES cells have been regarded as a powerful platform or tool for developmental studies, drug screening, diseases treatment, tissue repair engineering, and regenerative medicine. However, two main limitations have impeded the application of ES cell-based therapy. First, ethical dilemma regarding the human embryo donation and destruction. Second, ES cells are incompatible with the immune system of patients. To circumvent these deficiencies, scientists worldwide have devoted to developing a variety of reprogramming techniques to reverse somatic cells into a stem cell-like state.[4] In 2006, Takahashi and Yamanaka[5] published a landmark discovery that reprogramming of somatic cells back to iPS cells could be achieved by retroviral transduction of four

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2210-8335
doi:10.1016/j.jcgg.2010.12.003

pluripotency-associated transcription factors—Oct3/4, Sox2, c-Myc, and Klf4. These iPS cells possessed morphological and molecular features that resemble those of ES cells, as well as gave rise to teratoma and germline-competent chimeras on injection into blastocysts. This amazing finding showed that cell fate could be manipulated by certain genes and was recently honored by many awards, including 2009's Albert Lasker Basic Medical Research Award and 2010's International Balzan Prize. Since this astonishing report, iPS cells are now generated by various ways, including kinds of exogenous genes delivery methods,[6–10] choosing multiple somatic cell sources,[11–15] and even by small compounds[16] to improve the efficiency of the reprogramming process.

2. Comparison of iPS cells with ES cells

Generally, fully reprogrammed iPS cells display numerous properties similar to those of ES cells. First of all, iPS cells are morphologically identical to ES cells and show infinite proliferation and self-renewal abilities. Several molecular and functional assays were set to evaluate the similarity of iPS cells to ES cells, including reactivation of self-renewal and pluripotency-associated genes, telomerase activity, X chromosome, and stage-specific embryonic surface antigens, suppression of somatic genes associated with cell of origin, silencing of exogenous factors, capabilities of *in vitro* differentiation, demethylation of promoters of pluripotency genes, and *in vivo* teratoma formation, chimera contribution, germline transmission, and tetraploid complementation.[7,17] A recent study demonstrated that patient-specific iPS cells from dermal fibroblasts of patients with long QT syndrome can differentiate into functional cardiac myocytes but still recapitulated the electrophysiological features of the disorder.[18] Therefore, the major advantage of iPS cells over ES cells is that iPS cells can be derived from a patient's own somatic cells, thereby avoiding immune rejection after transplantation and the ethical concerns raised by using ES cells.

3. Advances in reprogramming techniques

Based on their pluripotent capability of differentiating into any functional cell type, iPS cells possess great potential for regenerative and therapeutic applications. However, the group led by Dr. Yamanaka also reported that these chimeras derived from mouse iPS cells and their progeny often develop tumors mainly because of reactivation of c-Myc transgene.[19] Thus, numerous approaches to generate iPS cells with lower tumorigenicity have been established. Several studies have shown that iPS cells generated without c-Myc virus demonstrated reduced tumor incidence in chimeric mouse, but the efficiency of iPS creation is significantly reduced.[20,21] To overcome this dilemma, Nakagawa et al.[22] found another member of Myc, L-Myc, which possessed stronger activity to generate iPS cells and less tumorigenic activity.

The use of genome-integrating retroviruses that are closely related with tumor formation was another major limitation of the original iPS cell generation techniques. Thus, reprogramming strategies with nonintegrating systems seems to be solutions to make iPS-based therapy feasible. In 2008, Stadtfeld et al.[23] established mouse iPS cells from fibroblasts and liver cells by nonintegrating adenoviruses carrying four defined factors, suggesting that insertional mutagenesis is not required for *in vitro* reprogramming. At the same time, Okita et al.[24] successfully generated iPS cells by transient transfection of two plasmids containing cDNAs encoding four factors, eliminating transgenic integration by the use of retroviruses. More recently, Somers et al.[25] and Carey et al.[26] individually described a "stem cell cassette" or a polycistronic virus, a single lentiviral vector composed of all four factors, was able to yield iPS with reduced insertional mutagenesis and viral reactivation. Another novel reprogramming technique using piggyBac transposon was published.[9,10,27] A polycistronic plasmid harboring four factors and piggyBac transposon was constructed and integrated into the genome in the presence of piggyBac transposase. As the reprogramming process achieved, the inserted fragment was easily removed by reexpressing transposase. The transposon-based method eliminates the use of virus, displays equivalent efficiencies to retroviral transduction, excises integrated sequences without genome alteration, and therefore represents a landmark progress toward therapeutically relevant virus-free iPS cells. To avoid introducing exogenous genetic materials, two amazing advances were reported. Zhou et al.[28] demonstrated that mouse fibroblasts could be fully reprogrammed by direct delivery of recombinant reprogramming proteins. In 2010, an impressive work conducted by Warren et al.[29] showed a strategy for reprogramming by administration of synthetic mRNAs that code for key factors and created RNA-iPS cells. Both techniques are safer, simpler, and faster approaches than the currently established genetic method.

4. Reactive oxygen species and stem cells differentiation

High efficiency of iPS cells reprogramming/differentiation is required in clinical application. Many studies have reported that reactive oxygen species (ROS) play a critical role in mediating iPS cells or stem cells reprogramming/differentiation.[30,31] Intracellular ROS serves as a second messenger in signaling transduction pathways. They are produced in vascular cells by a number of oxidases, such as the NADPH oxidases and xanthine oxidase, lipoxygenases, cytochrome p450, and uncoupling of the mitochondrial respiratory chain.[32] iPS cells have similar function in stress defense mechanisms and mitochondrial regulation with human ES cells.[33] Francisco et al.[34] had revealed that high glucose promoted stem cell differentiation into cardiomyocyte by activating NADPH oxidase as well as increasing intracellular ROS level. Ji et al.[35] had reported ROS-enhanced stem cell differentiation via mediating extracellular signal-regulated kinase/c-Jun *N*-terminal kinase, P38 mitogen-activated protein kinase, and protein kinase B. Furthermore, Varum et al.[36] had shown that attenuating the mitochondrial respiratory chain can increase pluripotency in human ES cells by facilitating intracellular ROS generation. Moreover, generation of ROS and the activities of antioxidant enzymes must be mainly manipulated to preserve the homeostasis of the intracellular redox status. Intracellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, play an important role to mitigate oxidative stress, such as SODs protect against superoxide-mediated cytotoxicity by catalyzing O^2 to form H_2O_2. SOD is inactivated by H_2O_2 formed by repressing of the superoxide anion.[37] Not only ROS level is activated but also intracellular antioxidant enzymes are mediated during differentiation. Chen et al.[38] had validated that intracellular antioxidant enzymes, mitochondrial mass, as well as oxygen consumption rate were increased during differentiation in human mesenchymal stem cells.

5. Clinical application of iPS cells

5.1. iPS cells in the diseases of central nervous system

The development of stem cell studies makes cell transplantation a promising therapy for the diseases of central nervous system, including stroke, traumatic brain injury, hypoxic encephalopathy, and degenerative disorders.[39] Parkinson's disease (PD) is the best candidate for the cell replacement therapy because only one group of cells are affected, which are dopaminergic neurons. The main pathology of PD is cellular loss of the substantia nigra pars compacta dopaminergic neurons that project to the striatum.[40] Clinical

signs of PD, which include rest tremor, rigidity, and bradykinesia, are evident when about 80% of striatal and 50% of nigral neurons are lost.[41] The first attempt of cell replacement therapy was to use fetal mesencephalic tissue, and the results were successful in the earliest reports.[39,42,43] However, adverse effects and limitations were revealed in the following studies, which included off-medication dyskinesia,[44–46] graft-induced inflammatory responses,[47] and limited tissue availability.[39]

Graft-induced dyskinesia may be caused by unfavorable composition of the fetal mesencephalic grafts. The fetal mesencephalic tissue includes not only dopaminergic but also nondopaminergic neurons.[39] The exclusion of serotonin and γ-aminobutyric acid neurons and enrichment of substantia nigra dopaminergic neurons will decrease the occurrence of dyskinesia.[47] Stem cells are ideal cell sources to achieve this goal. Recent evidence has shown that dopaminergic neurons derived from ES cells and bone marrow-derived neural progenitors are functional when grafted into parkinsonian rats.[48,49] Several methods are able to improve the effectiveness of midbrain dopaminergic neuron generation from stem cells, including manipulating transcription factor (e.g., Nurr1, Pitx3, or Lmx1a), coculture with astrocytes, and using fluorescence-activated cell sorting.[47] The ability of deriving large quantities of correctly differentiated dopamine neurons makes stem cells a good cell sources for transplantation in PD.

Cell replacement therapy is more complicated for stroke, brain injury, and other degenerative diseases, such as Alzheimer's disease. The difficulties are because of variable cell types involved, which include neurons, astrocytes, oligodendrocytes, and endothelial cells of blood vessels.[50] ES cells have been demonstrated to have good developmental potential and significant survival rate after transplantation into the brain.[51] Transplantation of ES cells also recovered behavioral dysfunction induced by middle cerebral arterial occlusion in an animal model.[52] However, the ethical consideration, the limited availability, and the possibility of immune rejection after transplantation restrict the accessibility of ES cells.

Because iPS cells are derived from the somatic cells, potential immune rejection and ethical consideration can be avoided. Recently, Wernig et al.[53] demonstrated that neurons and glial cells could be derived from iPS cells *in vitro*, and that transplantation of iPS cell-derived neurons into brain was able to improve behavior in a rat model of PD. We also demonstrated an efficient method to differentiate iPS cells into astrocyte-like and neuron-like cells, which displayed functional electrophysiological properties. Our *in vivo* study showed that direct injection of iPS cells into damaged areas of rat cortex significantly decreased the infarct size, improved the motor function, attenuated inflammatory cytokines, and mediated neuroprotection after middle cerebral artery occlusion. Subdural injection of iPS cells with fibrin glue was as effective as the direct-injection method and provided a safer choice for cell replacement therapy.[54]

Teratoma or tumor formation is a major adverse effect of cell transplantation using ES or iPS cells.[55] One of the methods to prevent teratoma/tumor formation is elimination of nonneural progenitors, which can be achieved by the elaboration of differentiation protocols that allow maximal homogeneity of the transplant[56] or by cell sorting before transplantation.[57] Exclusion of poorly differentiated ES or iPS cells can also reduce the rate of teratoma or tumor formation.[58] Some antioxidants may prevent tumorigenesis after cell transplantation. Resveratrol, a natural polyphenol antioxidant, is demonstrated that it can inhibit teratoma formation *in vivo*.[59] Our recent study also found that docosahexaenoic acid can inhibit teratoma formation in addition to promoting dopaminergic differentiation in iPS cells in PD-like rats.[60] It has been only two years since the development of iPS cells. Enhancement of effectiveness and eliminating adverse effects of this cell transplantation therapy required more extensive studies.

5.2. iPS cells in cardiovascular diseases

In the aging population of a modern world, cardiovascular diseases are major medical problems because they usually cause morbidity and mortality.[1] The treatments of cardiovascular diseases include medication, surgical intervention, rehabilitation, exercise programs, and transplantation.[61] There are several side effects, complications, and limitations of transplantation therapy, such as immunological reaction, infection, and limited availability.[62] A new hope in cardiovascular regenerative medicine has been revealed since Doetschman et al.[63] successfully induced mouse ES cells differentiating into cardiomyocytes *in vitro* in 1985. Many studies had reported facilitated differentiation from ES cells or iPS cells into cardiomyocytes, endothelial vascular cells, and smooth muscle cells.[64,65] In animal models, cardiovascular regeneration therapy markedly attenuated ventricular wall thinning as well as enhanced contractility of cardiomyocytes postligation of the left anterior descending artery,[66] restored the function of heart and electric stability after myocardial infarction,[67] and enriched the formation of small capillaries and venules.[68]

5.3. iPS cells in lung diseases

Acute lung injury (ALI) is characterized by neutrophil accumulation in the lungs, interstitial edema, disruption of epithelial integrity, and leakage of proteins into the alveolar space.[69–72] Infection, associated with endotoxemia and blood loss are frequent predisposing factors to the development of ALI[69]; and in experimental settings, endotoxemia produces ALI. Neutrophils play a central role in this acute pulmonary inflammatory process as their elimination can prevent the development of ALI.[73] The neutrophils present in the lungs during ALI produce inflammatory mediators, including cytokines, such as interleukin-6 and macrophage inflammatory peptide-2, and demonstrate increased activation of transcriptional regulatory factors, including nuclear factor-κB (NF-κB).[73–76]

Binding elements for NF-κB are present in the enhancer/promoter regions of cytokine genes, such as interleukin-1β, macrophage inflammatory peptide-2, and tumor necrosis factor-α, as well as other important immunoregulatory molecules, such as intercellular adhesion molecule-1 and complement C4 protein.[77] Inhibition of NF-κB activation prevents endotoxin-induced increases in proinflammatory cytokine expression in the lungs.[76]

iPS cell administration improved the impairment of pulmonary function in endotoxin-induced ALI, including airway resistance (enhanced pause), lung tidal volumes, and arterial partial oxygen pressure levels. Hypoxemia is the major symptom and sign of ALI, no matter whether in the mice model or in human cases. The effect of iPS cell treatment was to rescue the hypoxemia, similar to another study using a therapeutic agent in an animal model of lung injury.[78] A recent study found that transplantation of human ES cells abrogated bleomycin-induced lung injury in mice and restored blood arterial oxygen saturation and lung tidal volume.[79] Our study showed that the intravenous injection of iPS cells led to recovery of the impairment of both airway resistance and lung tidal volume induced by the instillation of endotoxin intratracheally. In a previous mice model of early ALI, most changes in bronchoalveolar lavage suggestive of acute pulmonary irritation were compatible with the changes in pulmonary function, such as airway resistance (enhanced pause) and tidal volume.[80] Thus, iPS cell therapy not only abolished endotoxin-induced lung injury in mice but also improved the changes in pulmonary physiological function. This

novel cellular therapy opened an era of cell-based transplantation by overcoming the immune rejection and the ethical controversy over the use of ES cells and mesenchymal stem cells.

5.4. iPS in liver diseases

Liver diseases and liver injuries are common health problems throughout the world. The loss of functional liver tissue after injury will activate a wound healing process aimed to repair and restore the integrity of the injured liver. Intense or uncontrollable insults could efface the healing response and result in end-stage liver disease, which is irreversibly associated with liver failure. Currently, orthotopic liver transplantation is the most effective therapy for acute and chronic liver failure. However, it is limited by shortage of donors, operative risk, lifelong use of immunosuppressive agents, and very high costs. The development and application of cell therapies has been attempted to treat different forms of liver diseases.[81–86] Cell therapy has been considered as a potential therapeutic alternative to orthotopic liver transplantation.[87–89] It has minimal invasive procedures and fewer surgical complications.[90–92] These cells, particularly the stem cell population, appeared very attractive and have gained considerable attention for its potential to supportive tissue regeneration. Besides, they have the potential to generate large amounts of donor cells available for transplant or to be stored for future use.

Although previous studies using stem cells in the treatment of liver diseases have shown beneficial effects, the underlying mechanisms accounting for their therapeutic effects have not been completely revealed. One of the possible explanations is that the transplanted stem cells generate cells that function as normal hepatocytes. However, it has been noticed that the percentage of liver repopulation remains very low despite efforts to improve cell engraftment. Another explanation is the indirect paracrine effects that initiated in the damaged liver after stem cell transplant.[93] Some soluble factors could have been secreted to facilitate the process of repair and regeneration. It is still unclear how these soluble factors regulate the recovery process in the injured liver after stem cell transplantation.

Currently, the therapeutic roles of iPS cell or iPS-derived hepatocytes (IDHs)-like cells for liver injury have gained increasing attention.[94,95] Si-Tayeb et al.[94] reported that human iPS cells from foreskin fibroblasts could be used to efficiently generate human hepatocyte-like cells. The IDHs-like cells displayed several hepatic functions, including albumin expression, accumulation of glycogen, metabolism of indocyanine green, accumulation of lipid, active uptake of low-density lipoprotein, synthesis of urea, and expressed the same hepatocyte mRNA fingerprint. However, the levels of expression of these enzymes were lower in most cases when compared with adult liver samples, suggesting that although hepatocyte-like cells derived from human iPS cells have differentiated to a state that supports many hepatic activities, they do not entirely recapitulate mature liver function. Similarly, it is not clear that whether iPS cells and IDHs have the homing characteristic of locating the area of acute hepatic failure and further can rescue the liver function.

6. Summary

In the past, scientists tried to ameliorate the injury through transplantation of target cells- or stem cells-derived precursors. However, it is hard to prepare enough amounts of target cells *in vitro* or to efficiently isolate differentiated cells from stem cell populations. The generation of iPS cells stands a better chance than other reprogramming procedures (somatic cell nuclear transfer, cell fusion, and so forth) of overcoming these issues, whereas a large number of iPS cells can be prepared *in vitro*. To date, iPS-derived strategies have been applied to four disease models, sickle cell anemia, PD, hemophilia A, and acute myocardial infarction. However, there still exist several questions to be answered, such as what are the detailed molecular mechanisms of reprogramming? Can iPS cells be generated solely by chemical compounds like epigenetic modifier without DNA transduction? How to improve the yield of iPS? In addition, to provide replacement cells for therapy, a new cell by lineage switching or direct conversion from a normal somatic cell should also be considered.[96] In conclusion, the iPS techniques open a new era for stem cell research and offer promising opportunities for patient-specific pluripotent cell-based regenerative medicine.

References

1. Sun Y. Myocardial repair/remodelling following infarction: roles of local factors. *Cardiovasc Res* 2009;**81**:482–90.
2. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981;**292**:154–6.
3. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* 1981;**78**:7634–8.
4. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;**282**:1145–7.
5. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–76.
6. Hanna J, Carey BW, Jaenisch R. Reprogramming of somatic cell identity. *Cold Spring Harb Symp Quant Biol* 2008;**73**:147–55.
7. Maherali N, Hochedlinger K. Guidelines and techniques for the generation of induced pluripotent stem cells. *Cell Stem Cell* 2008;**3**:595–605.
8. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 2009;**136**:964–77.
9. 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;**458**:766–70.
10. 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**:363–9.
11. 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;**4**:16–9.
12. Liao J, Cui C, Chen S, Ren J, Chen J, Gao Y, et al. Generation of induced pluripotent stem cell lines from adult rat cells. *Cell Stem Cell* 2009;**4**:11–5.
13. Liu H, Zhu F, Yong J, Zhang P, Hou P, Li H, et al. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell* 2008;**3**:587–90.
14. 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;**131**:861–72.
15. Sun N, Longaker MT, Wu JC. Human iPS cell-based therapy: considerations before clinical applications. *Cell Cycle* 2010;**9**:880–5.
16. 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**:301–12.
17. Colman A, Dreesen O. Induced pluripotent stem cells and the stability of the differentiated state. *EMBO Rep* 2009;**10**:714–21.
18. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med* 2010;**363**:1397–409.
19. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007;**448**:313–7.
20. Wernig M, Meissner A, Cassady JP, Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2008;**2**:10–2.
21. Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol* 2007;**25**:1177–81.
22. Nakagawa Masato, Koyanagi Michiyo, Tanabe Koji, Takahashi Kazutoshi, Ichisaka Tomoko, Aoi Takashi, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nature Biotechnology* 2008;**26**:101–6.
23. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 2008;**322**:945–9.
24. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008;**322**:949–53.
25. Somers A, Jean JC, Sommer CA, Omari A, Ford CC, Mills JA, et al. Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. *Stem Cells* 2010;**28**:1728–40.
26. Carey BW, Markoulaki S, Hanna J, Saha K, Gao Q, Mitalipova M, et al. Reprogramming of murine and human somatic cells using a single polycistronic vector. *Proc Natl Acad Sci USA* 2009;**106**:157–62.

27. 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;**458**:771–5.
28. 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;**4**: 381–4.
29. 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;**7**:618–30.
30. Sauer H, Rahimi G, Hescheler J, Wartenberg M. Role of reactive oxygen species and phosphatidylinositol 3-kinase in cardiomyocyte differentiation of embryonic stem cells. *FEBS Lett* 2000;**476**:218–23.
31. Schmelter M, Ateghang B, Helmig S, Wartenberg M, Sauer H. Embryonic stem cells utilize reactive oxygen species as transducers of mechanical strain-induced cardiovascular differentiation. *FASEB J* 2006;**20**:1182–4.
32. Papaharalambus CA, Griendling KK. Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. *Trends Cardiovasc Med* 2007;**17**:48–54.
33. Armstrong L, Tilgner K, Saretzki G, Atkinson SP, Stojkovic M, Moreno R, et al. Human induced pluripotent stem cell lines show stress defense mechanisms and mitochondrial regulation similar to those of human embryonic stem cells. *Stem Cells* 2010;**28**:661–73.
34. Crespo FL, Sobrado VR, Gomez L, Cervera AM, McCreath KJ. Mitochondrial reactive oxygen species mediate cardiomyocyte formation from embryonic stem cells in high glucose. *Stem Cells* 2010;**28**:1132–42.
35. Ji AR, Ku SY, Cho MS, Kim YY, Kim YJ, Oh SK, et al. Reactive oxygen species enhance differentiation of human embryonic stem cells into mesendodermal lineage. *Exp Mol Med* 2010;**42**:175–86.
36. Varum S, Momcilovic O, Castro C, Ben-Yehudah A, Ramalho-Santos J, Navara CS. Enhancement of human embryonic stem cell pluripotency through inhibition of the mitochondrial respiratory chain. *Stem Cell Res* 2009;**3**:142–56.
37. Jewett SL, Rocklin AM, Ghanevati M, Abel JM, Marach JA. A new look at a time-worn system: oxidation of CuZn-SOD by H2O2. *Free Radic Biol Med* 1999;**26**:905–18.
38. Chen CT, Shih YR, Kuo TK, Lee OK, Wei YH. Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells. *Stem Cells* 2008;**26**:960–8.
39. Lindvall O, Kokaia Z, Martinez-Serrano A. Stem cell therapy for human neurodegenerative disorders-how to make it work. *Nat Med* 2004;(10 Suppl): S42–50.
40. Samii A, Nutt JG, Ransom BR. Parkinson's disease. *Lancet* 2004;**363**:1783–93.
41. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 1991;**114**(Pt. 5):2283–301.
42. Lindvall O, Hagell P. Clinical observations after neural transplantation in Parkinson's disease. *Prog Brain Res* 2000;**127**:299–320.
43. Kordower JH, Freeman TB, Snow BJ, Vingerhoets FJ, Mufson EJ, Sanberg PR, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med* 1995;**332**:1118–24.
44. Olanow CW, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol* 2003;**54**:403–14.
45. Hagell P, Piccini P, Bjorklund A, Brundin P, Rehncrona S, Widner H, et al. Dyskinesias following neural transplantation in Parkinson's disease. *Nat Neurosci* 2002;**5**:627–8.
46. Freed CR, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001;**344**:710–9.
47. Hedlund E, Perlmann T. Neuronal cell replacement in Parkinson's disease. *J Intern Med* 2009;**266**:358–71.
48. Yang D, Zhang ZJ, Oldenburg M, Ayala M, Zhang SC. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in parkinsonian rats. *Stem Cells* 2008;**26**:55–63.
49. Glavaski-Joksimovic A, Virag T, Chang QA, West NC, Mangatu TA, McGrogan MP, et al. Reversal of dopaminergic degeneration in a parkinsonian rat following micrografting of human bone marrow-derived neural progenitors. *Cell Transplant* 2009;**18**:801–14.
50. Zhang ZG, Chopp M. Neurorestorative therapies for stroke: underlying mechanisms and translation to the clinic. *Lancet Neurol* 2009;**8**:491–500.
51. Takahashi K, Yasuhara T, Shingo T, Muraoka K, Kameda M, Takeuchi A, et al. Embryonic neural stem cells transplanted in middle cerebral artery occlusion model of rats demonstrated potent therapeutic effects, compared to adult neural stem cells. *Brain Res* 2008;**1234**:172–82.
52. Yanagisawa D, Qi M, Kim DH, Kitamura Y, Inden M, Tsuchiya D, et al. Improvement of focal ischemia-induced rat dopaminergic dysfunction by striatal transplantation of mouse embryonic stem cells. *Neurosci Lett* 2006;**407**:74–9.
53. 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 USA* 2008;**105**:5856–61.
54. Chen SJ, Chang CM, Tsai SK, Chang YL, Chou SJ, Huang SS, et al. Functional improvement of focal cerebral ischemia injury by subdural transplantation of induced pluripotent stem cells with fibrin glue. *Stem Cells Dev* 2010;**19**: 1757–67.
55. Erdo F, Buhrle C, Blunk J, Hoehn M, Xia Y, Fleischmann B, et al. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. *J Cereb Blood Flow Metab* 2003;**23**:780–5.
56. Brederlau A, Correia AS, Anisimov SV, Elmi M, Paul G, Roybon L, et al. Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of in vitro differentiation on graft survival and teratoma formation. *Stem Cells* 2006;**24**:1433–40.
57. Chung S, Shin BS, Hedlund E, Pruszak J, Ferree A, Kang UJ, et al. Genetic selection of sox1GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. *J Neurochem* 2006;**97**:1467–80.
58. Tabar V, Panagiotakos G, Greenberg ED, Chan BK, Sadelain M, Gutin PH, et al. Migration and differentiation of neural precursors derived from human embryonic stem cells in the rat brain. *Nat Biotechnol* 2005;**23**:601–6.
59. Kao CL, Tai LK, Chiou SH, Chen YJ, Lee KH, Chou SJ, et al. Resveratrol promotes osteogenic differentiation and protects against dexamethasone damage in murine induced pluripotent stem cells. *Stem Cells Dev* 2010;**19**: 247–58.
60. Hung CW, Liou YJ, Lu SW, Tseng LM, Kao CL, Chen SJ, et al. Stem cell-based neuroprotective and neurorestorative strategies. *Int J Mol Sci* 2010;**11**: 2039–55.
61. Christie JD, Edwards LB, Aurora P, Dobbels F, Kirk R, Rahmel AO, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fifth official adult lung and heart/lung transplantation report–2008. *J Heart Lung Transplant* 2008;**27**:957–69.
62. Augoustides JG, Riha H. Recent progress in heart failure treatment and heart transplantation. *J Cardiothorac Vasc Anesth* 2009;**23**:738–48.
63. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J Embryol Exp Morphol* 1985;**87**:27–45.
64. Iacobas I, Vats A, Hirschi KK. Vascular potential of human pluripotent stem cells. *Arterioscler Thromb Vasc Biol* 2010;**30**:1110–7.
65. Xie CQ, Huang H, Wei S, Song LS, Zhang J, Ritchie RP, et al. A comparison of murine smooth muscle cells generated from embryonic versus induced pluripotent stem cells. *Stem Cells Dev* 2009;**18**:741–8.
66. Kofidis T, de Bruin JL, Hoyt G, Ho Y, Tanaka M, Yamane T, et al. Myocardial restoration with embryonic stem cell bioartificial tissue transplantation. *J Heart Lung Transplant* 2005;**24**:737–44.
67. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 2007;**25**: 1015–24.
68. Li Z, Wu JC, Sheikh AY, Kraft D, Cao F, Xie X, et al. Differentiation, survival, and function of embryonic stem cell derived endothelial cells for ischemic heart disease. *Circulation* 2007;**116**(11 Suppl):I46–54.
69. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000;**342**:1334–49.
70. Chollet-Martin S, Jourdain B, Gibert C, Elbim C, Chastre J, Gougerot-Pocidalo MA. Interactions between neutrophils and cytokines in blood and alveolar spaces during ARDS. *Am J Respir Crit Care Med* 1996;**154**(3 Pt 1): 594–601.
71. Goodman RB, Strieter RM, Martin DP, Steinberg KP, Milberg JA, Maunder RJ, et al. Inflammatory cytokines in patients with persistence of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1996;**154**(3 Pt 1):602–11.
72. Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer JM. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992;**145**:1016–22.
73. Abraham E, Carmody A, Shenkar R, Arcaroli J. Neutrophils as early immunologic effectors in hemorrhage- or endotoxemia-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2000;**279**:L1137–1145.
74. Parsey MV, Tuder RM, Abraham E. Neutrophils are major contributors to intraparenchymal lung IL-1 beta expression after hemorrhage and endotoxemia. *J Immunol* 1998;**160**:1007–13.
75. Shenkar R, Abraham E. Mechanisms of lung neutrophil activation after hemorrhage or endotoxemia: roles of reactive oxygen intermediates, NF-kappa B, and cyclic AMP response element binding protein. *J Immunol* 1999;**163**: 954–62.
76. Xing Z, Jordana M, Kirpalani H, Driscoll KE, Schall TJ, Gauldie J. Cytokine expression by neutrophils and macrophages in vivo: endotoxin induces tumor necrosis factor-alpha, macrophage inflammatory protein-2, interleukin-1 beta, and interleukin-6 but not RANTES or transforming growth factor-beta 1 mRNA expression in acute lung inflammation. *Am J Respir Cell Mol Biol* 1994;**10**: 148–53.
77. Foo SY, Nolan GP. NF-kappaB to the rescue: RELs, apoptosis and cellular transformation. *Trends Genet* 1999;**15**:229–35.
78. Treml B, Neu N, Kleinsasser A, Gritsch C, Finsterwalder T, Geiger R, et al. Recombinant angiotensin-converting enzyme 2 improves pulmonary blood flow and oxygenation in lipopolysaccharide-induced lung injury in piglets. *Crit Care Med* 2010;**38**:596–601.
79. Wang D, Morales JE, Calame DG, Alcorn JL, Wetsel RA. Transplantation of human embryonic stem cell-derived alveolar epithelial type II cells abrogates acute lung injury in mice. *Mol Ther* 2010;**18**:625–34.

80. Pauluhn J. Comparative assessment of early acute lung injury in mice and rats exposed to 1,6-hexamethylene diisocyanate-polyisocyanate aerosols. *Toxicology* 2008;**247**:33–45.
81. Kawashita Y, Guha C, Yamanouchi K, Ito Y, Kamohara Y, Kanematsu T. Liver repopulation: a new concept of hepatocyte transplantation. *Surg Today* 2005;**35**:705–10.
82. Horslen SP, Fox IJ. Hepatocyte transplantation. *Transplantation* 2004;**77**:1481–6.
83. Fox IJ, Roy-Chowdhury J. Hepatocyte transplantation. *J Hepatol* 2004;**40**:878–86.
84. Ito M, Nagata H, Miyakawa S, Fox IJ. Review of hepatocyte transplantation. *J Hepatobiliary Pancreat Surg* 2009;**16**:97–100.
85. Weber A, Groyer-Picard MT, Franco D, Dagher I. Hepatocyte transplantation in animal models. *Liver Transpl* 2009;**15**:7–14.
86. Puppi J, Dhawan A. Human hepatocyte transplantation overview. *Methods Mol Biol* 2009;**481**:1–16.
87. Keeffe EB. Liver transplantation: current status and novel approaches to liver replacement. *Gastroenterology* 2001;**120**:749–62.
88. Lee LA. Advances in hepatocyte transplantation: a myth becomes reality. *J Clin Invest* 2001;**108**:367–9.
89. Ott M, Schmidt HH, Cichon G, Manns MP. Emerging therapies in hepatology: liver-directed gene transfer and hepatocyte transplantation. *Cells Tissues Organs* 2000;**167**:81–7.
90. Kakinuma S, Nakauchi H, Watanabe M. Hepatic stem/progenitor cells and stem-cell transplantation for the treatment of liver disease. *J Gastroenterol* 2009;**44**:167–72.
91. Yoshimi A, Nannya Y, Ueda K, Asano D, Yamamoto G, Kumano K, et al. Successful hematopoietic stem cell transplantation from an HLA-identical sibling in a patient with aplastic anemia after HLA-haploidentical living-related liver transplantation for fulminant hepatitis. *Biol Blood Marrow Transplant* 2009;**15**:389–90.
92. Navarro-Alvarez N, Soto-Gutierrez A, Kobayashi N. Stem cell research and therapy for liver disease. *Curr Stem Cell Res Ther* 2009;**4**:141–6.
93. Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, et al. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. *Gastroenterology* 2008;**134**:2111–21. 2121 e1–e3.
94. Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology* 2010;**51**:297–305.
95. Espejel S, Roll GR, McLaughlin KJ, Lee AY, Zhang JY, Laird DJ, et al. Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice. *J Clin Invest* 2010;**120**:3120–6.
96. Gurdon JB, Melton DA. Nuclear reprogramming in cells. *Science* 2008;**322**:1811–5.