REVIEW ARTICLE

Induced Pluripotent Stem Cells: History, Properties and Potential Applications

N Nordin, PhD*,*, M I Lai, PhD**,***, A Veerakumarasivam, PhD*,***,*****, R Ramasamy, PhD**,***,****, S Abdullah, DPhil*,***,****, W Y Wendy-Yeo, MSc*,****, R Rosli, PhD*,***,****

*Department of Obstetrics & Gynaecology, **Department of Pathology, ***Stem Cell Research Laboratory, Faculty of Medicine & Health Sciences, ****Institute of Bioscience, Universiti Putra Malaysia and *****Perdana University Graduate School of Medicine, Perdana University, 43400 Serdang UPM, Selangor, Malaysia

SUMMARY

The development of induced pluripotent stem cells (iPSCs) has been met with much enthusiasm and hailed as a breakthrough discovery by the scientific and research communities amidst the divisive and ongoing debates surrounding human embryonic stem cells (hESC) research. The discovery reveals the fact that embryonic pluripotency can be generated from adult somatic cells by the induction of appropriate transcriptional factor genes essential for maintaining the pluripotency. They provide an alternative source for pluripotent stem cells, thus representing a powerful new research tool besides their potential application in the field of regenerative medicine. In this review, the historical background of iPSCs generation will be discussed together with their properties and characteristics as well as their potential therapeutic applications.

KEY WORDS:
Induced pluripotent stem cells, Reprogramming genes, Epigenetics status, Pluripotency markers

INTRODUCTION

The discovery on the ability of somatic cells to be reprogrammed into embryonic state-like cells has marked a remarkable event in the history of stem cell development. These cells that have undergone the reprogramming procedure termed as induced pluripotent stem cells (iPSCs) have been shown to be greatly similar to the embryonic stem cells (ESCs). The rank of ESCs at the highest level of the stemness hierarchy justifies their wide differentiation potential. This unique pluripotent property has placed them as the most suitable candidate for clinical applications and laboratory settings. They also offer a beneficial model system to study mammalian embryogenesis and disease processes[1]. Unfortunately, despite the remarkable advantages offered by ESCs in addressing the advances in human medicine, there have been major concerns and controversies that have hindered their use in clinical settings. Therefore, in this review the limitations of ESCs will be discussed prior to addressing the alternative source for pluripotent stem cells, the iPSCs.

Limitations of Embryonic Stem Cells (ESCs)

The policies on human ESC research solely relied on the basis for the regulation of stem cell research which includes the source of the stem cells, objective of research and the symbolic moral right of the embryo[2]. Ethical controversy in ESC research arises due to current methods to produce embryonic stem cell lines that require the destruction of living human embryos. The ESC lines currently available for studies are derived from blastocyst generated for *in vitro* fertilization (IVF). The main ethical consideration is raised upon the morality of destroying embryos based on the argument that human life begins when an egg is fertilised. The moral objection remains even though only surplus embryos from *in-vitro* fertilization (IVF) have been used, and the research promises great potential benefits to humankind.

Our national guidelines for stem cell research and therapy confine the use of ESCs for research purpose using only surplus embryos from IVF. The government's stand is also in line with religious regulatory boards whereby the National Fatwa Committee on Assisted Reproductive Technology (APT) allows the utilisation of excess embryos which are derived from IVF however it imposed a halt on somatic cell nuclear transfer (SCNT)[2].

Secondly, the safety of existing ESC lines also implicates a major concern in therapeutic usage. Most ESCs are grown in heterogeneous cultures with irradiated mouse fibroblasts as a feeder cell layer[3]. However, such mixed culture could be classified as xenotransplants under the Food and Drug administration (FDA), thus limiting the possibility for their use in clinical studies[4]. It has been found that many human ESC lines undergo genetic and epigenetic changes. Hence, the genetic stability of ESCs remains an issue to be verified. Furthermore, due to its genetic instability, it is of high risk to utilise undifferentiated ESCs for therapeutic purposes which may contribute to malignant transformation[5]. Bongso and his colleagues in their extensive review on this issue also believe that the formation of tumour has been a major obstacle as the tumour will still develop even though ES cell-differentiated derivatives were injected into a normal or immune-compromised animal[6].

Another important limitation of ESCs is the immunorejection problem as a result of mismatch between the donor and the recipient cells upon transplantation. A global storage for varieties of HLA typed stem cell lines for compatible match for specific patients is being established to overcome this problem[7,8,9]. However, this approach also has been hindered

This article was accepted: 27 March 2011
Corresponding Author: Norshariza Nordin, Universiti Putra Malaysia, Obstetrics & Gynaecology, Universiti Putra Malaysia, Serdang, Selangor 43400 Malaysia Email: shariza@medic.upm.edu.my

with the realistic number of human ESC lines required in finding the HLA-matched lines[6].

For these reasons, there is an increasing interest in developing alternative mechanisms for generating ESC lines or even non-embryonic sources of pluripotent stem cells. Some works have been initiated to develop methods for therapeutic cloning via transferring somatic nuclei into enucleated oocytes (SCNT) or embryonic stem cell cytoblasts[10]. The accomplishment of SCNT confers the exploitation of the intracellular environment towards modification of cellular epigenetics and reprogramming to establish pluripotent stem cells[11]. Although the genetic instability factors were lowered via this method, yet other ethical issues pertaining to the morality and political issues are still viable. Therefore, the novel finding by Japanese researchers that the pluripotency of ESCs could be created from adult somatic cells by the induction of four genes that are normally expressed in ESCs[12] has received a lot of attentions. These are the induced pluripotent stem, iPSCs.

Induced Pluripotent Stem Cells (iPSCs)

In essence, iPSCs are mature cells that have been genetically reprogrammed (Table I) to an embryonic stem cell–like state through ectopic introduction of transcriptional factor genes critical for maintaining the properties of ESCs[12]. This reversion of adult cells to a state resembling ESCs offers unprecedented potential to generate patient- and disease-specific tissues for targeted disease research, as well as drug screening methods for the development of new therapies.

The iPSCs are believed to be similar to embryonic stem cells in terms of their morphology, cell behavior, gene expression, epigenetic status and differentiation potential both in culture and *in vivo*[13]. Although these cells meet the criteria for pluripotent stem cells, it is yet unclear whether iPSCs and ESCs differ in clinically significant ways. Hence, this is an area of investigation actively being pursued. In this paper, we underline the historical background of the generation of iPSCs and highlight their properties and characteristics as well as their potential therapeutic applications.

Historical Background of iPSCs Technology

First generation

The announcement by Shinya Yamanaka and his team at Kyoto University in 2006 in which they reported for the first time, the successful reprogramming of adult mouse fibroblasts into iPSCs[12] set the stage for this revolutionary technology. The remarkable feat was finding a combination of reprogramming factors that actually worked, albeit at modest efficiency. The approach employed a retrovirus to transduce mouse fibroblasts with the selected genes and cells were isolated by antibiotic selection of Fbx15 positive cells. Unfortunately, this iPSC line showed DNA methylation errors compared to original patterns in ESC lines and failed to produce viable chimeras when injected into developing embryos[12].

Second generation

In 2007, Yamanaka's group along with two other independent research groups from Harvard, MIT and UCLA showed reprogramming of mouse fibroblasts into iPSCs from which viable chimeras were produced[14,15,16]. As in the earlier work, these cell lines were also derived from mouse fibroblasts by retroviral mediated reactivation of the same four endogenous pluripotent factors *(Oct4, Sox2, Klf4 and c-Myc)*, but a different marker for detection was utilized. *Nanog*, an important gene in ESCs and which has been shown to be a major determinant of cellular pluripotency was used instead of *Fbx*[15].

Human iPSCs

Intense investigations in this area continued and resulted in yet another mile stone for iPSCs research when later in 2007, Thomson and Yu at the University of Wisconsin-Madison and Yamanaka and colleagues at Kyoto University independently reported the successful creation of iPSCs in adult human cells[17,18]. Based on their earlier work in a mouse model, Yamanaka and colleagues refined their approach and successfully used the same four genes with a retroviral system. Thomson and Yu revealed the use of two alternative factors, *NANOG* and *LIN28* to facilitate the reprogramming process using a lentiviral system. These human ES cell-like cells also expressed markers of ESCs and were capable of differentiating into cell types of all three germ layers[17].

Properties and Characterisation of iPSCs

Despite the fact that various methods have been successfully applied to derive iPSCs from different cellular sources (Table II), the identity and characteristics of the established iPSCs are very much similar to the naturally-isolated pluripotent stem cells, such as ESCs. Here we will discuss the similarities of iPSCs in comparison to ESCs following four main aspects; cellular morphology, genetic profiles, epigenetic status and differentiation potential (pluripotency).

Cellular Morphology

Regardless of the method of choice and the number of reprogramming genes involved in generating the human and mouse iPSC lines, most of them exhibit similar morphology as classic ESCs. They mostly form tightly packed and flat colonies with high nucleo-cytoplasmic ratios, defined borders and prominent nucleoli. In addition, iPSCs established from dermal fibroblasts or bone marrow-derived mesenchymal cells from patients with specific disease such as Down syndrome, Duchenne muscular dystrophy, Parkinson disease and type 1 diabetes mellitus also demonstrated similar cell morphology[19]. Morphology of iPSCs, derived from mouse embryonic fibroblast with four reprogramming genes, and ESCs are also indistinguishable at ultrastructural level when viewed under electron microscopy, showing the similarities between these two populations of pluripotent cells[20].

Genetic Profiles

The established human and mouse iPS cell lines also demonstrate a stable genomic integrity, which is a crucial aspect in generating a high-quality iPSCs. Genomic stability is critically important for any therapeutic application as genomic modifications may result in development of certain disease. A number of studies have revealed the stable karyotype for mouse[15] as well as human[17,18] even though some abnormalities were also detected in a few lines[21]. Nonetheless, majority of the lines demonstrate a normal karyotype; 40 and 46 chromosomes for mouse and human, respectively. However, recent study conducted by a group of

Table I: Reprogramming Strategies used to induce the reprogramming of adult somatic cells into pluripotent stem cells. A review on these strategies has been recently published[71].

Strategies		Type
DNA Methods	Viral delivery system	Adenovirus[19,54]
		Retrovirus[15,18,27,55]
		Lentivirus[23,56]
	Conditional system and transposon	Cre-loxP recombination, piggyback transposon[57,58]
	Non-viral system	Episomal plasmids[59]
Non-DNA methods	Protein mediated	Delivery of the reprogramming proteins directly onto the cells[26]
		Wnt3a conditioned medium[60]
	Manipulation of cell culture conditions	Lower amount of oxygen[61]
	Histone deacetylase (HDAC) inhibitor	Valproic acid (VPA) increases reprogramming[55]

Table II: Sources of Adult Somatic Cells for generation of iPSCs

Sources	Authors
Fibroblasts	17,20
Stomach cells	62
Liver cells	23,62
Neural progenitor cells	63,64
Lymphocytes	63
B- cells	65
Keratinocytes	22
Human blood	66,67
Human cord blood	68,69
Human adipose tissues	70

researchers in Spain revealed that prolonged culture of human iPSCs has repeatedly resulted in chromosomal abnormalities[22]. Similar to human ESCs, culturing of human iPSCs needs to be carefully monitored for induction of genetic abnormalities in culture condition.

Another important similarity of iPSCs to ESCs is reactivation of mouse telomerase reverse transcriptase (Tert) and also telomerase activity[18,23,24]. These studies showed that iPSCs can maintain telomere length which clearly indicate the ability of these cells, as ESCs, to undergo unlimited cell proliferation *in vitro*. Population doubling time of human iPSCs[18] were similar to the reported doubling time of human ESCs[25]. Single cell survival assay conducted by Zhou and colleagues also revealed that mouse protein iPSCs also can be clonally expanded demonstrating that, similar to ESCs, these cells are clonogernic[26].

Most studies also revealed that iPSC lines express most key marker genes for ESCs regardless of the method of choice or the number of reprogramming genes involved. Using RT-PCR and immnofluorescence techniques, most groups show that the established iPSCs express stage-specific embryonic antigen (SSEA-1 for mouse, SSEA-3 and -4 for human), tumour-related antigen (TRA)-1-60, TRA-1-81, and alkaline phosphatase (AP). In addition, the cell lines also express many markers for undifferentiated ESCs including Oct4, Sox2, Nanog, growth and differentiation factor 3 (GDF3), reduced expression 1 (Rex1), embryonic cell specific gene 1 (ESG1) and telomerase reverse transcriptase (TERT)[18,27,28,23,24].

Gene expression arrays have been applied by several groups to analyze the global gene expression patterns in iPSCs in comparison to different cell populations including ESCs and varieties of somatic cells[12,15,16,29]. DNA microarrays analyses demonstrated that both mouse and human iPS cell lines have similar, but not identical, global gene-expression patterns to their respective ESCs[21]. Zhou and colleagues[26] also observed that the expression in protein-iPSCs was similar to mouse ESCs. Microarray analysis conducted by Takahashi *et al.*[18] revealed only 1,267 genes out of 32,266 genes analyzed were detected with >5 fold difference between human iPSCs and human ESCs. In another study, Lowry and co-researchers found that the expression of pluripotency markers, OCT4, SOX2 and REX1 was two-fold lower in human iPSCs than that in human ESCs[30]. However, there are possibilities that these variations might be due to differences in culture, as observed in human ES cell-culture[27], rather than due to incomplete reprogramming[21]. Recent studies in 2010 also emphasized on the need to have proper assessments on genetic stability and the use of efficient culture conditions that enhance the genetic stability of the established iPSC and ESC lines. Some degree of genetic instability have been discovered both in iPSCs and human ESCs[31,32] .

Epigenetic status

It is known that there is a huge difference between the epigenetic status of a somatic cell and pluripotent stem cell. Thus, determination of the epigenetic status in iPSCs would offer the meaningful approach to assess the completion of the reprogramming and the epigenetic modifications, which have the essential role in controlling the activity of genes and regulatory elements in the genome. One aspect of the epigenetic modifications is DNA methylation which plays crucial role in gene regulation. Promoters of pluripotent gene markers are demethylated in ESCs. Several groups have used bisulfite genomic sequencing analyses to evaluate the methylation statuses of cytosine guanine dinucleotides (CpG) in the promoter regions of the key markers for pluripotency. Takahashi and colleagues discovered that the CpG in the

promoter regions of OCT4, NANOG and REX1 were highly demethylated indicating the activity of these promoters in human iPSCs. The CpG in the promoters were highly methylated in human dermal fibroblast[18]. In another study, Zhou *et al.* [26] also found that the promoters in *Oct4* and *Nanog* were demethylated in mouse protein-iPSCs as they were in mouse ESCs, while the promoters were hypermethyated in mouse embryonic fibroblast (MEFs). These studies, therefore, show the reactivation of the pluripotency transcription programmme in the iPSC lines.

Another aspect of epigenetic modification involves histone modification status. Several groups have used chromatin-immunoprecipitation (ChIP) or followed by hybridization to microarrays (ChIP-Chip) to analyze the histone methylation status at histone H3 lysine 27 trimethylation (H3K27me3) and histone H3 lysine 4 trimethylation (H3K4me3). They found that H3K4me3 was methylated while H3K27me3 was demethylated in the promoter regions of Oct4, Sox2 and Nanog in iPSCs[12,16,18,29]. These results show that the epigenetic status in iPSCs is similar to that of ESCs[33].

Differentiation potential

An important characteristic of pluripotent stem cells is their ability to differentiate into the cells/tissues representative of the three primary germ layers, the ectoderm, endoderm and mesoderm. In general, both human and mouse iPSCs are subjected to differentiate through the formation of three dimensional aggregates called embryoid bodies (EBs)[18,26]. Attached EBs have been shown to develop into variety of cells with morphology close to neuronal cells, cobblestone-like cells and epithelial cells. Immunocytochemistry revealed the cells to be positive for β-tubulin class III (a marker for ectoderm), glial fibrillary acidic protein (GFAP, ectoderm), α-smooth muscle actin (α-SMA, mesoderm) desmin (mesoderm), α-fetoprotein (AFP, endoderm) and vimentin (mesoderm). Once differentiated, the expression of pluripotency-associated markers decreased, indicating that the iPSCs are able to differentiate into the three primary germ layers.

In vitro differentiation and teratoma formation have been widely used to assess the developmental potential of iPSCs, especially the mouse iPSCs. Several groups have observed that mouse iPSCs have a developmental potential close to that of ESCs. Histological analysis also showed that the iPSCs were able to develop teratoma consisting of the derivatives of the three primary germ layers[12,15,16,26,29].

Induced pluripotent stem cells have been successfully differentiated into a number of different differentiated cell types[34] including neurons[35], haematopoietic and endothelial cells[36], pancreatic-insulin producing cells[37], hepatocyte-like cells[38] and retinal cells[39]. In addition, iPSCs also have been shown to differentiate into functional cells. Zhang *et al.*[40] have managed to differentiate human iPSCs into beating cardiomyocytes and multiple cell types including nodal, atrial and ventricular cardiomyocytes. Another group has successfully differentiated human iPSCs into functional cardiomyocytes that expressed cardiac markers such as Nkx 2.5, GATA4 and atrial natriuretic peptide[41].

In recent studies, three independent groups have successfully generated viable adult mice from mouse iPSCs through tetraploid complementation assays[42,43,44] demonstrating the full developmental potential of mouse iPSCs. These studies demonstrate the potential use of iPSCs in therapeutic application without the controversial issue of ESCs.

Applications, Challenges & Future Directions of IPSCs

The explosive interest in iPSCs has generated numerous technological advancements and with it greater understanding of these cells as described in previous subsections. In this subsection, we discuss the application of iPSCs as well as issues that need to be addressed before the full potential of iPSCs can be tapped into. iPSCs is revolutionizing regenerative medicine through the facile derivation of patient-specific stem cells for research or clinical purposes. They appear to be key for the materialization of personalized medicine by allowing a patient's own cells to become the source of therapeutic tissues. Key areas that have immense iPSCs application are in pharmaco-toxicological screening, disease modeling and autologous cell transplantation.

Pharmaco-toxicological screening

In vitro pharmaco-toxicological evaluations are imperative to determine the efficacy of the drug/compound as well as the safety of consumer and patients, while reducing the number of tests on vertebrates. Tests based on human cells ameliorate interspecies variations and as such predict more precisely adverse effects on the human body. However, the ethical issues of the derivation of human ESCs remain controversial. iPSCs point to a way out of this dilemma, since these cells have apparently very similar characteristics as ESCs and could serve as a basis for the development of toxicity tests. In addition, in an era where we are moving towards tailor-made management, disease and patient specific iPSC seems to be an ideal model for drug and toxicity screening[45].

The ability to mimic the pathophysiological characteristics of the disease, promises a cell-based platform that will accelerate the validation of novel small molecules and drug candidates. The identification of population-specific copy number variations (CNVs) and single-nucleotide polymorphisms (SNPs), thus make the utility of specific-iPSCs becomes immense[46]. In particular, iPSCs from individuals representing highly polymorphic variants in metabolic genes and different ethnic groups will provide pharmaceutical development and toxicology studies a unique opportunity to revolutionise predictive drug toxicology assays.

Disease modelling

The isolation of human iPSCs offers a new strategy for modelling human disease, especially in rare and poorly characterised diseases as well as better characterisation of more common diseases. For instance in familial dysautonomia (FD), a fatal peripheral neuropathy disorder, the derivation of patient-specific FD-iPSCs and the subsequent directed differentiation highlighted tissue-specific mis-splicing of a specific gene (IKBKAP). The mis-splicing resulted in transcriptomic dysregulation and marked defects in neurogenic differentiation and migration phenotype. In addition to the directed differentiation into cells of all three germ layers, these FD-IPSCs were also used for the assessment of candidate drugs' potency in reversing these defects[47].

iPSC-derived hepatic endoderm cells exhibited hepatic

morphological and active functional physiological properties, thus generating an efficient model for the dissection of human liver disease[48]. The development of motor neuron cell lines from patients with Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disorder not only allows for potential autologous cell transplantation but also increased understanding of ALS pathogenesis[49]. iPSCs also provide a new vaccination avenue. Whereby, iPSCs derived from patient's somatic cells were promoted to an immune cell fate and subsequently *in vitro* antigen presenting and processing to produce memory B cells that secrete functional antibodies to different pathogens. These cells were then transplanted back into the patient[50].

Potential Clinical Applications

Induced pluripotent stem cells have already been differentiated into various functional cell types[42,48,51,52]. The correction of defects in humanised Fanconi anaemia mice through the combination of gene targeting and direct reprogramming has been demonstrated[51]. Most pertinent was the ability of these disease-specific iPSCs to give rise to haematopoietic progenitors of the myeloid and erythroid lineages that were phenotypically normal. Thus, the iPSCs were able to rescue the disease phenotype when transplanted into the donor. Correspondingly, iPSCs-derived dopaminergic neurons were able to alleviate Parkinson's phenotype upon transplantation in adult rat brains[52]. In this instance, committed neural cells were separated from contaminating pluripotent cells to minimise the risk of tumour formation from the transplanted cells.

Challenges & Future Directions

Many proof-of-concept experiments provide insights to how iPSCs can be used for the generation of disease-corrected, patient-specific cells with a massive potential for cell therapy applications. Nonetheless, there are multiple concerns that need to be arrested before iPSCs broader clinical applications can be extended. Amongst these concerns are the consistent and efficient routine generation of human iPSCs without DNA integration into patient's genome, inferior efficacy of human iPSCs manipulation relative to murine derivatives, biased generation of certain and not all desired cell types through directed differentiation of iPSCs, the heterogeneity of iPSC-derived cells and lack of routine high-throughput comprehensive characterisation of human iPSC and iPSC-derived cells for quality control purposes. The assessment of relative potency of human iPSCs to ESCs is hampered by the inability to use some of the gold-standard assays of pluripotency used for mouse equivalents such as chimerism, germline transmission.

In addition, the characterisation of the molecular divergence between iPSCs and ESCs has been limited at best. Although ESCs and iPSCs share similar global gene expression profiles, iPSCs have a unique recurrent gene expression signature regardless of origin or the method by which they were generated. The unique gene expression signature extends to miRNA expression and has been attributed to differential promoter binding by the reprogramming factors. Nevertheless, it is reassuring that these iPSCs do not harbour any specific chromosomal microaberrations and that genomic instability is not a major concern[53].

Whilst mutation by viral integration poses a considerable technical limitation, recent advancements in non-viral strategies may provide an answer to this obstacle. In drug screening, a better understanding of the quality and homogeneity of the iPSC and iPSC-derived cell types are paramount. For instance, when investigating active compounds that could improve specific phenotypes, it is important to be assured that the disease-specific iPSCs are differentiated into the specific affected cell type. In addition, it is imperative to differentiate the actual disease phenotype that the screen is targeting from reprogramming and culture induced phenotypes. The completion of a comprehensive high-resolution genotype-phenotype map will accelerate human iPSCs utility in the production of new disease models and drug development, consequently revolutionizing regenerative medicine.

CONCLUDING REMARKS

The derivation of patient-specific cell lines from individuals with disease causing sporadic and inherited genetic defects has the potential to serve as a human cellular model. By replicating key molecular aspects, iPSCs will lead to a better understanding of the pathogenesis. The iPSCs and the derivate cell lines can be potentially used for high-throughput drug and toxicity screening. Ultimately, once the technical, safety, and regulatory concerns related to clinical applications of iPSCs have been appropriately addressed, new avenues for tailor-made stem cell-based restorative therapies for a myriad of diseases will materialise. In light of the population-specific CNV and SNP variations, Malaysia should join the bandwagon in an effort to create patient and disease-specific iPSCs especially towards diseases and genotypic variations that are more prevalent in this part of the world.

REFERENCES

1. Lensch M, Daheron L, Schlaeger T. Pluripotent stem cells and their niches. Stem Cell Rev 2006; 2(3): 185-201.
2. Guidelines for stem cell research and therapy, M.o.H.M. Medical Development Division, Editor. 2009: Kuala Lumpur. p. 70.
3. Martin M, Muotri A, Gage F, Varki A. Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat Med 2005; 11(2): 228-32.
4. Food and Drug Administration: PHS Guideline on Infectious Disease Issues in Xenotransplantation. Washington DC, Department of Health and Human Services. 2001; Available from: http://www.fda.gov/cber/gdlns/xenophs0101.htm.
5. Jensen J, Hyllner J, Björquist P. Human embryonic stem cell technologies and drug discoveries. J Cell Physiol 2009; 219(3): 513-9.
6. Fong CY, Gauthaman K, Bongso A. Teratomas from pluripotent stem cells: A clinical hurdle. J Cell Biochem 2010; 111(4): 769-81.
7. Nakajima F, Tokunaga K, Nakatsuji N. Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the Japanese population for use in cell transplantation therapy. Stem Cells. 2007; 25(4): 983-5.
8. Condic ML, Rao M. Regulatory issues for personalized pluripotent cells. Stem Cells 2008; 26(11): 2753-8.
9. Osafune K, Caron L, Borowiak M, *et al*. Marked differences in differentiation propensity among human embryonic stem cell lines. Nat Biotechnol 2008; 26(3): 313-5.
10. Wakayama T, Tabar V, Rodriguez I, Perry A, Studer L, Mombaerts P. Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. Science 2001; 292(5517): 740-3.
11. Brambrink T, Hochedlinger K, Bell G, Jaenisch R. ES cells derived from cloned and fertilized blastocysts are transcriptionally and functionally indistinguishable. Proc Natl Acad Sci U S A 2006; 103(4): 933-8.
12. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4): 663-76.
13. Marchetto M, Yeo G, Kainohana O, Marsala M, Gage F, Muotri A.

Transcriptional signature and memory retention of human-induced pluripotent stem cells. PLoS ONE 2009; 4(9): e7076.
14. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448(7151): 313-7.
15. Wernig M, Meissner A, Foreman R, *et al*. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 2007; 448(7151): 318-24.
16. Maherali N, Srihadran R, Xie W, *et al*. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell 2007; 1(1): 55-70.
17. Yu J, Vodyanik M, Smuga-Otto K, *et al*. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007; 318(5858): 1917-20.
18. Takahashi K, Tanabe K, Ohnuki M, *et al*. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131(5): 861-72.
19. Park I, Arora N, Huo H, *et al*. Disease-specific induced pluripotent stem cells. Cell 2008; 134(5) : 877-86.
20. Zeuschner D, Mildner K, Zaehres H, Schöler H. Induced Pluripotent Stem Cells at Nanoscale. Stem Cells Dev 2010; 19(5): 615-20.
21. Amabile G, Meissner A. Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol Med 2009; 15(2): 59-68.
22. Aasen T, Raya A, Barrero M, *et al*. Efficient and rapid generation of induced pluripotent stem cells from human keratinocyte. Nat Biotechnol 2008; 26(11): 1276-84.
23. Stadtfeld M, Brennand K, Hochedlinger K. Reprogramming of pancreatic beta cells into induced pluripotent stem cells. Curr Biol 2008; 18(12): 890-4.
24. Park I, Lerou P, Zhao R, Huo H, Daley G. Generation of human-induced pluripotent stem cells. Nat Protoc 2008; 3(7): 1180-6.
25. Cowan C, Klimanskaya I, McMahon J, *et al*. Derivation of embryonic stem-cell lines from human blastocysts. N Engl J Med 2004; 350(13): 1353-56.
26. Zhou H, Wu S, Joo J, *et al*. Generation of induced pluripotent stem cells using recombinant proteins. Cell Stem Cell 2009; 4(5): 381-4.
27. Nakagawa M, Koyanagi M, Tanabe K, *et al*. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 2008; 26(1): 101-6.
28. 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(11): 1269-75.
29. Mikkelsen T, Hanna J, Zhang X, *et al*. Dissection direct reprogramming through integrative genomix analysis. Nature 2008; 454(7200): 49-55.
30. Lowry W, Richter L, Yachechko R, *et al*. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 2008; 105: 2883-88.
31. Mayshar Y, Ben-David U, Lavon N, *et al*. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. Cell Stem Cell. 2010;7(4) : 521-31.
32. Laurent L, Wong E, Li G, *et al*. Dynamic changes in the human methylome during differentiation. Genome Res. 2010; 20(3): 320-31.
33. Pan G, Tian S, Nie J, *et al*. Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cell. Cell Stem Cell 2007; 1(3): 299-312.
34. Das AK, Pal R. Induced pluripotent stem cells (iPSCs): the emergence of a new champion in stem cell technology-driven biomedical applications. J Tissue Eng Regen Med 2010; 4(6): 413-21.
35. Chambers S, Fasano C, Papapertrou E, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPSCs by dual inhibition of SMAD signaling. Nat Biotechnol 2004; 27(3): 275-80.
36. Choi K, Yu J, Smuga-Otto K, *et al*. Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. Stem Cells 2009; 27(3): 559-67.
37. Zhang D, Jiang W, Liu M, *et al*. Highly efficient differentiation of human ES cells and iPSCs into mature pancreatic indulin-producing cells. Cell Res 2009; 19(4): 429-38.
38. Song Z, Cai J, Liu Y, *et al*. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Res 2009; 19(11): 1233-42.
39. Meyer J, Shearer R, Capowski E, *et al*. Modeling early retinal development with human embryonic and induced pluripotent stem cells. Proc Natl Acad Sci U S A 2009; 106(39): 16698-703.
40. Zhang J, Wilson G, Soerens A, *et al*. Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 2009; 104(4): e30-41.
41. Tanaka T, Tohyama S, Murata M, *et al*. *In vitro* pharmacologic testing using human induced pluripotent stem cell-derived cardiomyocytes. Biochem Biophys Res Commun Cell 2007; 385(4): 495-502.
42. Boland M, Hazen J, Nazor K, *et al*. Adult mice generated from induced pluripotent stem cells. Nature 2009; 461(7260): 91-4.
43. Kang L, Wang J, Zhang Y, Kou Z, Gao S. iPSCs can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell 2009; 5(2): 135-8.
44. Zhao X, Li W, Lu Z, *et al*. iPSCs produce viable mice through tetraploid complementation. Nature 2009; 461(7260): 86-90.
45. Chakraborty C, Shah KD, Cao WG, Hsu CH, Wen ZH, Lin CS. Potentialities of induced pluripotent stem (iPS) cells for treatment of diseases. Curr Mol Med 2010; 10(8): 756-62.
46. Redon R, Ishikawa S, Fitch K, *et al*. Global variation in copy number in the human genome. Nature 2006; 444(7118): 444-54.
47. Lee G, Papapetrou E, Kim H, *et al*. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature 2009; 461(7262): 402-6.
48. Sullivan G, Hay D, Park I, *et al*. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology 2010; 51(1): 329-35.
49. Papadeas S, Maragakis N. Advances in stem cell research for Amyotrophic Lateral Sclerosis. Curr Opin Biotechnol 2009; 20(5): 545-51.
50. Li J, Gao G, Yuan T. Cell based vaccination using transplantation of iPSC-derived memory B cells. Vaccine 2009; 27(42): 5728-9.
51. Hanna J, Wernig M, Markoulaki S, *et al*. Treatment of sickle cell anemia mouse model with iPSCs generated from autologous skin. Science 2007; 318(5858): 1920-3.
52. Wernig M, Zhao J, Pruszak J, *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; 105(15): 5856-61.
53. Chin M, Mason M, Xie W, *et al*. Induced pluripotent stem cells ad embryonic stem cells are distinguished by gene expression signatures. Cell Stem Cell 2009; 5(1): 111-23.
54. Zhou W, Freed C. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells 2009; 27(11): 2667-74.
55. 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(11): 1269-75.
56. Soldner F, Hockemeyer D, Beard C, *et al*. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009; 136(5): 964-77.
57. 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(7239): 771-5.
58. Woltjen K, Michael I, Mohseni P, *et al*. PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009; 458(7239): 766-70.
59. Yu J, Hu K, Smuga-Otto K, *et al*. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009; 324(5928): 797-801.
60. Marson A, Foreman R, Chevalier B, *et al*. Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell 2008; 3(2): 132-5.
61. Page R, Ambady S, Holmes W, *et al*. Induction of stem cell gene expression in adult human fibroblasts without transgenes. Cloning Stem Cells 2009; 11(3): 417-26.
62. Aoi T, Yae K, Nakagawa M, *et al*. Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 2008; 321(5889): 699-702.
63. Kim J, Zaehres H, Wu G, *et al*. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 2008; 454(7204): 646-50.
64. Eminli S, Utikal J, Arnold K, Jaenisch R, Hochedlinger K. Reprogramming of neural progenitor cells into induced pluripotent stem cells in the absence of exogenous Sox2 expression. Stem Cells 2008; 26(10): 2467-74.
65. Hanna J, Markoulaki S, Schorderet P, *et al*. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. Cell 2008; 133(2): 250-64.
66. Loh Y, Agarwal S, Park I, *et al*. Generation of induced pluripotent stem cells from human blood. Blood 2009; 113(22): 5476-9.
67. Ye Z, Zhan H, Mali P, *et al*. Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders. Blood 2009;114(27): 5473-80.
68. Haase A, Olmer R, Schwanke K, *et al*. Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 2009; 5(4): 434-41.
69. Giorgetti A, Montserrat N, Aasen T, *et al*. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. Cell Stem Cell 2009; 5(4): 353-7.
70. Sun N, Panetta N, Gupta D, *et al*. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc Natl Acad Sci U S A 2009; 106(37): 15720-5.
71. Lai MI, Ramasamy R, Wendy WY, Nordin N, Rosli R, Veerakumarasivam A and Abdullah S. Advancements in Reprogramming Strategies for the Generation of Induced Pluripotent Stem Cells. Journal of Assisted Reproduction and Genetics. Doi: 10.1007/s10815-11-9552-6.