

iPS and ES Cells: Do Both Roads Lead to Rome?

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Abstract: The seminal report from Takahashi and Yamanka in 2006 describing the reprogramming of somatic cells to induced pluripotent stem (iPS) cells [1] marked the beginning of a new field of research, resulting in hundreds of publications in a short 3 years. Among other things, the promise of iPS cells in cell therapy circumvents many of the ethical concerns associated with embryonic stem (ES) cell research, and autologous patient-specific cells can be generated. Nonetheless, the jury is still out on the extent to which iPS cells and ES cells are functionally equivalent. This review focuses on the genetic and functional comparisons between these two cells types.

Keywords: Embryonic stem cells, induced pluripotent stem cells, antibodies, differentiation, insulin, cardiomyocyte, motor neurons, dopaminergic neurons, hepatocytes, hematopoietic cells, retinal pigmented epithelium cells.

CHARACTERIZATION OF iPS CELLS

One of the challenges of iPS cell research is to carefully establish standards and minimum criteria for the identification and characterization of iPS cells. As the strategies for generating iPS cells evolve from the introduction of transcription factors *via* viral vector methods through to small molecules, and optimal sources of cells for reprogramming are identified, a consensus set of assays has to be agreed upon for relevant and meaningful comparative data to be generated. Otherwise, the consequence could be ambiguity arising from a myriad of irrelevant and disparate results generated from unstandardized cell lines and methods of characterization.

The criteria used to characterize iPS cells are very similar to that employed for ES cell research. For example, the seminal and subsequent papers reporting the generation of human iPS cells [2, 3], describe them as exhibiting many of the hallmark indicators of human ES cells [4, 5] (Table 1). Despite the similarities there are, however, differences between iPS cells and their ES cell counterparts.

Typically, assessment of gross morphology is used for initial isolation of iPS cells. Colonies of compact cells exhibiting a high nucleus to cytoplasmic ratio resembling human ES cell cultures will appear 3-4 weeks after reprogramming. In addition, iPS cells and ES cells have comparable growth rates and doubling times [2, 3], and express cell surface markers such as stage-specific embryonic antigen (SSEA)-3, SSEA-4, Tra-1-60, Tra-1-81; transcription factors OCT3/4 and NANOG. Moreover, iPS cells typically have elevated telomerase and alkaline phosphatase activities (Table 1). These phenotypes have been observed for iPS cells generated from healthy donors and patients with a variety of genetic diseases. The latter includes disorders with either Mendelian or complex inheritance such

as adenosine deaminase deficiency-related severe combined immunodeficiency (ADA-SCID), Shwachman-Bodian-Diamond syndrome (SBDS) and Gaucher disease (GD) type III [6].

Our group has raised and characterized a panel of monoclonal antibodies (mAbs) specific to cell surface markers on human ES cells [7]. We have recently compared the reactivity of our panel of mAbs to iPS cell lines (ES4SKIN and ESIMR90) [2], 21 day embryoid bodies (EB) derived from iPS cells, their respective parental fibroblast lines and undifferentiated human ES cells, and found that reactivity of all the mAbs towards iPS and human ES cells was similar (unpublished data). Furthermore, all but 2 of the antibodies had no reactivity to 21 day EB or the parental fibroblast lines. Uniquely, one of the clones from our panel of mAb, mAb 84, not only binds to podocalyxin (PODXL) on human ES cells, it also selectively kills undifferentiated human ES cells *via* pore formation, resulting in membrane damage and leading to oncotic cell death [8]. When tested on iPS cells, the efficiency of cell killing by mAb 84 was comparable for iPS cells and human ES cells (>90-95%) while absent for EB and the fibroblast lines (unpublished data).

At the genetic level, one crucial difference between iPS cells and human ES cells is the introduction/integration and expression of genes essential for the reprogramming process. ES cells are directly derived from the inner cell mass of the blastocyst and do not require additional perturbation of the genome. iPS cells by-and-large continue to maintain a normal karyotype (46 chromosomes) after prolonged culture (e.g. >7 months) [9]. It should however be noted that like human ES cells, continuous passaging of iPS cells as a single cell culture (e.g. ~13 passages [9]) can result in karyotype changes especially involving chromosomes 17 and 20. Thus, monitoring karyotype stability is necessary for extended culture of both iPS cells and human ES cells.

Clonality may be another important criterion for establishing iPS cell lines. After reprogramming, iPS cells generated under identical conditions are by no means

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Table 1. Characterization of iPS Cells and Human ES Cell

	Cell Types								
	iPS Cells						human ES cells		
	Yu, 2007 [2]	Takahashi, 2007 [3]	Park, 2008 [6]	Park, 2008 [17]	Aasen, 2008 [9]	Huangfu, 2008 [13]	Thomson, 1998 [5]	Reubinoff, 2000 [4]	Heins, 2006 [75]
Morphology									
High nucleus to cytoplasmic ratio	✓	✓	✓	✓	✓	✓	✓	✓	✓
Immunocytochemistry/FACS									
Surface markers									
SSEA-3	✓	✓	✓	✓	✓	-	✓	-	✓
SSEA-4	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tra-1-60	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tra-1-81	✓	✓	✓	✓	✓	✓	✓	-	✓
Transcription Factors									
Oct4	-	-	✓	✓	✓	✓	-	-	✓
Nanog	-	✓	✓	✓	✓	✓	-	-	-
Alkaline Phosphatase activity	✓	✓	✓	✓	✓	✓	✓	✓	✓
Telomerase activity	✓	✓	-	-	-	-	✓		✓
Microarray/qRT-PCR	✓	✓	-	✓	✓	✓	-	✓	-
Embryoid body formation	✓	✓	✓	✓	✓	✓	-	-	✓
Teratoma formation	✓	✓	✓	✓	✓	✓	✓	✓	✓
Normal karyotype	✓	✓	✓	✓	✓/✕	✓	✓	✓	✓

homogeneous. Cells are at different stages of reprogramming and risk integration and insertional mutagenesis, which render them refractory to differentiation with increased potential to form immature teratomas [10]. Masaki and colleagues observed that even alkaline phosphatase (ALP)-positive colonies can be categorized into 40 groups based on the gene expression pattern of 8 marker genes – Nanog, TDGF1, Dnmt3b, Zfp42, FoxD3, GDF3, CYP26A1 and Tert [11]. Screening resulted in the isolation of 3 iPS cell lines that were positive for all 8 marker genes (only 4 out of 162 ALP+ clones expressed the 8 marker genes ~0.02%). Importantly, these findings support the need to definitively isolate and characterize *bona fide* iPS cells while removing aberrantly reprogrammed cell populations. Unfortunately, clonal selection has to be performed against the background of poor survival of stem cells when passaged as single cells. To this end, Rho-associated kinase (ROCK) inhibitor could be used, with single cell survival previously shown to improve by approximately 8-9 fold at day 7 post-seeding [12].

Other comparisons between iPS and human ES cells include analysis of the methylation status for promoters of ES-cell specific genes, telomerase activity, gene-specific quantitative or semi-quantitative RT-PCR and genome-wide gene expression profiling. Methylation status of cytosine guanine dinucleotides (CpG) in the promoter regions of pluripotent genes such as OCT4, NANOG and REX1 on iPS cells, the parental fibroblasts and human ES cells were compared using bisulfite genomic sequencing [3, 13]. Consistently, the promoter regions of these genes were

predominantly unmethylated in iPS cells and human ES cells while they were highly methylated in the parental fibroblast lines [3, 13]. Another similarity between iPS cells and human ES cells is high telomerase activity. Interestingly, Marion and colleagues observed that after reprogramming, telomere elongation occurred as a consequence of increased telomerase activity [14]. At early passages, the telomere length is intermediate between the parental fibroblast and ES cells. At later passages, the telomere length is comparable between iPS cells and ES cells. In addition, the conformation of the telomere heterochromatin and a reduced density of histone heterochromatin marks (H3K9m3 and H4K20m3) were similar between the 2 cell types [14]. Similarly, using ChIP-chip technique, Maherali and colleagues found that promoter regions of >900 ESC signature genes in iPS cells were reset to the same epigenetic state (bivalent domains) as their ES cell counterparts [15].

The expression of ES cell specific genes such as OCT-4, NANOG, REX-1, KLF1, hTERT, GDF-3 has been analyzed using qRT-PCR. The expression of these genes is elevated in iPS cells compared to the parental fibroblast cells, and comparable to human ES cells [16, 17]. Several groups have utilized DNA microarrays to compare the global gene expression profile of iPS cells, the non-reprogrammed fibroblasts, and ES cells. Based on the gene set defined by the International Stem Cell Initiative [18], Masaki and colleagues found that 3 human iPS cell lines generated by them clustered with a panel of human ES cell lines but were separated from their parental fibroblast cells [11]. Similar results were also observed in separate studies by Yu and

Takahashi where iPS cells and human ES cell gene expression profiles were by-and-large similar [2, 3]. It is however interesting to note that there are variations between human ES and iPS cells; and also within different clones of iPS cells. Out of the 32266 transcripts compared by Takahashi *et al.*, ~4% (1267 genes) were either up- or down-regulated by >5-fold between iPS cells and ES cells [3]. Similarly, Chin *et al.* observed that there was a distinct gene expression signature between early and late passages of human iPS cells [19]. Early passage iPS cells clustered more closely with the parental fibroblast whilst late passage iPS cells resembled hESC. The authors suggested that at early passages, iPS cells are probably incompletely reprogrammed – either due to inefficient silencing of fibroblast-specific genes or incomplete induction of hESC-specific genes. However, when the cultures are extended, the majority of the differences between human ES cells and iPS cells are resolved [19]. This phenomenon is unique to iPS cells despite the route of reprogramming and this variability was not observed between early and late passages of human ESC.

Studies of *in vitro* and *in vivo* differentiation are useful for testing stem cell pluripotency. iPS cells have been differentiated *in vitro*, either spontaneously to embryoid bodies followed by real-time PCR analysis for expression of genes from the 3 germ layers or directed towards specific lineages [20]. Unlike mouse iPS cells where pluripotency can be demonstrated beyond doubt through the generation of germline chimeras, the equivalent experiment cannot be performed for human iPS cells. The best alternative is to test teratoma formation in SCID mice. Like human ES cells, iPS cells can form teratomas *in vivo* after reprogramming [2, 3]. However, it should be noted that teratoma formation does not guarantee pluripotency as there are instances of mouse ES-like cells that form teratomas but do not produce germline chimeras [10]. Ellis and colleagues suggest that perhaps a non-teratoma forming iPS cell line may be a “desirable” outcome as this removes the risk of residual *in vivo* tumorigenicity after transplantation of cells [21]. Nonetheless, iPS cells must retain the capacity to efficiently generate a desired cell/tissue type and hence a greater understanding of their behavior is necessary.

FUNCTIONAL APPLICATIONS OF iPS CELLS

A salient question about iPS cells is whether they can be differentiated into clinically relevant cell types. Given the similarity between human ES and iPS cells, the large body of work on lineage-specific differentiation of ES cells is informative for iPS cell differentiation (Table 2). Once determined, differentiated cells could theoretically be considered for autologous cell transplantation and tissue engineering, with information to be gleaned about *in vivo* development, disease progression and drug screening from *in vitro* modeling [22].

Neuronal Cells

Neurodegenerative diseases such as Parkinson’s disease result from the deterioration of neurons in the brain and spinal cord. The ability for endogenous neurons to regenerate is limited, with a loss of or damage to neurons having a devastating effect on the individual. Clearly the

derivation of functional and clinically-compliant neuronal cell types *in vitro* will be of great therapeutic benefit. Methods for differentiating human ES cells into neural precursors (NPs) is an area of intense research [23, 24], more recently extending to iPS cell differentiation. Comparative studies of ES and iPS neuronal derivatives are important to determine the latter’s regenerative potential and ultimately their use for modeling neurodevelopment and neurological disorders [25, 26] and as patient-specific immuno-compatible cells for transplantation therapy.

Motor neurons are required for the control of essential voluntary movements including breathing, swallowing, speaking, walking, and overall body movements. Degeneration of motor neurons results in diseases such as primary lateral sclerosis, amyotrophic lateral sclerosis (also known as Lou Gehring’s disease), and spinal muscular atrophy. Several groups using a variety of cell clones and lineage specific protocols have achieved the derivation of electrophysiologically active motor neurons from iPS cells [27-29]. Although the differentiation of neuronal subtypes from iPS and human ES cells appear to follow developmental cues in a similar fashion, the overall differentiation efficiency of some, but not all, iPS clones remain lower than that in human ES cells. These differences could be attributed to the clonal variations across cell lines [29].

The production of disease-relevant cell types from patient-specific stem cells will enable researchers to better study the etiology and progression of the disease *in vitro*, and successfully derive cells for autologous transplantation. To this end, human iPS cells from patients suffering from motor neuron diseases amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) have been generated [30, 31]. The iPS cells were then further differentiated to the relevant neuronal cell types, which importantly, still maintained the cognate mutations in the disease-causing gene. Since disease progression can be recapitulated in these patient-derived cells, these iPS cells can aid in the study of factors that contribute to the disease. In addition, disease-specific human iPS cells will be useful in drug screens for potential xenobio-tic candidates. Indeed, the SMA-iPS cells demonstrated the expected increase in survivin motor neuron 1 after treatment with valproic acid and tobramycin [31]. In a separate study, neural crest precursors from familial dysautonomia iPS clones were used to screen candidate drugs that can reverse the effects of the disease-causing I κ B kinase complex-associated protein and one candidate, kinetin, resulted in partial restoration of the disease phenotype [32].

The generation of dopamine neurons from a human iPS line and from skin biopsies of Parkinson’s patients have also been reported [33, 34]. Importantly, the authors were able to achieve viral-free reprogramming of the skin fibroblasts *via* Cre-recombinase excisable vectors, thus eliminating the risk of re-expression of the reprogramming factors [33]. Both dopaminergic neurons of mouse and human iPS origins can also be successfully transplanted into the 6-OHDA lesioned Parkinson’s rats and result in long term survival and functional rescue of the disease phenotype [34, 35]. However the authors cautioned that cell therapy may not represent an end-all solution as the cause of neural degeneration in

Table 2. Differentiated Cell Types Derived from Mouse iPS and Human iPS Cells

Differentiated Cells	Reference	Input	Disease Phenotype		Comparable to ESC		Remarks
			iPS Source	Correction	Efficiency	Function	
Motor Neurons	Dimos, 2008 [30]	Human skin fibroblasts	ALS	-	Yes	NR	
	Chambers, 2008 [28]	Human fetal lung fibroblasts	-	-	Yes	NR	
	Karumbayarm, 2009 [27]	Human dermal foreskin fibroblast	-	-	Yes ¹	Yes	¹ iPS cells had lower efficiency during neural specification, but not for motoneuron patterning
	Ebert, 2009 [31]	Human fibroblasts	Type I –SMA	-	NR	Yes	
	Hu, 2010 [29]	Human fibroblasts	-	-	Yes/No ²	Yes	² Some but not all iPS lines display lower differentiation efficiency
Dopaminergic Neurons	Chambers, 2008 [28]	Human fetal lung fibroblasts	-	-	Yes	NR	
	Wernig, 2008 [35]	Mouse embryonic fibroblasts	-	6-OHDA PD rats	NR	Yes	
	Soldner, 2009 [33]	Human skin biopsy	Parkinson's	-	Yes ³	NR	³ Reprogramming factor-free human iPSCs
	Cai, 2010 [34]	Human fibroblasts	-	6-OHDA PD rats	Yes	Yes	
Neural Crest Precursors	Lee, 2009 [32]	Human fibroblasts	Familial Dysautonomia	-	NR	Yes	
Cardiomyocytes	Sckenke-Layland, 2008 [39]	Mouse embryonic fibroblasts	-	-	Yes	Yes	
	Mauritz, 2008 [40]	Mouse embryonic fibroblasts	-	-	Yes/No ⁴	Yes	⁴ more beatings in mouse ES cell aggregates, but mouse iPS-and mouse ES cell-cardiomyocytes have comparable function
	Narazaki, 2008 [38]	Mouse fibroblasts	-	-	Yes	Yes	
	Zhang, 2008 [41]	Human fibroblasts	-	-	Yes ⁵	Yes	⁵ differences between clones
	Zwi, 2009 [42]	Human fibroblasts	-	-	Yes	Yes	

(Table 2) Contd.....

Differentiated Cells	Reference	Input	Disease Phenotype		Comparable to ESC		Remarks
			iPS Source	Correction	Efficiency	Function	
Smooth Muscle Cells	Skenke-Layland, 2008 [39]	Mouse embryonic fibroblasts	-	-	Yes	Yes	
	Xie, 2009 [44]	Mouse tail-tip fibroblasts	-	-	Yes/No ⁶	Yes	⁶ one iPS line did not survive differentiation
	Lee, 2009 [45]	Human aortic vascular smooth muscle cells	-	-	No	NR ⁷	⁷ iPS-smooth muscle cells were functionally similar to human aortic smooth muscle cells
	Lian, 2010 [46]	Human fibroblasts ⁸	-	Mouse hind limb ischemia	NR	NR	⁸ Smooth muscle cells were derived from iPS-mesenchymal stem cells
Endothelial Cells	Skenke-Layland, 2008 [39]	Mouse embryonic fibroblasts	-	-	Yes	Yes	
	Narazaki, 2008 [38]	Mouse fibroblasts	-	-	Yes	NR	
	Xu, 2009 [60]	Mouse tail-tip fibroblasts	-	Hemophilia	NR	Yes	
	Choi, 2009 [55]	Human fibroblasts	-	-	Yes	NR	
	Taura, 2009 [76]	Human fibroblasts	-	-	Yes	NR	
	Lian, 2010 [46]	Human fibroblasts ⁹	-	Mouse hind limb ischemia	NR	NR	⁹ Endothelial cells were derived from iPS-mesenchymal stem cells
Insulin-Producing Cells	Zhang, 2009 [49]	Human fibroblasts	-	-	NR	Yes	
	Tateishi, 2008 [48]	Human foreskin fibroblasts	-	-	Yes	Yes/No ¹⁰	¹⁰ one iPSC-derived cells had decreased response to glucose stimulation
Hepatocytes	Li, 2009 [53]	Mouse embryonic fibroblasts	-	-	Yes	Yes	
	Gai, 2010 [52]	Mouse tail tip fibroblasts	-	-	NR	NR	
	Hanna, 2007 [69]	Mouse tail-tip fibroblasts	Sickle Cell Anemia	Sickle Cell Anemia	NR	-	
	Skenke-Layland, 2008 [39]	Mouse embryonic fibroblasts	-	-	Yes	NR	
	Raya, 2009 [70]	Human dermal fibroblasts	Fanconi Anemia	-	Yes	-	
	Choi, 2009 [54, 55]	Human fibroblasts	-	-	Yes	NR	
	Ye, 2009 [58]	Human CD34+ cells	Myeloproliferative disorders	-	NR	NR	
Dendritic Cells	Feng, 2010 [56]	Human fibroblasts	-	-	No	No	
	Senju, 2009 [57]	Mouse embryonic fibroblasts	-	-	NR	Yes	

(Table 2) Contd.....

Differentiated Cells	Reference	Input	Disease Phenotype		Comparable to ESC		Remarks
			iPS Source	Correction	Efficiency	Function	
Retinal Cells	Osakada, 2009 [63]	Human fibroblasts	-	-	Yes/No ¹¹	NR	¹¹ One iPS line could not generate pigment cells
	Meyer, 2009 [65]	Human fibroblasts	-	-	No	NR	
	Buchholz, 2009 [61]	Human fibroblasts	-	-	Yes ¹²	Yes ¹²	¹² Efficiency and function also similar to fetal retinal cells
	Hirami, 2009 [62]	Mouse and human fibroblasts	-	-	Yes/No ¹³	NR	¹³ One human iPS line did not differentiate efficiently; mouse iPS cells had lower differentiation efficiency compared with mouse ES cells
	Feng, 2010 [56]	Human fibroblasts	-	-	No	No	
	Parameswar, 2010 [64]	Mouse fibroblasts	-	-	NR	NR	
Adipocytes	Taura, 2009 [68]	Human skin fibroblasts	-	-	Yes	Yes	

NR denotes Not Reported.

Parkinson's is still unknown, thus the transplanted cells may still be subject to the same genetic and/or disease-causing factors.

Cardiovascular Cell Types

Myocardial infarction is an ischemic event where blood perfusion through the heart is unable to keep up with the demand, eventually leading to the loss of cardiomyocytes [36]. As mature cardiomyocytes are terminally differentiated cells, they have limited regenerative potential, thus there is a need to repair cardiomyocytes or supply them from an external source [37]. iPS cells are an attractive source of cardiomyocytes since autologous derivation of syngeneic cardiomyocytes ensures compatibility with the host immune system. The ability of iPS-cardiomyocytes to incorporate into the heart and become integrated into the natural electrical impulses is among other challenges that need to be addressed before ES-derived cardiomyocytes can reach the clinic.

Several groups have reported the successful differentiation of functional cardiomyocytes from mouse ES cells and iPS cells. Using the early endothelial marker Flk-1 to enrich for cardiac cells followed by a systematic differentiation protocol, cardiomyocytes, arterial, venous, smooth muscle, and lymphatic endothelial cells have been generated [38, 39]. Cardiac cells could also be differentiated by forming mouse iPS cell aggregates. However, mouse ES cells-derived aggregates exhibited a larger percentage of contracting areas when compared to mouse iPS aggregates, whose peak percentage of contracting areas was also delayed [37]. In general, efficiency and lineage marker expression of mouse iPS cell- and ES cell cardiac derivatives were not statistically different regardless of the differentiation method. More importantly, extensive physiological characterizations of mouse iPS cell- and ES cell-cardiomyocytes showed that they were phenotypically indistinguishable in

terms of beating rates and the ability to generate Ca^{2+} transients and membrane potentials. Furthermore, the mouse iPS and ES cell-smooth muscle cells displayed similar contraction patterns after carbachol treatment and comparable uptake of acetylated low-density lipoprotein [38-40]. Interestingly, Mauritz *et al.* reported that the level of pluripotent markers NANOG and OCT3/4 were downregulated more slowly in mouse iPS cells during differentiation, which possibly contributed to the later appearance of the mesoderm-like phenotype in these cells [40].

Cardiomyocytes derived from human ES and iPS cells have been shown to be similar and also functionally variable within clones of each cell type [41, 42]. Even within the same study where two ES cell and two iPS cell lines were compared side-by-side, one ES cell line displayed higher beating percentage over the other ES cell and iPS cell lines. However, all lines expressed similar levels of cardiac-specific genes, comparable percentage of nodal, atrial, and ventricular-like action potentials, action potential durations, amplitudes, diastolic potentials, and functional β -adrenergic signaling [41, 42]. Therefore, cardiomyocytes derived from either human ES or iPS cells may be functionally similar, and variations could be attributed to the intrinsic heterogeneity between cell lines. As reported in mouse iPS cells, pluripotency markers OCT4 and NANOG were not down regulated in iPS cells as rapidly as in ES cells, possibly due to the persistent expression of these transgenes during differentiation [41].

Smooth muscle cells are involved in the maintenance of vascular tone, thus the balance of a heterogeneous population of smooth muscle cells is important for the control of blood pressure and distribution of blood flow [43]. Part of the pathogenesis of cardiovascular diseases involves the dysregulation of differentiation and dedifferentiation of smooth muscle cells, and models to study this process *in vitro* are lacking. Smooth muscle cells have been obtained

from both mouse and human iPS sources. In the report by Xie *et al.* the authors compared smooth muscle cells derived from two mouse iPS cell lines and two mouse ES cell lines [44]. One mouse iPS cell line did not survive the differentiation, but the remaining iPS and ES cell lines differentiated with equal efficiency and were determined to be functionally responsive to carbachol and caffeine treatment. In a slightly different approach, Lee and colleagues reprogrammed human aortic smooth muscle cells to iPS cells, then re-differentiated them to smooth muscle cells [45]. Both of the human iPS-smooth muscle cells and human ES-smooth muscle cells highly expressed the smooth muscle markers α and γ -smooth muscle actin. A functional demonstration of iPS-derived vascular cells was carried out where human iPS-derived mesenchymal stem cells induced vascular and muscle regeneration, leading to the attenuation of limb ischemia in mice [46]. In fact, iPS-mesenchymal stem cells stained significantly higher for smooth muscle and endothelial cells, and had prolonged engraftment survival as compared to bone marrow-derived mesenchymal stem cells, further demonstrating the *in vivo* utility of iPS-derived cells in tissue repair [46].

Insulin-Secreting Cells

One treatment for diabetes is to transplant insulin-secreting pancreatic β -cells into patients [47]. However, since the supply of donor pancreas is often limiting, there is a need for other means of generating insulin-secreting cells. There is much ongoing research in the derivation of pancreatic β -cells from ES cells, with a few recent studies focusing on iPS cells. Stepwise differentiation protocols have been used to generate insulin-secreting cells [48, 49]. In the study by Teteishi *et al.*, two human iPS cell clones and one human ES cell line were subject to differentiation and both showed similar gene and protein expression of appropriate stage-specific markers. C-peptide and glucagon were also detectable in cells derived from human iPS cells and human ES cells; however when stimulated with high glucose, one human iPS clone responded poorly with regards to insulin secretion, while the other human iPS clone secreted a comparable amount of insulin as the human ES cell-derived clone [48]. It is also important to note that although human ES cell- and human iPS cell-derived pancreatic cells are capable of insulin secretion upon glucose stimulation, they still are unable to achieve levels comparable to that of adult human β -cells, thus more efficient differentiation protocols still need to be established.

Hepatocytes

Patients suffering from end stage-liver disease have to await transplantation as the adult liver is unable to replace the loss in liver mass by regenerating sufficient hepatocytes. Primary hepatocytes are a potential source for transplantation but the demand for them greatly exceeds the supply. In addition, primary hepatocytes have limited proliferative potential and sufficient amounts are difficult to generate. Therefore, the use of stem cells as a source of hepatocytes is a promising option [50, 51]. Murine hepatocytes expressing mature hepatic markers such as albumin and alpha-fetoprotein have been derived from mouse iPS and ES cells with comparable efficiency [52, 53]. In addition, these

mouse iPS-hepatocytes exhibit relevant functional characteristics of liver cells including cytoplasmic glycogen storage, LDL uptake, and urea production. Moreover, the iPS-derived liver cells could be successfully grafted into mouse recipient livers [52].

Hematopoietic Cells

Multipotent hematopoietic stem cells can give rise to cells of the myeloid and lymphoid lineages that play important roles in the immune system. The ability to study human variants of these cells has been limited by the availability of bone marrow cells, from which the hematopoietic stem cells originate. The induction of mature hematopoietic cells from mouse and human iPS cells bearing similar marker expression and morphology as those derived from their ES counterparts has been achieved by separate groups. However, these studies differ in their assessment of the functional characteristics of the resulting differentiated cells [54-57]. In particular, the study by Feng and colleagues saw a significant reduction in the survival and expansion of the hemangioblast (a bipotent progenitor population), endothelial, and retinal pigmented epithelium cell population when compared with human ES cell-derivatives [56]. The differences in differentiation potential of iPS and ES cells highlight the need for more stringent evaluation criteria for iPS cells.

Patient-specific hematopoietic cells have also been derived from reprogrammed peripheral blood CD34+ cells that present with myeloproliferative disorders [58]. The iPS-hematopoietic progenitors recapitulated the disease phenotype of increased erythropoiesis and offer the potential to further investigate myeloproliferative disorder pathogenesis.

Hemophilia A is a monogenic coagulation disorder caused by deficiencies of Factor VIII and is also a candidate target for gene therapy [59]. Although gene therapy approaches have been used to correct the defect, it has been met with limited success mainly due to the response from host immune cells and the insufficient production of a sustained amount of the gene product. By injecting mouse iPS cells that were differentiated to progenitor and mature endothelial cells into an irradiated mouse model of hemophilia, Xu *et al.* demonstrated a correction of the disease phenotype [60]. The mature endothelial cells expressed characteristic markers of the endothelial lineage and importantly, also expressed Factor VIII. When the injected mice were challenged to a tail-clip bleeding assay, these mice survived for more than 3 months, as compared to the non-injected counterparts, which survived for a few hours. Analysis of the pathological features such as hemoglobin levels, reticulocyte count and morphology, and urine concentration defect showed improvement in the gene-corrected iPS cell-injected mice [60]. The question remains whether the transplanted cells are able to effect long-term correction of the hemophilia phenotype.

Retinal Pigmented Epithelium Cells

In retinal degenerative diseases, vision can be severely impaired by damage to retinal photoreceptors (RPE). The ability to generate immune-compatible RPE cells from autologous iPS cells represents a solution to replace the

diseased cells. To that end, several groups have successfully generated retinal cells including retinal progenitors, retinal pigmented epithelium, retinal ganglion cells, cone, and rod photoreceptors from mouse and human iPS cells [56, 61-65]. A comparison between human iPS cells reprogrammed using three- or four-factors did not yield considerable differences in differentiation efficiency and function [63], neither did reprogramming using “Yamanaka” or “Thomson factors” [61-64]. However, considerable variability was found amongst iPS cell lines in their ability to generate Pax6⁺ cells, a marker for early precursors of retinal pigmented epithelium [65]. In most cases, apart from the expression of cell-type appropriate markers, further characterization of the iPS-retinal cells is still required to determine the functional utility of these cells in rescuing a diseased phenotype.

Adipocytes

Lipodystrophy is a medical condition characterized by the loss of adipose tissue and is linked to conditions such as insulin-resistance, hepatomegaly, and hypermetabolism [66]. The etiology of this disease is not well understood, and significant differences exist between adipocyte development in mice and humans [67]. Human iPS-adipocytes could therefore serve as a model to study the progression of lipodystrophy. Generation of adipocytes from 4 human iPS cell clones was reported and compared with the differentiation potential of 2 separate human ES cell lines [68]. Human iPS- and ES cell-adipocytes were similar in terms of marker expression and lipid accumulation potential, although variations existed within each cell type [68].

iPS Cells in Gene Therapy

One of the difficulties in bringing gene therapy into the clinic is the risk of immune rejection. With autologous iPS cells, this problem can at least be circumvented. Studies have demonstrated the possibility of correcting or complementing a mutated gene in using iPS cell-based methods.

Ideal targets for gene therapy are single-gene disorders such as sickle cell anemia, where the repair of one gene can correct the disease phenotype. In that respect, Hanna and colleagues were able to show that wild-type iPS-hematopoietic progenitor cells can be derived from the diseased mouse by replacing the diseased β -globin genes by the healthy allele [69]. When the normal iPS-hematopoietic progenitor cells were injected into irradiated sickle mice, the treated mice showed improvement in all pathological features of sickle cell anemia, with decreased anisocytosis and normal red cell counts among other parameters, thereby demonstrating that disease-corrected iPS cells can indeed be used *in vivo* to treat a disease phenotype [69].

Fanconi Anemia (FA) is caused by chromosomal instability of any of the genes in the FA pathway, resulting in decreased numbers of hematopoietic cells. Using dermal fibroblasts of FA patients, Raya *et al.* corrected the FA phenotype by genetic complementation followed by reprogramming into iPS cells [70]. Interestingly, only FA-corrected cells could be reprogrammed into human iPS cells, showing that FA restoration is indispensable for reprogramming to occur [70]. Unlike studies where mouse iPS-derived cells can be tested in an animal model, the

difficulty in demonstrating therapeutic application of these FA-human iPS cells hematopoietic progenitors in an *in vivo* model remains a challenge.

CONCLUDING REMARKS

Many challenges lie ahead before iPS cells can be brought into the clinic. Nonetheless, the research community has learned much about the properties of these cells in a relatively short period of time. Interestingly, several studies have shown that differentiated cells can be directly transdifferentiated into neurons and insulin-producing cells, obviating the need for reprogramming [71, 72]. Nevertheless, the question remains: are ES cells and iPS cells equivalent and can they be employed in the same manner? From the studies carried out so far, the answer may be a reserved *yes*. It is worthy to note that, in more than one case, marked differences have been observed between differentiated progenies from iPS and ES cells [29, 40, 44, 56, 62, 63]. However, dissimilarities in the differentiation potential across ES cell lines have also been reported [73, 74]. Therefore the question of whether these differences are attributed to within or across cell types awaits the establishment of more systematic and standard approaches to iPS cell characterization before it can be solved.

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REFERENCES

- [1] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; 126(4): 663-76.
- [2] Yu J, Vodyanik MA, Smuga-Otto K, *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; 318(5858): 1917-20.
- [3] 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.
- [4] Reubinoff BE, Pera MF, Fong CY, *et al.* Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat Biotechnol* 2000; 18(4): 399-404.
- [5] Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282(5391): 1145-7.
- [6] Park IH, Arora N, Huo H, *et al.* Disease-specific induced pluripotent stem cells. *Cell* 2008; 134(5): 877-86.
- [7] Choo AB, Tan HL, Ang SN, *et al.* Selection against undifferentiated human embryonic stem cells by a cytotoxic antibody recognizing podocalyxin-like protein-1. *Stem Cells*. 2008; 26(6): 1454-63.
- [8] Tan HL, Fong WJ, Lee EH, *et al.* mAb 84, a cytotoxic antibody that kills undifferentiated human embryonic stem cells via oncosis. *Stem Cells* 2009; 27(8): 1792-801.
- [9] Aasen T, Raya A, Barrero MJ, *et al.* Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 2008; 26(11): 1276-84.
- [10] Yamanaka S. A fresh look at iPS cells. *Cell* 2009; 137(1): 13-7.
- [11] Masaki H, Ishikawa T, Takahashi S, *et al.* Heterogeneity of pluripotent marker gene expression in colonies generated in human iPS cell induction culture. *Stem Cell Res* 2007; 1(2): 105-15.
- [12] Watanabe K, Ueno M, Kamiya D, *et al.* A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat Biotechnol* 2007; 25(6): 681-6.

- [13] 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.
- [14] Marion RM, Strati K, Li H, *et al.* Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell* 2009; 4(2): 141-54.
- [15] Maherali N, Sridharan R, Xie W, *et al.* Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 2007; 1(1): 55-70.
- [16] 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.
- [17] Park IH, Zhao R, West JA, *et al.* Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008; 451(7175): 141-6.
- [18] Adewumi O, Aflatoonian B, Ahrlund-Richter L, *et al.* Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat Biotechnol* 2007; 25(7): 803-16.
- [19] Chin MH, Mason MJ, Xie W, *et al.* Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 2009; 5(1): 111-23.
- [20] Lowry WE, Richter L, Yachechko R, *et al.* Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci USA* 2008; 105(8): 2883-8.
- [21] Ellis J, Bruneau BG, Keller G, *et al.* Alternative induced pluripotent stem cell characterization criteria for *in vitro* applications. *Cell Stem Cell* 2009; 4(3): 198-9.
- [22] Rubin LL. Stem cells and drug discovery: the beginning of a new era? *Cell* 2008; 132(4): 549-52.
- [23] Erceg S, Ronaghi M, Stojkovic M. Human embryonic stem cell differentiation toward regional specific neural precursors. *Stem Cells* 2009; 27(1): 78-87.
- [24] Dhara SK, Stice SL. Neural differentiation of human embryonic stem cells. *J Cell Biochem* 2008; 105(3): 633-40.
- [25] Crook JM, Kobayashi NR. Human stem cells for modeling neurological disorders: accelerating the drug discovery pipeline. *J Cell Biochem* 2008; 105(6): 1361-6.
- [26] Phillips BW, Crook JM. Pluripotent human stem cells: a novel tool in drug discovery. *BioDrugs* 2010; 24(2): 99-108.
- [27] Karumbayaram S, Novitsch BG, Patterson M, *et al.* Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells*. 2009; 27(4): 806-11.
- [28] Chambers SM, Fasano CA, Papapetrou EP, *et al.* Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* 2009; 27(3): 275-80.
- [29] Hu BY, Weick JP, Yu J, *et al.* Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci USA* 2010; 107(9): 4335-40.
- [30] Dimos JT, Rodolfa KT, Niakan KK, *et al.* Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 2008; 321(5893): 1218-21.
- [31] Ebert AD, Yu J, Rose FF Jr, *et al.* Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 2009; 457(7227): 277-80.
- [32] Lee G, Papapetrou EP, Kim H, *et al.* Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 2009; 461(7262): 402-6.
- [33] 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.
- [34] Cai J, Yang M, Poremsky E, *et al.* Dopaminergic neurons derived from human induced pluripotent stem cells survive and integrate into 6-ohda lesioned rats. *Stem Cells Dev* 2010; 19: 1017-23.
- [35] Wernig M, Zhao JP, 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 USA* 2008; 105(15): 5856-61.
- [36] Thygesen K, Alpert JS, White HD, *et al.* Universal definition of myocardial infarction. *Circulation* 2007; 116(22): 2634-53.
- [37] Ahuja P, Sdek P, MacLellan WR. Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev* 2007; 87(2): 521-44.
- [38] Narazaki G, Uosaki H, Teranishi M, *et al.* Directed and systematic differentiation of cardiovascular cells from mouse induced pluripotent stem cells. *Circulation* 2008; 118(5): 498-506.
- [39] Schenke-Layland K, Rhodes KE, Angelis E, *et al.* Reprogrammed mouse fibroblasts differentiate into cells of the cardiovascular and hematopoietic lineages. *Stem Cells* 2008; 26(6): 1537-46.
- [40] Mauritz C, Schwanke K, Reppel M, *et al.* Generation of functional murine cardiac myocytes from induced pluripotent stem cells. *Circulation* 2008; 118(5): 507-17.
- [41] Zhang J, Wilson GF, Soerens AG, *et al.* Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009; 104(4): e30-41.
- [42] Zwi L, Caspi O, Arbel G, *et al.* Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation* 2009; 120(15): 1513-23.
- [43] Xu Q. Stem cells and transplant arteriosclerosis. *Circ Res* 2008; 102(9): 1011-24.
- [44] Xie CQ, Huang H, Wei S, *et al.* A comparison of murine smooth muscle cells generated from embryonic versus induced pluripotent stem cells. *Stem Cells Dev* 2009; 18(5): 741-8.
- [45] Lee TH, Song SH, Kim KL, *et al.* Functional recapitulation of smooth muscle cells via induced pluripotent stem cells from human aortic smooth muscle cells. *Circ Res* 2009; 106(1): 120-8.
- [46] Lian Q, Zhang Y, Zhang J, *et al.* Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice. *Circulation* 2010; 121(9): 1113-23.
- [47] White SA, Shaw JA, Sutherland DE. Pancreas transplantation. *Lancet* 2009; 373(9677): 1808-17.
- [48] Tateishi K, He J, Taranova O, *et al.* Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 2008; 283(46): 31601-7.
- [49] Zhang D, Jiang W, Liu M, *et al.* Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res* 2009; 19(4): 429-38.
- [50] Kung JW, Forbes SJ. Stem cells and liver repair. *Curr Opin Biotechnol* 2009; 20(5): 568-74.
- [51] Ogawa S, Miyagawa S. Potentials of regenerative medicine for liver disease. *Surg Today* 2009; 39(12): 1019-25.
- [52] Gai H, Nguyen DM, Joon Moon Y, *et al.* Generation of murine hepatic lineage cells from induced pluripotent stem cells. *Differentiation* 2010; 79(3): 171-81.
- [53] Li W, Wang D, Qin J, *et al.* Generation of functional hepatocytes from mouse induced pluripotent stem cells. *J Cell Physiol* 2010; 222(3): 492-501.
- [54] Choi KD, Vodyanik MA, Slukvin, II. Generation of mature human myelomonocytic cells through expansion and differentiation of pluripotent stem cell-derived lin-CD34+CD43+CD45+ progenitors. *J Clin Invest* 2009; 119(9): 2818-29.
- [55] Choi KD, Yu J, Smuga-Otto K, *et al.* Hematopoietic and endothelial differentiation of human induced pluripotent stem cells. *Stem Cells* 2009; 27(3): 559-67.
- [56] Feng Q, Lu SJ, Klimanskaya I, *et al.* Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. *Stem Cells* 2010; 28: 704-12.
- [57] Senju S, Haruta M, Matsunaga Y, *et al.* Characterization of dendritic cells and macrophages generated by directed differentiation from mouse induced pluripotent stem cells. *Stem Cells* 2009; 27(5): 1021-31.
- [58] 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.
- [59] Kaufman RJ. Advances toward gene therapy for hemophilia at the millennium. *Hum Gene Ther* 1999; 10(13): 2091-107.
- [60] Xu D, Alipio Z, Fink LM, *et al.* Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. *Proc Natl Acad Sci USA* 2009; 106(3): 808-13.
- [61] Buchholz DE, Hikita ST, Rowland TJ, *et al.* Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. *Stem Cells* 2009; 27(10): 2427-34.
- [62] Hirami Y, Osakada F, Takahashi K, *et al.* Generation of retinal cells from mouse and human induced pluripotent stem cells. *Neurosci Lett* 2009; 458(3): 126-31.
- [63] Osakada F, Jin ZB, Hirami Y, *et al.* *In vitro* differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. *J Cell Sci* 2009; 122(Pt 17): 3169-79.

- [64] Parameswaran S, Balasubramanian S, Babai N, *et al.* Induced pluripotent stem cells (iPSCs) generate both retinal ganglion cells and photoreceptors: therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration. *Stem Cells* 2010; 28: 695-703.
- [65] Meyer JS, Shearer RL, Capowski EE, *et al.* Modeling early retinal development with human embryonic and induced pluripotent stem cells. *Proc Natl Acad Sci USA* 2009; 106(39): 16698-703.
- [66] Hegele RA, Joy TR, Al-Attar SA, *et al.* Thematic review series: Adipocyte Biology. Lipodystrophies: windows on adipose biology and metabolism. *J Lipid Res* 2007; 48(7): 1433-44.
- [67] Arner P. Resistin: yet another adipokine tells us that men are not mice. *Diabetologia* 2005; 48(11): 2203-5.
- [68] Taura D, Noguchi M, Sone M, *et al.* Adipogenic differentiation of human induced pluripotent stem cells: comparison with that of human embryonic stem cells. *FEBS Lett* 2009; 583(6): 1029-33.
- [69] Hanna J, Wernig M, Markoulaki S, *et al.* Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 2007; 318(5858): 1920-3.
- [70] Raya A, Rodriguez-Piza I, Guenechea G, *et al.* Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* 2009; 460: 53-9.
- [71] Murtaugh LC, Melton DA. Genes, signals, and lineages in pancreas development. *Annu Rev Cell Dev Biol* 2003; 19: 71-89.
- [72] Vierbuchen T, Ostermeier A, Pang ZP, *et al.* Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 463(7284): 1035-41.
- [73] 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.
- [74] Wu H, Xu J, Pang ZP, *et al.* Integrative genomic and functional analyses reveal neuronal subtype differentiation bias in human embryonic stem cell lines. *Proc Natl Acad Sci USA* 2007; 104(34): 13821-6.
- [75] Heins N, Lindahl A, Karlsson U, *et al.* Clonal derivation and characterization of human embryonic stem cell lines. *J Biotechnol* 2006; 122(4): 511-20.
- [76] Taura D, Sone M, Homma K, *et al.* Induction and isolation of vascular cells from human induced pluripotent stem cells--brief report. *Arterioscler Thromb Vasc Biol* 2009; 29(7): 1100-103.

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