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REVIEW

Thinking outside the liver: Induced pluripotent stem cells for hepatic applications

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Abstract

The discovery of induced pluripotent stem cells (iPSCs) unraveled a mystery in stem cell research, after identification of four re-programming factors for generating pluripotent stem cells without the need of embryos. This breakthrough in generating iPSCs from somatic cells has overcome the ethical issues and immune rejection involved in the use of human embryonic stem cells. Hence, iPSCs form a great potential source for developing disease models, drug toxicity screening and cell-based therapies. These cells have the potential to differentiate into desired cell types, including hepatocytes, under *in vitro* as well as under *in vivo* conditions given the proper microenvironment. iPSC-derived hepatocytes could be useful as an unlimited source, which can be utilized in disease modeling, drug toxicity testing and producing autologous cell therapies that would avoid immune rejection and enable correction of gene defects prior to cell transplantation. In this review, we discuss the induction methods, role of reprogramming factors, and characterization of iPSCs, along with hepatocyte differentiation from iPSCs and potential applications. Further, we discuss the location and detection of liver stem cells and their role in liver regeneration. Although tumor formation and genetic mutations are a cause of concern, iPSCs still form a promising source for clinical applications.

Key words: Liver stem cells; Hepatocytes; Disease modeling; Drug toxicity; Clinical applications; Patient-specific induced pluripotent stem cell-derived hepatocytes

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INTRODUCTION

Patients suffering from chronic end-stage liver disease are currently receiving inadequate treatment due to the lack of organ donors for transplantation[1]. Alternatively, cell-based therapies are gaining importance as supportive therapy. Hepatocytes (adult, fetal) and liver stem cells form promising sources for cellular therapies in the treatment of liver diseases. However, inadequate proliferation, ethical issues and scanty numbers limit their applicability[2-5]. Therefore, it is essential to think outside the liver in favor of generating hepatocytes for drug screening, disease modeling and cell therapy applications. Identification of four reprogramming transcription factors revolutionized stem cell research in generating induced pluripotent stem cells (iPSCs). iPSCs generated from somatic cells can be utilized not only for cell-based therapies, but also for disease modeling and drug toxicity screening. Patient-specific iPSCs can be generated by reprogramming and differentiating somatic cells from the patient into the desired cell type. Key advantages of iPSCs over current transplantation approaches are that

they form an unlimited potential source and are patient-specific. In addition, the possibility of correcting genetic defects in liver diseases is currently under investigation[6].

The identification of patient-specific pluripotent stem cells has long been an important goal for scientists working in the field of stem cells. In 2006, Takahashi *et al*[7] first reported that forced expression of four transcription factors [octamer-binding transcription factor (Oct) 3/4, SRY box-containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4) and c-Myc] reprogrammed mouse somatic fibroblasts into embryonic stem cell (ESC)-like colonies, which were termed iPSCs. Later, human induced pluripotent stem cells (hiPSCs) were generated from embryonic, neonatal and adult fibroblasts[8-10]. In addition, derivation of patient-specific iPSCs for various diseases/disorders has also been reported[11-15]. Recently, several groups have investigated the possibilities of disease modeling using patient-derived iPSCs[6,16-23]. Apart from all these applications, hepatocytes derived from iPSCs will have larger implications in drug toxicity studies. Before iPSCs, several approaches were used to reprogram the differentiated cells to a pluripotent state. In the beginning, patient-specific human embryonic stem cells (hESCs) were derived using somatic cell nuclear transfer or therapeutic cloning. This technique requires the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a nuclear transfer embryo. The objective of this technique is to produce pluripotent hESCs that carry the nuclear genome of the patient and then induce them to differentiate into cells which may be transplanted back into the patient[24-28]. Another method is the fusion of fibroblasts with ESCs[29,30]. However, the therapeutic application of either approach has been experiencing both ethical and technical difficulties, summarized in Table 1.

METHODS TO GENERATE iPSCs

It was demonstrated that somatic cells can be re-programmed into pluripotent stem cells by ectopic expression of four transcription factors, namely Oct4, Klf4, Sox2, and c-Myc, using four independent retroviral vectors[7]. This achievement revolutionized stem cell research. Initially, iPSCs were derived from somatic cells by the retroviral or lentiviral transduction of transcription factors in which transgenes are randomly inserted into the genome of the hosts. Such integration of transgenes has the risk of tumorigenicity[31]. Later, trials to omit transgenic insertion of c-Myc resulted in low reprogramming efficiency and did not eliminate the risk of tumor formation[32], as overexpression of Oct3/4 and Klf4 can also cause tumor formation[33]. In Table 2, we have summarized the advantages and disadvantages of various strategies used for inducing iPSCs generation[7,8,9,32,34-49]. Furthermore, combining all four factors (Oct4, Klf4, Sox2, and c-Myc) into a single vector allowed derivation of iPSCs with a single lentiviral stem cell cassette containing a loxP sequence in the long terminal repeat (LTR)[43]. Following this, transgenes were removed using Cre-mediated excision. Although it left an incomplete LTR in the iPS genome, this method minimized the genomic alteration[44]. A transposon system encoding a reprogramming cassette has also been used for iPSC induction. The transduction of a plasmid-based transposon vector can integrate into the host genome with the help of transposase, and induces iPSC colony formation. The re-expression of the transposase after the establishment of iPSCs recognizes the terminal repeat of the integrated transposon vector, and excises it from the genome. The excision of the transposon does not leave a footprint in most cases, so it maintains the original endogenous sequences[45,46,50,51]. Several techniques have been used for obtaining transgene-free iPSCs.

The first integration-free iPSCs were generated from adult mouse hepatocytes using non integrating adenoviral vectors. However, this required repeated transduction to maintain transgene expression[34,38]. Another technique used is transduction with the Sendai virus, an RNA virus, to deliver the reprogramming factors[35]. The Sendai virus does not integrate into the genome, but working with this system requires more than 15 passages to eliminate viral transgene expression. This complexity limits the general use of this method[48]. Transient transfection of plasmids, episome-based DNA vectors and minicircle vectors has been used to generate transgene-free iPSCs. Mouse embryonic fibroblasts were reprogrammed by repeated transfection with two plasmid constructs carrying the reprogramming factors; the first plasmid expressed c-Myc, while the second expressed the other three factors Oct4, Klf4 and Sox2[36]. Furthermore, experiments with non-integrating episomal vectors have also been successful in iPSC generation[16]. Similarly, minicircle vectors lack the bacterial origin of replication and antibiotic resistance gene and offer higher transfection efficiencies and more prolonged transgene expression as compared to regular plasmids[52]. Moreover, iPSCs have been established by the direct delivery of recombinant reprogramming proteins[38] and small molecules[39]. More recently, one research group has utilized synthetic mRNA molecules to reprogram human fibroblasts to pluripotency and stimulate them into myogenic cells[32]. However, reprogramming using modified RNAs is technically difficult, sensitive to reagents and requires labor-intensive procedures. The efficiency of iPSC induction using transgene-free methods is lower than that with retrovirus vectors, possibly due to low transduction efficiency and unstable expression. Therefore, it is essential to develop methods that require less time and have higher efficiency of reprogramming involving viral and transgene-free techniques to generate iPSCs.

REPROGRAMMING FACTORS

Takahashi *et al*[7] used a combination of four nuclear reprogramming factors, such as Oct4, Sox2, c-Myc and Klf4, for generating iPSCs from mice and reported an efficiency of 0.02%. Simultaneously, the Thomson group used a slightly different combination of factors, namely Oct4, Sox2, Nanog and Lin28, to reprogram human somatic cells at a similar efficiency (0.02%)[9]. Subsequently,

Table 1 New approaches to reprogramming of differentiated cells to a pluripotent state

Method	Results of reprogramming	Drawbacks	Ref.
Transfer of the nucleus from a somatic cell to an enucleated oocyte	The somatic cell nucleus is reprogrammed in the oocyte, and a whole organism develops as a result. Patient-specific hESCs can be derived	Low efficiency. Developmental abnormalities in cloned animals. Ethical and legal restrictions	[24-28]
Fusion of ESCs with differentiated cells	Hybrids of differentiated cells and ESCs display all properties of pluripotent cells	Cell hybrids lack a normal diploid chromosome set	[29,30]
Reprogramming of somatic cells to a pluripotent state can be generated by the ectopic expression of 4 transcription factors, Oct4, Klf4, Sox2 and c-Myc	Somatic cells regain a pluripotent state and become similar in properties to ESCs	Low efficiency of iPSC derivation. Viral integration. Tumor formation	[7]

ESCs: Embryonic stem cells; hESCs: Human embryonic stem cells; iPSC: Induced pluripotent stem cell; Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4.

Table 2 Various induction methods to generate induced pluripotent stem cells

Methods	Advantages	Disadvantages	Ref.
Retroviral vectors	High efficiency	Genome integration, dividing target cells needed	[7-9,32,41,42]
Lentiviral vectors	High efficiency, target cells need not be dividing	Genome integration	[47-49]
Lentiviral vectors with Cre/Lox	High efficiency	Minimize genomic integration	[43,44]
Piggyback transposon	Precise deletion is possible	Minimize genomic integration, laborious	[45,46]
Viral vectors	No genome integration	Low efficiency	[34-37]
Adenoviral vectors			
Sendai vectors			
DNA vectors			
Plasmid vectors			
Episomal vectors			
Minicircle vectors			
Protein transduction	No genome integration	Low efficiency	[38]
Small molecules	No genetic modification	Low efficiency	[39]
Synthetic mRNA	No genetic modification, high efficiency	Multiple rounds of transfection are needed	[40]

researchers have started to identify new reprogramming factors and usage of minimum factors for generating safe iPSCs. iPSCs have been established by 3 transcriptional factors without c-Myc (Oct3/4, Klf4, SOX2) at an efficiency of 0.002%[53,54]. It was also shown that using only Oct4 and Klf4 was enough to reprogram murine NSCs at an efficiency of 0.11%[55]. More recently, the forced expression of Oct4 alone was shown sufficient to reprogram murine NSCs, at a low efficiency of 0.014%[56]. However, the efficiency of iPSC generation has been significantly reduced with usage of minimum factors for generating safe iPSCs. The *Oct4*, *Sox2*, and *Nanog* genes code for transcription factors that activate the genes and signaling pathways responsible for the establishment and maintenance of the pluripotent state and repress the genes responsible for differentiation[57,58]. Others have reported that the expression of *Oct4* and *Sox2* genes is absolutely essential for iPSC generation. In addition, the products of the *Nanog, c-Myc, Klf4* and *Lin28* genes seem to act as catalysts which accelerate the reprogramming[59]. In Table 3, we have summarized the role of various reprogramming factors for iPSC generation[60-66].

Recently, molecules have been used in combination with reprogramming factors to improve the efficiency of iPSC generation, including cotransduction of the catalytic subunit of human telomerase, human telomerase reverse transcriptase, along with SV40 large T antigen, or the repression of the *Ink4a/Arf* locus (encoding cell cycle-dependent kinase inhibitors), or repression of the p53/p21 pathway. These efforts have led to dramatic increases in the efficiency of reprogramming[10,67-69].

CHARACTERIZATION OF iPSCs

The hiPSCs generated can be characterized for their pluripotency, as shown in Figure 1. In addition, assessment of their epigenetic status, silencing of transgene expression and DNA fingerprinting need to be established for confirmation. Assessment of pluripotency of iPSCs can be performed by checking the expression of protein and genes of Oct4, Sox2, Nanog, as well as for SSEA-1 (mouse) or SSEA-3/-4 and TRA-1-60/-81 (human) using flow cytometry, immunocytochemistry and reverse transcription-polymerase chain reaction (PCR) methods[70]. The pluripotent nature of iPSCs is routinely tested by two methods. The first is to determine the *in vitro* differentiation ability of iPSCs, where iPSCs can be allowed to differentiate spontaneously *in vitro* to form embryoid bodies. These embryoid bodies can be assessed for three embryonic germ layers, *i.e.*, mesoderm, endoderm and ectoderm. The second is to determine the *in vivo* differentiation ability of iPSCs[71], where iPSCs can be injected into adult immune-deficient mice (SCID mice). In the host animal, injected iPSCs can form tumors called teratomas.

Table 3 Role of reprogramming factors for induced pluripotent stem cell generation

Reprogramming factors	Description	Function	Ref.
Oct4	Octamer binding transcription factor 4	This transcription factor plays a role in embryonic development, especially during early embryogenesis, and it is necessary for embryonic stem cell pluripotency	[7]
Sox2	SRY box 2	In embryonic stem cells, Sox2 and Oct3/4 often co-occupy target genes, including own promoters. These proteins cooperate regulatory feedback loops to maintain pluripotency	[60]
Klf4	Kruppel-like factor 4	This transcription factor plays a role in upregulation of pluripotency gene *Nanog* and the modification of chromatin structure to facilitate the binding of Oct3/4 and Sox2 to their sequences. Klf4 itself is an oncogenic factor. This gene is over expressed in a variety of tumor types associated with advanced cancer	[61-63]
c-Myc	Proto oncogene protein	An oncogene that induces global histone acetylation, allowing Oct3/4 and Sox2 to bind to their specific target loci	[60,63]
Nanog	Homeo box transcription factor	A transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells	[64]
Lin28	RNA binding protein Lin28	The *Lin28* gene codes for an RNA-binding protein that selectively blocks the processing of microRNAs of the let-7 family, and possibly certain other microRNAs in ESCs, to prevent their differentiation	[65,66]

ESCs: Embryonic stem cells; Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4.

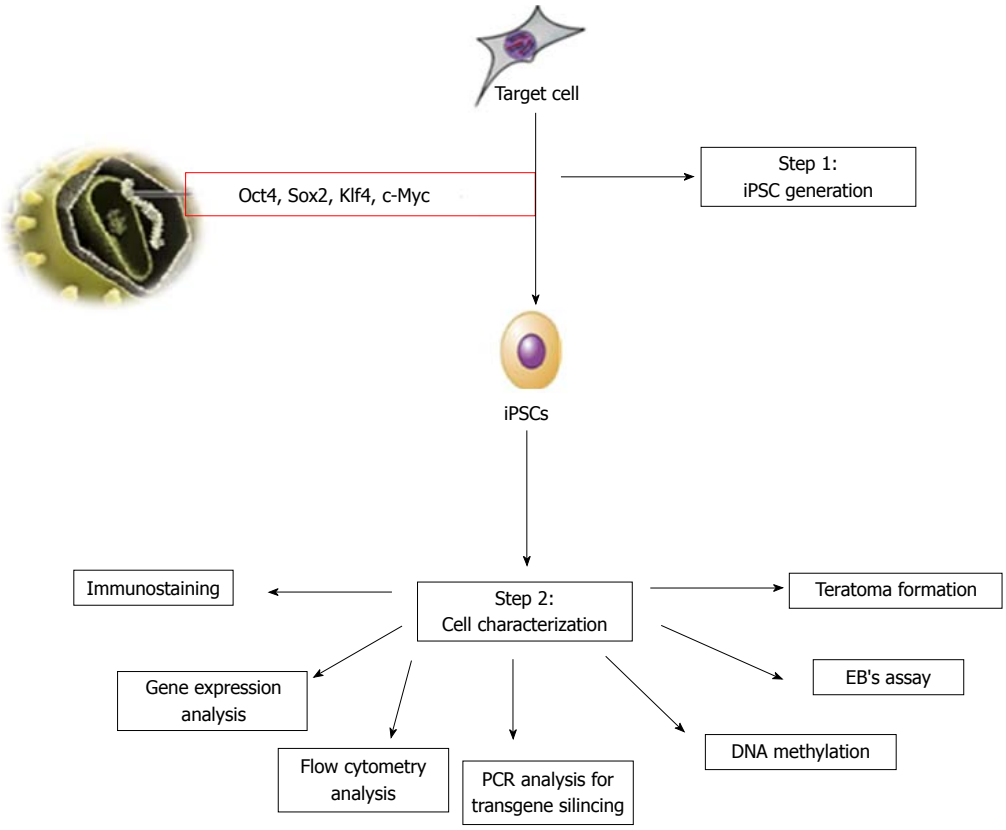

Figure 1 Flow diagram of generation and characterization of human induced pluripotent stem cells. Induced pluripotent stem cells (iPSCs) are derived through the introduction of stem cell factors into fibroblasts. After that, assessment of pluripotency of iPSCs can be studied by expression of protein and genes using various techniques such as immunocytochemistry, flow cytometry and reverse transcription-polymerase chain reaction (PCR) methods, respectively. *In vitro* and *in vivo* differentiation ability of iPSCs can be studied by embryoid body assay (EB assay) and teratoma formation assay, respectively. In addition, PCR analysis is required to demonstrate silencing of transgene expression in iPSCs and DNA methylation to confirm reprogramming of somatic cells. Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4.

In addition to pluripotency assessment, it is important to confirm the silencing of exogenous transgene expression. PCR analysis can be used to demonstrate silencing of retro/lentiviral transgene expression using virus-specific primers[70]. Further, DNA fingerprinting can be performed to confirm iPSCs are genetically matched to their parental somatic cells. DNA methylation analysis of the *Oct4*, *Sox2* and *Nanog* promoter regions using bisulfite sequencing can be used to reveal the different epigenetic states of the cells. Thus, the methylation status of promoter regions of pluripotency genes confirms successful reprogramming[70].

Table 4 Differentiation protocols for induced pluripotent stem cell-derived hepatocytes

Ref.	Species	Differentiation protocol	Remarks
Sullivan *et al*[78]	Human	Activin A, Wnt3a (3 d), Activin A (2 d), DMSO (3 d), HGF, OSM (6 d)	Generated functional hepatocyte-like cells from human-iPSCs
Song *et al*[79]	Human	Activin A (3 d), FGF4, BMP-2 (4 d), HGF, KGF (6 d), OSM, Dex (5 d) then OSM, Dex, N2B27 (3 d)	iPSCs had fewer expressed liver-enriched genes compared with human hepatocytes
Si-Tayeb *et al*[80]	Human	Activin A (5 d), bFGF, BMP-4 (5 d), HGF (5 d), OSM (5 d)	Transplanted hepatocyte-like cells into the lobe of newborn mice and demonstrated homing of donor cells
Liu *et al*[81]	Human	Activin A (5 d), FGF4, HGF (5 d), Single Quotes (lonza), FGF4, HGF, OSM, Dex (10 d)	Human hepatocyte-derived iPSCs are able to differentiate into functional hepatocytes
Takata *et al*[82]	Human	Activin A (3 d), HGF (5 d), OSM (5 d)	Generated hepatocyte-like cells from iPSCs using three growth factors in a short time
Gai *et al*[83]	Mouse	Activin A, Wnt3 (6 d), bFGF, DMSO (3 d), HGF, DMSO (9 d), HGF, OSM, DMSO (7 d)	Generated hepatocytes from iPSCs
Iwamuro *et al*[84]	Mouse	Activin A, bFGF (3 d), HGF (5 d)	Generated hepatocyte-like cells from iPSCs

iPSCs: Induced pluripotent stem cells; DMSO: Dimethyl sulfoxide; HGF: Hepatocyte growth factor; OSM: Oncostatin M; Dex: Dexamethasone; FGF4: Fibroblast growth factor-4; BMP: Bone morphogenetic protein; KGF: Keratinocyte growth gactor; bFGF: Basic fibroblast growth factor.

GENERATION OF HEPATOCYTES FROM iPSCs

To date, many protocols have been used to differentiate iPSCs into desired cell types. However, different iPSC lines have different outcomes under identical culture conditions. iPSC lines have a propensity to produce certain lineages or cell types when allowed to differentiate spontaneously, indicating that choosing a proper clone is also essential in differentiating iPSCs into a specific lineage[72-74]. A major issue in differentiation is to obtain hepatocytes from pluripotent stem cells that have an adult phenotype, and which stably express liver-like functions and reflect those *in vivo* functions[75]. Recently, a number of protocols have been developed to derive hepatocytes from hiPSCs. These protocols for hepatocyte generation are hampered by inefficient differentiation and maturation that lead to low yield and heterogeneous cell populations in cultures[76]. Recently, a homogenous population of hepatocytes from pluripotent stem cells has been isolated by sorting for surface asialoglycoprotein receptor marker; however, these enriched cells are found to retain immature fetal liver characteristics[77]. In Table 4, we have summarized various protocols used to differentiate hepatocytes from iPSCs[78-84]. Even after enriching the hepatocytes from culture prior to transplantation, the risk of teratoma formation may arise due to the presence of a few undifferentiated iPSCs. Therefore, further enriching hepatocytes using negative selection against pluripotent cells could be useful to avoid teratoma formation. Figure 2 summarizes the strategy on differentiation of human iPSCs into hepatocytes. Figure 3 depicts the hepatocytes generated from hiPSCs in our laboratory.

POTENTIAL APPLICATIONS OF iPSC-DERIVED HEPATOCYTES

iPSCs represent a promising source of hepatocytes for a wide range of applications, including disease modeling, drug toxicity testing and cell transplantation (Figure 4).

Disease modeling

iPSCs represent a novel tool for *in vitro* disease modeling. Traditionally, researchers rely on animal models, hepatic immortalized cell lines, or short-lived primary hepatocyte cultures to understand the mechanisms and pathogenesis of diseases and testing of drug candidates[85-87]. Each of these has limitations in functionality, reproducibility and availability. Disease-specific iPSCs derived from patients suffering from specific diseases may provide a more relevant model system because their properties closely resemble those found in the patient's own system, without the need for genetic manipulation. Several groups have successfully derived a wide range of iPSCs from patients with diseases[88] and inherited liver diseases[21]. These cells can be used as models to study the pathogenesis, disease mechanism(s) and possible cure for liver disorders. Therefore, human iPSC-derived hepatocytes could generate more accurate predictions of human physiological responses than animal models. iPSC-derived hepatocytes will overcome these limitations and provide a reliable source of highly reproducible and readily available human hepatocytes for disease modeling in pre-clinical drug development.

Drug toxicity screening

Hepatotoxicity is the most common side effect of new candidate drugs under clinical trial, and is the leading cause of post approval drug recalls; for example, bromfenac and troglitazone[89]. The development of liver toxicity screening technologies utilizing iPSC-derived hepatocytes would allow investigation into the effects of single nucleotide polymorphisms on drug metabolism and toxicity[90]. An example of this is warfarin, a drug for which polymorphisms in cytochrome P-450 2C9 create problems with obtaining an appropriate pharmacotherapeutic range[91]. iPSC-derived hepatocytes could remain viable in culture for several months, enabling the assessment of acute and chronic toxicity of drugs due to their pluripotent ability. Drug toxicity assays will be performed in petri dishes which require small amounts of compound for a hepatic cytotoxicity profile. Terminally differentiated he-

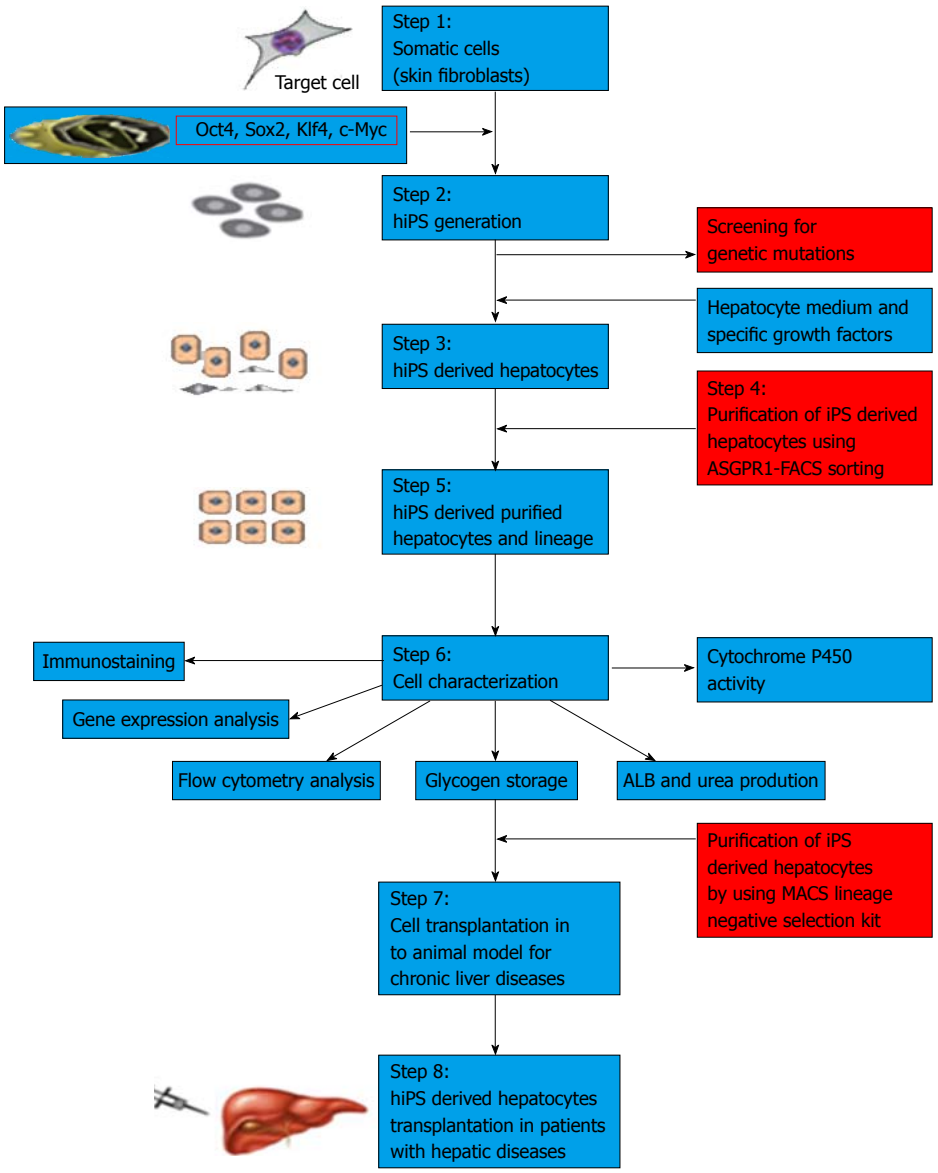

Figure 2 Flow diagram showing the strategy for human induced pluripotent stem cell-derived hepatocyte clinical applications. Steps 1 and 2, human induced pluripotent stem cells (hiPSCs) are generated from somatic cells using reprogramming techniques and screened for mutations; Step 3, hiPSCs are differentiated into hepatocytes using specific growth factors and medium; Step 4, enrichment of hiPSC-derived hepatocytes; Steps 5 and 6, characterization of enriched iPSC-derived hepatocytes for protein expression, gene expression and functional assays. Before clinical transplantation, hiPSC-derived hepatocytes are enriched again using negative selection against pluripotent cells to avoid teratoma formation; Step 7, transplantation of enriched hiPSC-derived hepatocytes into chronic liver failure animal model; Step 8, hiPSC-derived enriched hepatocytes could be transplanted into liver disease patients. Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4; ASGPR: Asialoglycoprotein receptor; FACS: Fluorescence activated cell sorting; MACS: Magnetic-activated cell sorting; ALB: Albumin.

patocytes with cytochrome P-3A4 functional activity and scale-up of iPSC-derived hepatocytes will help in pharmaceutical industry drug toxicity applications.

Patient-specific iPSC-derived hepatocytes for cell transplantation

Liver transplantation represents the only way to treat patients suffering from chronic liver failure, but this is associated with numerous problems, including shortage of donors, high cost, rejection and complications. Transplantation of hepatocytes derived from hiPSCs could represent an alternative cell source for liver failure and inborn liver diseases. The important issue is the generation of safe and functional cell types for therapy. Indeed, the cell sources of iPSCs influence the safety of the established iPSCs. It has been demonstrated that hiPSCs retain certain gene expressions of the parent cells, and this suggests that iPSCs of different origins may possess different capacities to differentiate. A complete study using various mouse iPSCs has demonstrated that the origin of the iPSCs has a profound influence on the tumor-forming propensities in a cell transplantation therapy model[92]. Mouse tail-tip fibroblast iPSCs (mesoderm origin) have shown the highest tumorigenic propensity, whereas gastric epithelial cells and hepatocyte iPSCs (both are endoderm origin) have shown lower propensities[93]. The recent evidence suggests that epigenetic memory of the somatic cell of origin is retained in the iPSCs, and

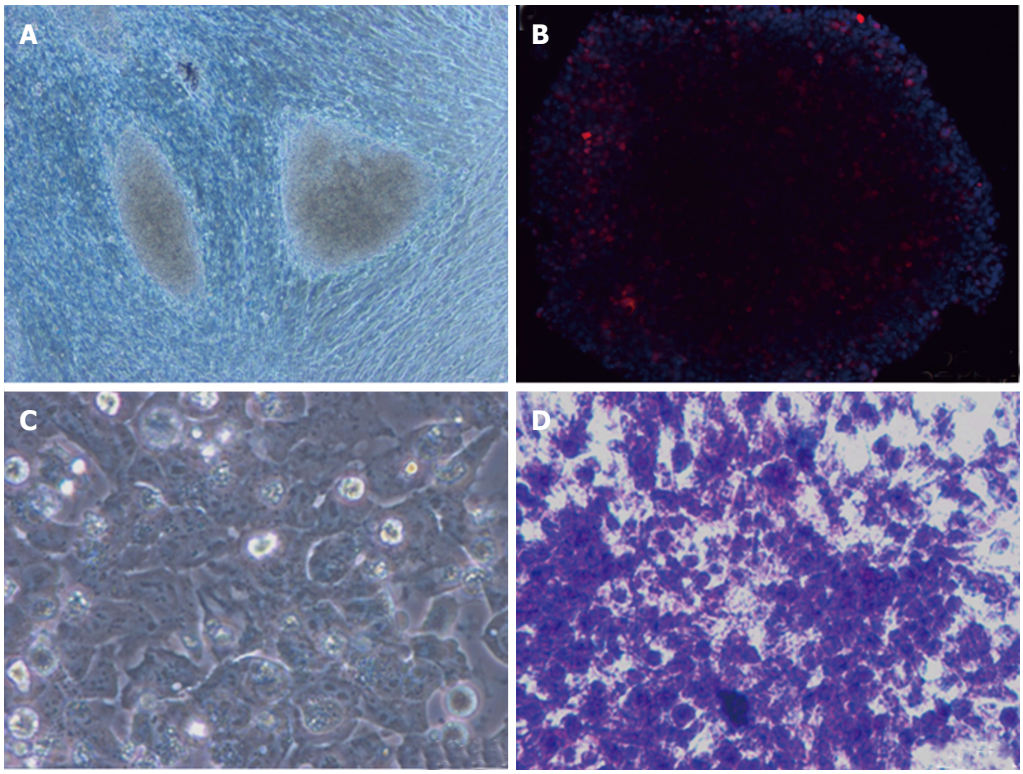

Figure 3 Human induced pluripotent stem cells generated from human foreskin fibroblasts using single lentiviral stem cell cassette kit (Millipore, United States) method. A, B: Human induced pluripotent stem cell (hiPSC) colonies resembling embryonic stem cells in morphology were observed, and iPSC with a flat, packed, tight colony morphology and a high nucleus to cytoplasm ratio (A, × 40) were positive for Oct4 marker on immunocytochemistry (B, × 200); C, D: hiPSCs were differentiated into hepatocytes. At day 13, these differentiated cells exhibited polygonal morphology (C, × 400) and showed pink color (glycogen storage) on periodic acid schiff staining (D, × 200).

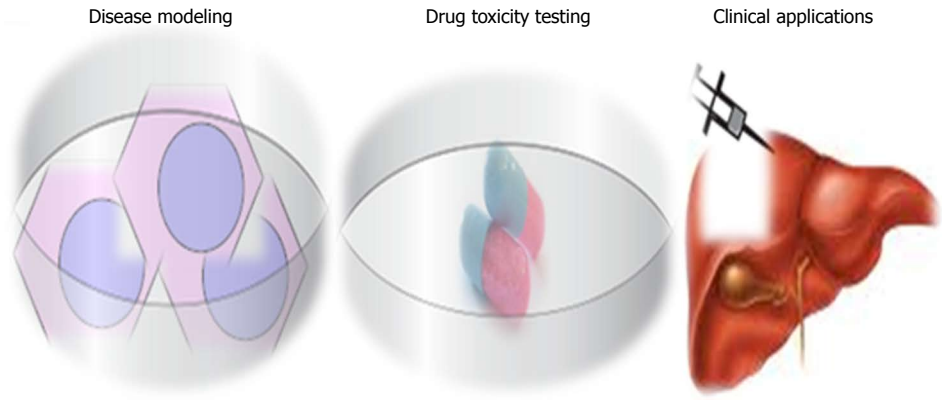

Figure 4 Flow diagram of potential applications of induced pluripotent stem cell-derived hepatocytes. Induced pluripotent stem cells (iPSCs) are capable of self-renewal and are able to differentiate into hepatocytes *in vitro*. iPSC-derived hepatocytes can be applied to disease modeling, drug toxicity screening assays, and clinical applications.

that may influence their directed differentiation potential into blood cells[94,81] or hepatocytes[92]. In the mouse, iPSCs have been generated from derivatives of all three embryonic germ layers, including mesodermal fibroblasts, epithelial cells of endodermal origin and ectodermal keratinocytes, whereas human iPSCs have been produced from mesoderm (fibroblasts and blood cells) or ectoderm (keratinocytes and neural stem cells) and endoderm (hepatocytes)[81]. It is therefore extremely important to establish human iPSC lines of multiple origins and thoroughly examine the source impact on both the safety issues and their differentiation potentials.

Recently, it has been demonstrated that iPSC-derived hepatocytes can restore liver function in an animal model of liver failure[95]. These results indicate the utility of hiPSC-derived hepatocytes as an alternative treatment for patients with end-stage liver disease. Researchers investigated and analyzed the potential of hiPSC-derived hepatocytes to model inborn liver diseases such as α_1-antitrypsin deficiency, familial hypercholesterolemia, glycogen storage disease type 1a, hereditary tyrosinemia, and Crigler-Najjar syndrome[6]. Genetic diseases of the liver modeled in hiPSC-

Table 5 Direct conversion approaches for specific cell types

Ref.	Key factors	Direct converted cell type
Vierbuchen *et al*[109]	Brn2, Ascl1, and Myt1l	Transdifferentiated mouse fibroblasts into functional neuronal cells
Ieda *et al*[110]	Gata4, Mef2c, and Tbx5	Transdifferentiated mouse dermal fibroblasts into cardiomyocyte-like cells
Szabo *et al*[111]	Oct4	Transdifferentiated human fibroblast cells into hematopoietic progenitors
Huang *et al*[112]	Gata4, Hnf1α and Foxa3, and inactivation of p19Arf	Transdifferentiated mouse tail-tip fibroblasts into hepatocyte-like cell

Brn2: Brain-2; Ascl1: Achaete-scute homolog 1; Myt1l: Myelin transcription factor 1; Gata4: GATA binding protein 4; Mef2c: Myocyte enhancer factor 2; Tbx5: T-box protein 5; Hnf1α: Hepatocyte nuclear factor 1α; Foxa3: Forkhead box protein A3; p19: protein p19; Oct: Octamer-binding transcription factor.

derived human hepatocytes create new opportunities to develop autologous cell transplantation therapy to correct genetic defects in liver diseases.

Liver stem cells

The liver has a massive regenerative capacity. When liver regeneration is impaired, oval shaped cells emerge and are implicated in liver tissue repair[96]. These cells are derived from the canals of Hering, which are located in the periportal region of the liver and account for 0.3%-0.7% of the liver mass[97]. In rodents, these liver progenitor cells are called oval cells, while in humans they are known as hepatic progenitor cells[98]. These cells are phenotypically similar to fetal hepatoblasts and also have a bipotent differentiation potential. Oval cells or hepatic progenitors are difficult to isolate because of the lack of definitive markers. Various markers have been used to identify oval cells in adult liver, including liver stem cell and hematopoietic markers, such as OV6, Thy-1, CD34, c-kit, and Sca-1[99]. Hepatic progenitors have been isolated from fetal liver using the specific surface marker, epithelial cell adhesion molecule (EPCAM). These $EPCAM^+$ cells showed positive for hepatic progenitor markers such as CD29, CD49f and CD90[86]. Clinical studies have identified and confirmed the efficacy of fetal liver hepatic progenitors in end-stage liver diseases[100]. However, the clinical application of this cell source is limited due to the difficulty in obtaining large numbers of fetal liver cells, as well as ethical and immune rejection issues. Another stem cell population found in the fetal liver is side population cells which represents another potential source of liver progenitor cells[101], but these cell numbers are very much fewer in fetal liver. There is increasing evidence in the literature suggesting that bone marrow is another source of hepatic progenitor cells[102,103]. Autologous bone marrow-derived stem cell transplantations have been performed in patients with liver diseases but it is difficult to assess overall clinical benefit from these therapies[104].

In support of a role in liver regeneration, oval cell activation has been detected in chronic liver injury caused by inflammation, chronic hepatic necrosis, chronic alcoholism induced cirrhosis and hepatitis models[105,106]. Although the full complements of signals required for oval cell activation are still unknown, both continuous metabolic stress and chemical hepatotoxic substances have been implicated as potential oval cell activators when hepatocyte proliferation is inhibited[105,106]. A recent study reported the production of a chemokine known as stromal derived factor-1α in the liver following tissue damage[107]. The role of liver stem cells in physiology, pathophysiology and therapy is not yet exactly known; therefore, it needs to be further investigated[108]. Although a number of successful techniques have been developed, stem cell-derived hepatocytes from adult, fetal and embryonic sources are found to retain immature fetal liver characteristics, which are not similar in primary hepatocyte functionality. Therefore, the elucidation of other key developmental factors and tissue culture environments, together with iPSC technology, are essential in order to obtain functional hepatocytes for hepatic applications.

DIRECT CONVERSION

Apart from the methods discussed above, overexpression of lineage-specific transcription factors in somatic cells is a new approach (direct conversion) to generate specific cell types including neurons, cardiomyocytes, blood progenitors and hepatocyte-like cells; as summarized in Table 5[109-112]. This method could be useful as an alternative approach for autologous cell-replacement therapies. Unlike iPSCs and ESCs, directly converted cells may not easily multiply in the lab since they do not have pluripotency properties. Therefore, this approach may have limitations. Choosing highly proliferative starting somatic cells is essential in the direct conversion approach.

CONCLUSION

Thinking outside the liver explores the potential of iPSCs as an unlimited source for *in vitro* disease modeling and for drug toxicity studies and clinical applications. Patient-specific iPSCs or custom-made iPSCs may have future promising implications without immuno rejection. However, iPSC technology has several technical issues to be addressed such as generation of iPSCs without viral integration, elimination of tumor formation and genetic mutations that need to be eliminated before the cells are put to clinical applications. Despite limitations, iPSC-derived hepatocytes are a very promising population for cell therapies in hepatology.

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