Review

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Induced pluripotent stem cells: A new era for hepatology

Samira Asgari[1,2], Behshad Pournasr[1], Ghasem Hosseini Salekdeh[3,4], Arefeh Ghodsizadeh[1,2] Michael Ott[5], Hossein Baharvand[1,6,*]

[1]*Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran;* [2]*Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran;* [3]*Department of Molecular Systems Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran;* [4]*Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran;* [5]*Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, and TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany;* [6]*Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran*

Stem cell transplantation has been proposed as an attractive alternative approach to restore liver mass and function. Recent progress has been reported on the generation of induced pluripotent stem (iPS) cells from somatic cells. Human-iPS cells can be differentiated towards the hepatic lineage which presents possibilities for improving research on diseases, drug development, tissue engineering, the development of bio-artificial livers, and a foundation for producing autologous cell therapies that would avoid immune rejection and enable correction of gene defects prior to cell transplantation. This focused review will discuss how human iPS cell advances are likely to have an impact on hepatology.

Introduction

Allogeneic liver transplantation is the only effective treatment available to patients with liver failure [84]. A serious shortage of liver donors in combination with the high risk of organ rejection mandates alternative therapeutic approaches. Moreover, operative damage and, in some cases, recurrence of pre-transplant diseases can be considered as additional obstacles [72]. The discovery of human embryonic stem (ES) cells [136] has raised the hopes for curing diseases that have poor prognoses. However, after more than a decade of research, several challenges related to ES cell safety, efficacy, and bioethics have not been sufficiently answered. For example, in 2009 the US Food and Drug Administration (FDA) approved a clinical trial of human ES cell-derived oligodendrocyte progenitors in spinal cord injury patients, but it was subsequently placed on hold pending further data regarding safety issues [50].

In a groundbreaking 2006 report, Yamanaka and co-workers surprised the scientific community when they demonstrated that both mouse embryonic fibroblasts and tail tip fibroblasts could be reprogrammed into a pluripotent state similar to that observed in ES cells [134]. This was achieved by the retroviral transduction of Oct4, Sox2, Klf4, and c-Myc genes. These reprogrammed cells were named induced pluripotent stem (iPS) cells. Subsequently Yamanaka's and Thomson's laboratories successfully reprogrammed human somatic cells into iPS cells [133,153]. Human-iPS cells have the hallmarks of ES cell attributes including: morphology, unlimited self-renewal, expression of key pluripotency genes, and a normal karyotype. Additionally, in human-iPS cells proof of functional differentiation into specialized cell lineages of all three embryonic germ layers [57,118,122,130,154,155] have been demonstrated. This wide differentiation potential provides fascinating possibilities and tools for the study of human development and genetic diseases, in addition to their use for drug discovery and regenerative medicine (reviewed in Ref. [95]) (Fig. 1).

In this review, we discuss the potentials of human-iPS cells and challenges of using these cells in hepatology.

Methods used for differentiation and enrichment of hepatocyte-like cells from human ES and iPS cells *in vitro*

The directed differentiation of human ES cells (iPS cell counterparts) into hepatocytes was first reported in 2003 when Rambhatla et al. used sodium butyrate to generate cells that could express hepatocyte markers [103]. Subsequent reports optimized this method and directed differentiation was achieved in human ES cells by the administration of various growth factors in a time dependent manner (Table 1). For example, it was shown that Activin A and Wnt3a synergistically elicit rapid and highly efficient differentiation of human ES cells into functional hepatic endoderm [44]. Recently, Basma et al. presented a simple and reproducible growth factor-mediated method to generate functional hepatocyte-like cells (HLCs) from human ES cells [9]. Transplantation of HLCs which were enriched based on asialoglycoprotein-receptor (ASGPR) expression improved the liver mouse model [9]. In addition to growth factors, a variety of protocols have been used for the differentiation of human ES cells towards the hepatic lineage; EB formation, co-culturing with hepatic and non-hepatic cell types, genetic manipulation, and epigenetic

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* Corresponding author. Address: Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, P.O. Box 19395-4644, Tehran, Iran. Tel.: +98 21 22306485; fax: +98 21 22310406.
E-mail addresses: Baharvand@royaninstitute.org, Baharvand50@yahoo.com (H. Baharvand).

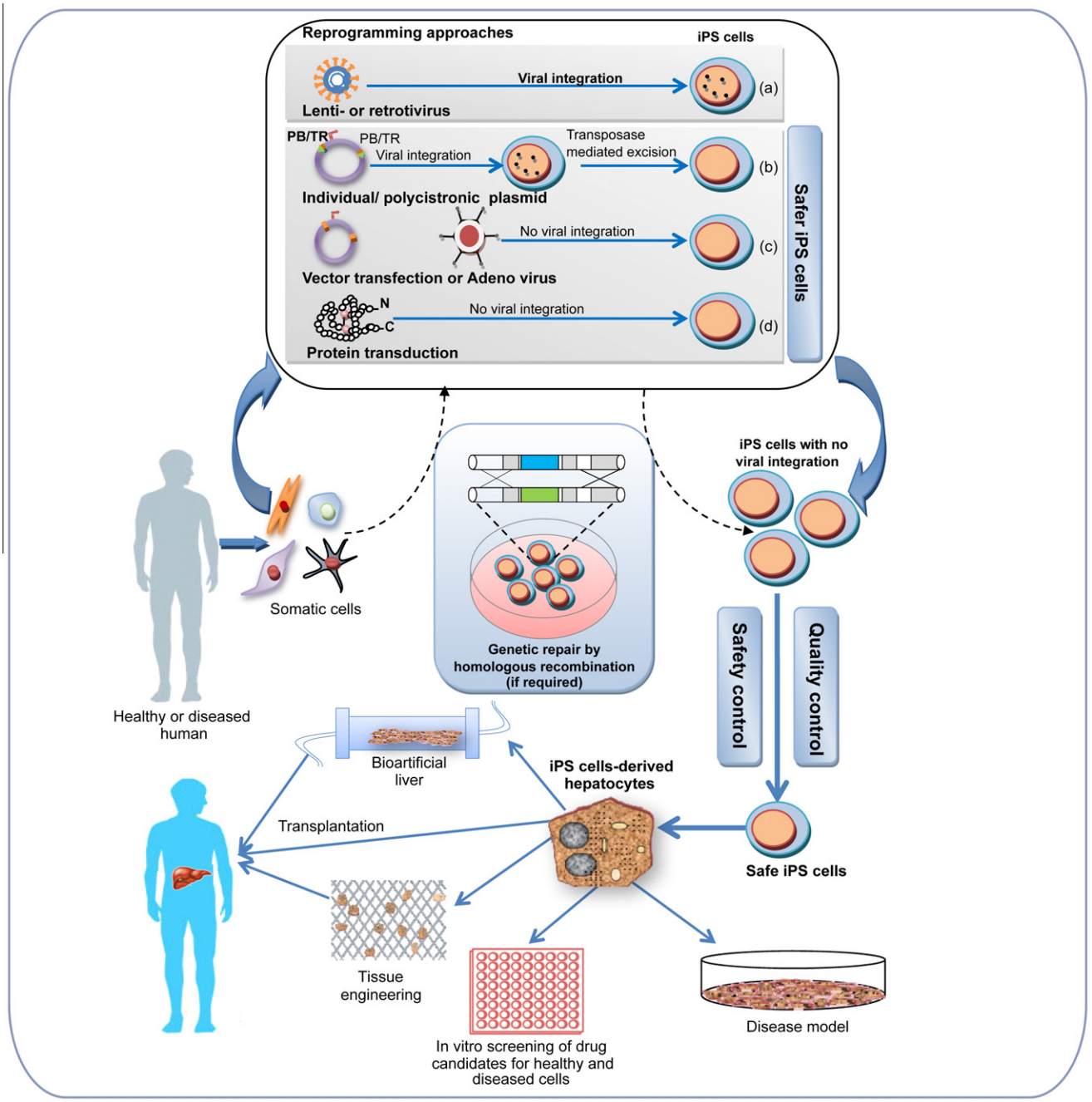

Fig. 1. Progress towards safer iPS cells and application of differentiated hepatocytes. Methods have evolved from conventional viral integration to virus-free strategies by transgene removal or the avoidance of viral integration. iPS cells have first been generated using integrating retroviral and lentiviral vectors to deliver reprogramming factors such as: OCT4, SOX2, KLF4, and c-MYC (A). Alternatively, iPS cells can be produced by the integration of reprogramming factors using the LoxP site containing plasmids, lentiviruses, or piggyBac transposon mediated gene transfer system followed by removal of transgene sequences from the host genome using Cre or transposase enzymes (B). Another strategy to generate iPS cells without viral integration used non-integrating viruses or plasmids to introduce reprogramming factors (C). Mouse and human iPS cell lines were also derived using vector or adenoviruses that transiently expressed Oct4, Sox2, Klf4, and c-Myc (C). It was also shown that virus-free mouse iPS cells could be generated using repeated plasmid transfections and by nucleofection of a single plasmid construct expressing Oct4, Sox2, Klf4, and c-Myc as a single polycistronic unit (C). Human-iPS cells were also recently generated completely free of vector and transgene sequences by transfection of a non-integrating episomal vector. Another recent strategy to create iPSCs without viral integration has been reported by protein transduction of the four reprogramming proteins fused with a cell-penetrating peptide (CPP) (D). Somatic or iPS cells can be used to treat disease by correction of the underlying genetic defect. The wild-type gene can be used to replace the defective gene by homologous recombination of somatic cells or iPS cells. The differentiated hepatic cell lineages provide fascinating possibilities and tools for the study of disease models, tissue engineering, and creation of bio-artificial livers, in addition to their use for drug discovery and regenerative medicine.

modifications (Table 1). Moreover, the application of small molecules has been used for the endodermal differentiation from ES cells [12]. It has been demonstrated that these molecules can regulate specific target(s) in signaling and epigenetic mechanisms and can manipulate cell fate without genetic alterations [69,110,147].

Table 1. Differentiation protocols that direct human ES and iPS cells toward hepatocytes.

Differentiation protocol	Hepatic features	Major result(s)	Ref.
Human ES cells			
Stage 1 (4 days): EB formation, NaBu (5 mM) or DMSO (1%), plated on Matrigel coated plate; **Stage 2 (10–11 days):** DMSO (1%) (4 days), NaBu (2.5 mM) (6–7 days); **Stage 3 (4 days):** NaBu (2.5 mM), HGF (2.5 ng/ml)	**RT-PCR analysis of** AFP, ALB, AAT, HNF4, AGPR, GATA4, TAT, C/EBPα, C/EBPβ; **IF analysis of** ALB, AAT, AFP, CK8, CK18, CK19. **PAS staining, EROD assay, BrdU incorporation**	The first report on differentiation of human ES cells into functional HLCs	[103]
Stage 1 (20 days): EB formation and culture in mouse primary hepatocyte CM; **Stage 2 (10 days):** Plated EB with or without GF including aFGF (100 ng/ml), bFGF (5 ng/ml), HGF (20 ng/ml), BMP4 (50 ng/ml)	**RT-PCR analysis of** AFP, apolipoprotein (A4,B,H,F), fibrinogen α,β,γ, ALB, ADH1C; **FACS analysis of** ALB-GFP	The first report to demonstrate the possibility of purifying differentiated HLCs by genetic manipulation with further culturing	[67]
Stage 1 (6 days): EB formation followed by plating on collagen I; **Stage 2 (8–43 days):** HGF (20 ng/ml), NGF (100 ng/ml), EGF (100 ng/ml), aFGF (100 ng/ml), bFGF (100 ng/ml), RA (1 μM), OSM (10 ng/ml), bovine insulin (0.126 U/ml), Dex (100 nmol), human insulin (0.126 U/ml)	**qRT-PCR analysis of** ALB, AAT; **IF analysis of** ALB, **Western blot analysis of** ALB; **urea synthesis**	The initial protocol for the differentiation of HLCs	[116]
Stage 1 (7–14 days): EB formation followed by plating on collagen 1 or either fibronectin, laminin, Matrigel, or uncoated; **Stage 2:** Serum-free media with or without HGF, OSM, aFGF, FGF7. (The time and concentrations were not available)	**qRT-PCR analysis of** AFP, ALB, CK18, HNF3β, HNF1, CK19, GATA4, CYP1A1, CYP1A2, CYP2B6, CYP3A4, ASGPR1; **IF analysis of** ALB, CK18, HNF3B, HNF1, **immunoblot analysis of** ALB, CK18, HNF1, ASGPR1; **urea production, ICG uptake, CYP activity**	Matrigel and collagen I had greater influence on HLC differentiation compared to laminin and fibronectin	[111]
Stage 1 (5 days): EB formation; **Stage 2 (20 days):** Plated on collagen I or 3D collagen scaffold, aFGF (100 ng/ml), HGF (20 ng/ml), OSM (10 ng/ml), Dex (0.1 μM), ITS	**RT-PCR analysis of** HNF3β, AFP, TTR, AAT, CK8, CK18, CK19, ALB, CYP7A1, TDO, TAT, G6P; **IF analysis of** ALB, CK18; **AFP & ALB production, urea synthesis, ALB production, *EM*** (ultra structure characteristics), **PAS staining, ICG uptake**	3D differentiation into HLCs made more functional hepatocytes compared to 2D cultures	[7]
Stage 1 (7 days): DMSO (1%); **Stage 2 (9 days):** HGF (2.5 or 10 ng/ml). **Stage 3 (4 days):** HGF (10 ng/ml), OSM (10 ng/ml)	**RT-PCR analysis of** AFP, TTR, HNF4α, AAT, ALB, TDO, C/EBPα, hTERT; **IF analysis of** AFP, HNF4α, ALB, HepPar1; **Western blot analysis of** Sox7, Sox17, c-Met, E-cad; **ICG uptake, PAS staining, ELISA** (ALB, AFP), **HPLC:** CYP3A4 activity	HGF promotes HLC differentiation in a dosedependent manner	[46]
Stage 1 (9 days): EB formation; **Stage 2 (10–14 days):** Plated on collagen I, IMDM, FBS (20%), Dex (100 nM). **Stage 3 (7 days):** Transduction with AAT-GFP lentivirus. **Stage 4:** GFP positive cell sorting	**RT-PCR analysis of** ALB, AAT, TF, AFP, CYP1B1, CYP1A1, CYP2B6, CYP3A4, CYP2C9, CYP2E1, ARG, G6P, HNF3|3, HNF4, C/EBPα, C/EBPβ, BMP2, BMP4, GATA4; **IF analysis of** ALB, AAT, AFP, CK18; **PAS staining, ICG uptake, ALB production, transplantation, ELISA** (human ALB in mouse blood)	The first to perform a successful transplantation of bioluminescence and GFP positive HLCs, to track their fate in the animal liver, and detect human **serum albumin in an animal model**	[27]
Stage 1 (3 days): Activin A (100 ng/ml) + 0% ITS (1 day), Activin A (100 ng/ml) + 0.1% ITS (1 day), Activin A (100 ng/ml) + 1% ITS (1 day); **Stage 2 (8 days):** FGF4 (30 ng/ml) + BMP2 (20 ng/ml). **Stage 3 (5 days):** HGF (20 ng/ml). **Stage 4:** OSM (10 ng/ml), Dex (0.1 μM)	**RT-PCR analysis of** AFP, ALB, CK8, CK18, AAT, HNF4α, PEPCK, TDO, TAT, CYP7A1, CYP2B6, CYP3A4, G6P, **qRT-PCR analysis of** ALB; **IF analysis of** Sox17, FoxA2, CK7, CK8, CK18, CK19, Ki67, AAT, ALB, AFP. **ALB production, LDL-uptake, PAS staining, ICG uptake, PROD assay, animal model transplantation, virus assay**	The first report testing the susceptibility of HLCs by HCV pseudovirus	[16]
Stage 1 (5 days): Activin A (100 ng/ml), 0.5% FBS (3 days), Activin A (100 ng/ml), 2% FBS or KOSR (2 days); **Stage 2 (6 days):** plated on Collagen I-coated plate, FGF4 (1 0 ng/ml),and HGF (10 ng/ml) (3 days), FGF4 (10 ng/ml), HGF (10 ng/ml), MDBK-MM, BSA (0.5 mg/ml) (3 days); **Stage 3 (9 days):** FGF4 (10 ng/ml), HGF (10 ng/ml), OSM (10 ng/ml), Dex (0.1 μM) (9 days)	**RT-PCR analysis of** AFP, ALB, AAT, CYP3A4, CYP7A1. **IF analysis of** Sox17, FOXA2, GATA4, Sox7, AFP, ALB, CD26, AAT, HNF4A, **Immunoblot analysis of** Sox17, FOXA2, GATA4, AFP. **FACS analysis of** CXCR4. **ICG uptake, PAS staining, ALB production, animal model transplantation**	Highly purified HLCs (70% ALB-positive cells). Transplantation and homing of DE into a mouse model were demonstrated	[3]

Table 1 (*continued*)

Differentiation protocol	Hepatic features	Major result(s)	Ref.
Stage 1 (3 days): NaBu (1 mM), Activin A (100 ng/ml) (1–2 days), NaBu (0.5 mM), Activin A (100 ng/ml) (2–3 days); **Stage 2 (7 days):** DMSO (1%); **Stage 3 (7 days):** HGF (10 ng/ml), OSM (20 ng/ml), L15, FBS (8.3%)	**RT-PCR analysis of** Nanog, hTERT, Brachy, GSC, Sox17, FoxA2, HNF4α, AFP, ALB, TAT, TTR, TDO, CAR, ApoF, PAX6, CXCR4, HNF1α, HNF1β, HNF6, CK7, CK18, CK19, Cyp3A4, CYP3A7, CYP2C19, PXR; **IF analysis of** FoxA2, HNF4α, AFP, ALB, HepPar, CK18, CK19, CD13, CYP3A, CPR, c-Met. **flow cytometry analysis of** CXCR4, **Western blot analysis of** FoxA2, HNF4α, AFP, ALB, AAT, c-Met, E-cad, CYP3A, CYP2D6; **Plasma proteins:** fibrinogen, fibronectin, A2M; **PAS staining, CYP activity measurement**	Reporting of basal metabolism activity and export proteins (alpha 2-macroglobulin, fibrinogen, and fibronectin) by HLCs	[45]
Stage 1 (7 days): aFGF (100 ng/ml), FGF4 (10 ng/ml); **Stage 2 (7 days):** HGF (20 ng/ml), FGF4 (10 ng/ml); **Stage 3:** HGF (20 ng/ml), OSM (10 ng/ml), Dex (0.1 μM), ITS	**RT-PCR analysis of** Nanog, HNF3β, HNF4β, C/EBPα, C/EBPβ, CK8, CK18, CK19, TTR, AFP, APOB, AAT, ALB, TDO, TAT, G6P, CYP7A1, **IF analysis of** ALB, CK18, HepPar1; **flow cytometry analysis of** ALB, CK18; **urea, ALB & AFP production, PAS staining, ICG uptake, LDL-uptake, TEM**	Differentiation into functional HLCs in a serum-free adherent culture condition	[8]
Stage 1 (2 days): EB formation; **Stage 2 (3 days):** Plated on 5% Matrigel-growth factor reduced, Activin A (100 ng/ml), bFGF (100 ng/ml); **Stage 3 (8 days):** DMSO (1%), HGF (100 ng/ml); **Stage 4:** Dex (0.1 μM)	**qRT-PCR analysis of** Nanog, SOX17, SOX7, ALB, AFP, CFV||, ASGR1, PDX1, Nestin, Brachury, Pax6, Nkx 2-5, G6P, UGT, oTc, BSEP; **IF analysis of** ALB, AFP, **FACS analysis of** ASGPR, **immunohistochemistry analysis of** AAT, ALB; **AAT & ALB production, animal model transplantation, EM, CYP activity, urea synthesis**	A simple and reproducible protocol for establishing functional HLCs and transplantation of ASGPR1 – enriched cells into Alb-uPA SCID mice	[9]
Stage 1 (2 days): EB formation; **Stage 2 (3 days):** Activin A (100 ng/ml), 1% FBS (in suspension). **Stage 3 (5 days):** Plated on irradiated feeder cells secreting bFGF in HepatoZYME medium; **Stage 4 (6 days):** HGF (20 ng/ml), OSM (10 ng/ml), Dex (0.1 μM)	**RT-PCR analysis of** Sox17, AFP, bFGF, ALB, CK18, CYP1B1; **IF analysis of** Sox17, ALB, AFP; **ICG uptake and release, PAS staining, ALB production, EM**	Induction to the early lineage stage of hepatic fate by co-culturing activin-derived endoderm with feeder cells that secreted bFGF	[102]
Stage 1 (3 days): Activin A (100 ng/ml) + 0% ITS. (1 day), Activin A (100 ng/ml) + 0.1% ITS (1 day), Activin A (100 ng/ml) + 1% ITS (1 day); **Stage 2 (5 days):** FGF4 (30 ng/ml) + BMP2 (20 ng/ml); **Stage 3 (5 days):** N-CAD$^+$ cell sorting, plated on STO feeder, HGF (20 ng/ml); **Stage 4 (5 days):** OSM (10 ng/ml), Dex (0.1 μM)	**RT-PCR analysis of** AFP, CK8, CK18, ALB, AAT, TAT, CYP2B6, CYP3A7, PEPCK, CK7; **IF analysis of** AFP, N-CAD, ALB, HNF4A, GATA4, FOXA2, AAT, CK7; **ALB secretion, PAS staining, ICG uptake, LDL uptake, PROD assay**	The first proliferative bipotential hepatic progenitor cells that have been reported by sorting N-CAD$^+$ cells	[156]
Stage 1: ES cells plated on fibronectin or FBS coated plate, Activin A (10 ng/ml), bFGF (12 ng/ml); **Stage 2 (3 days):** Ly294002 (1 μM), Activin A (100 ng/ml), BMP4 (10 ng/ml), bFGF (20 ng/ml); **Stage 3 (5 days):** FGF10 (50 ng/ml) (3 days), FGF10 (50 ng/ml), RA (0.1 μM), SB431542 (1 uM) (2 days); **Stage 4 (10 days):** HGF (50 ng/ml), FGF4 (30 ng/ml), EGF (50 ng/ml)	**RT-PCR analysis of** Bra, MixL1, Sox17, Sox7, Lhx1, GATA6, CXCR4, Eomes, Hex, **microarray analysis; IF analysis of** Sox17, FoxA2, GATA4, N-cad, AAT, ALB, CK8, CK18; **flow cytometry analysis of** CXCR4, ASGPR, LDLR, c-met, CD49f; **albumin secretion, CYP activity, PAS staining, LDL uptake, ICG uptake, animal transplantation**	Combination of high dosages of Activin, BMP4, bFGF in addition to PI3K inhibitor, Ly294002, lead to a more efficient DE. HLC transplantation in a mouse model with transient block of liver growth, thus allowing for better engraftment of the transplanted cells	[139]
Stage 1 (1-5 days): Activin A (100 ng/ml), bFGF (4 ng/ml) or Wnt3a (50 ng/ml). FBS concentration 0% first day, 0.2% following days; **Stage 2 (6–17 days):** BMP4 (100 ng/ml), bFGF (4 ng/ml). Some experiments: aFGF (100 ng/ml), bFGF (5 ng/ml), BMP2 (50 ng/ml), BMP4 (200 ng/ml); **Stage 3:** Dex (0.1 μM), OSM (10 ng/ml), HGF (20 ng/ml), singleQuots (Lonza)	**RT-PCR analysis of** Sox17, HNF3b, CXCR4, AFP, AAT; **IF analysis of** ALB, AAT, AFP, CK7, CK8, CK18, Ck19. CXCR4, CYP1A2, CYP3A4, EpCAM, HNF3b, HNF1a, HNF4a, Sox7, ICAM1, MRP2, Sox17, LFABP, **Western blot analysis of** AAT, AFP, CYP3A, MRP2, OATP2; **ICG uptake, urea production, PAS staining, CYP activity** (Midazelam, Diclofenac, Phenacetin)	A more realistic DE by a combination of Activin A and bFGF (excluding extra-embryonic endoderm)	[13]

(*continued on next page*)

Table 1 (*continued*)

Differentiation protocol	Hepatic features	Major result(s)	Ref.
Stage 1 (3 days): Activin A (100 ng/ml) (2 days), Activin A (100 ng/ml), B27, NaBu (0.5 mM); **Stage 2 (11–15 days):** Splitting cells in collagen I-coated plate or non-splitting cells, FGF4 (20 ng/ml), HGF (20 ng/ml), BMP2 (10 ng/ml), BMP4 (10 ng/ml) (1 day). The same supplement, DMSO 0.5% (10–14 days); **Stage 3:** FGF4 (20 ng/ml), HGF (20 ng/ml), OSM (50 ng/ml), Dex (100 nM), 0.5% DMSO	**RT-PCR analyses of** CYP1A 1, CYP1B1, CYP2A6, CYP2A7, CYP2B6, CYP2C8, CYP2C19, CYP2B1, CYP7A1, UGT1A3, UGT1A6, UGT1A8, UGT1A10 and **IF analyses of** CXCR4, SOX17, FOXA2, ALB, AAT, AFP, ASGPR, nuclear receptor, nuclear transporter, MRP1, OATP2, AhR, CAR, PXR, CPR, LXR; **flow cytometry analysis of CXCR4,** SOX17, FOXA2, AFP, ALB, AAT, ASGPR; **Western blot analysis of** CYP1A2, CYP3A4, CYP2C9, CYP2D6, UGT1A1, UGT2B7, GST P1-1, GST A1-1; **albumin secretion, ICG uptake, metabolic activity, drug metabolism**	Homogenous population of HLCs (90% albumin positive, 60% ASGPR positive, and 70% AAT positive) which indicates comparable functionality to primary hepatocytes	[28]
	Human-iPS cells		
Stage 1 (3 days): Activin A (100 ng/ml); **Stage 2 (4 days):** FGF4 (30 ng/ml), BMP2 (20 ng/ml); **Stage 3 (6 days):** HGF (20 ng/ml), KGF (20 ng/ml); **Stage 4 (6 days):** OSM (20 ng/ml), Dex (0.1 μM). **Stage 5 (3 days):** OSM (20 ng/ml), Dex (0.1 μM), N2, B27	**RT-PCR analysis of** AFP, Alb, CK8, CK18, CK19, PEPCK, HNF4α, HNF6, CEBPα, GATA4, HEX; **IF analysis of** Sox17, Sox7, CDX2, Foxa2, ALB, AFP, AAT, CYP7A4; **urea synthesis, PAS staining, ELISA:** ALB production, **CYP45:** inductive activity by drug	Hepatoblast expansion step by KGF and FGF10	[122]
Stage 1 (5 days): Activin A (100 ng/ml), Wnt3 (25 ng/ml) (3 days), Activin A (100 ng/ml) (2 days); **Stage 2:** DMSO 1%; **Stage 3:** L15 medium, tryptose phosphate broth (8.3%), FBS (8.3%), hydrocortisone (10 μM), insulin (1 μM), HGF (10 ng/ml), OSM (20 ng/ml)	**RT-PCR analysis of** AFP, Cyp7A1, HNF4a, Cyp1A2, Cyp3A4; **IF analysis of** albumin, E-cadherin, **ELISA:** AFP, TTR, fibrinogen, fibronectin; **CYP activity:** CYP1A2, CYP3A4	The first report on the generation of functional HLCs from human-iPS cells	[130]
Stage 1 (5 days): Activin A (100 ng/ml); **Stage 2 (5 days):** bFGF (10 ng/ml), BMP4 (20 ng/ml); **Stage 3 (5 days):** HGF (20 ng/ml); **Stage 4 (5 days):** OSM (20 ng/ml)	**Oligonucleotide array analysis; IF analysis of** FOXA2, SOX17, GATA4, FOXA2, HNF4a, AFP, ALB; **flow cytometry analysis of** ALB; **PAS staining, ICG uptake, LDL uptake, urea synthesis**	Transplantation of HLCs into the lobe of newborn mice and with demonstration of homing	[118]
This protocol was similar to [139]	**qRT-PCR analysis of** HNF4a, AFP, ALB; **IF analysis of** HNF4a, AFP, CK8, CK18	Hepatic lineage differentiation protocol for ES cells that generated HLCs from iPS cells	[139]
Stage 1 (5 days): Activin A (100 ng/ml), 0.5% FBS or 1% KOSR; **Stage 2 (10 days):** trypsinized and plated on collagen I-coated plate, FGF4 (10 ng/ml), HGF (10 ng/ml) (2 days), minimal Madin–Darby bovine kidney maintenance medium, FGF4 (10 ng/ml), HGF (10 ng/ml); **Stage 3 (10 days):** SingleQuotes (lonza), FGF4 (10 ng/ml), HGF (10 ng/ml), OSM (10 ng/ml), Dex (0.1 μM)	**Flow cytometry analysis of** CXCR4; **IF analysis of** AFP, ALB, AAT, CYP3A4; **PAS staining; CYP activity:** CYP1A2, CYP3A4	Generation of HLCs from hepatocyte-derived iPSc	[71]

a2M: alpha-2-Macroglobulin, AAT: Alpha-1-Antitrypsin, aFGF: acidic Fibroblast Growth Factor, AFP: Alpha Feto Protein, AGPR: Aasialo Glyco Protein receptors, ADH1C: Alcohol dehydrogenase 1C, ALB: Albumin, APOB: Apolipoprotein B, ARG: Arginase, bFGF: basic fibroblast growth factor, BrdU: Bromo deoxyuridine, BSEP: Bile salt export pump, BMP: Bone morphogenetic protein, C/EBP: CCAAT enhance binding protein, CM: Conditioned media, CAR: Constitutive androstane receptor, CF VII: Coagulation Factor VII, CK: Cytokeratin, CM: Conditioned media, c-Met: Mesenchymal-epithelial transition factor, CPR: Cytochrome P450 reductase, DE: Definitive endoderm, DEX: Dexamethasone, DMSO: Dimethyl sulfoxide, EB: Embryoid body, E-cad: E-cadherin, EGF: Epidermal growth factor, ELISA: Enzyme linked immunosorbent assay, EM: Electron microscopy, EROD: Ethoxyresorufin-O-deethylase, FACS: Flow cytometry activated cell sorting, FBS: Fetal bovine serum, FGF4: Fibroblast growth factor, GF: Growth factor, G6p: Glucose 6-phosphatase, GFP: Green fluorescence protein, GSC: Goosecoid, HGF: Hepatocyte growth factor, HLC: Hepatocyte-like cell, HNF: Hepatocyte nuclear factor, ICG: Indocyanin green, IF: Immuno fluorescence, ITS: Insulin/transferrin/selenium, KGF: Keratinocyte growth factor, Ly294002: PI3 kinase inhibitor, N-CAD: N-cadherin, NGF: Nerve growth factor, NaBu: Sodium butyrate, OTC: Ornithine transcarbamylase, OSM: OncostatinM, PAS: Periodic acid-Schiff, PEPCK: Phospho enol pyruvate carboxykinase, PROD: Pentoxyresorufin, RA: Retinoc acid, SB431542: TGF-beta inhibitor, TAT: Tyrosine amino transferase, TDO: Tryptophan-2,3-dioxygenase, TEM: Transmission electron microscopy, TERT: Telomerase reverse transcriptase, TF: Transferin, TTR: Transthyretin, UGT: Bilirubin-UDP glucuronosyl transferase.

It was shown that HLCs could also be generated from human-iPS cells [71,118,122,130,139] (Table 1), demonstrating the efficacy of these approaches with pluripotent stem cells of diverse origins. Here, the term *in vitro* HLCs indicates some of the properties of mature hepatocytes (Table 1). The characteristics of stem cell-derived hepatocytes produced from various differentiation protocols have been critically reviewed [47,107]. The differentiated cells should be assessed by comparing them with primary liver-derived

cells for morphology and the expression of a set of proteins such as α-fetoprotein and albumin. However, ultimately, *in vitro* proof that mature HLCs have been produced need demonstration of functional hepatocyte properties such as nutrient processing, detoxification, plasma protein synthesis, and engraftment, after transplantation into a suitable animal model [107].

Human-iPS cells as disease models

The use of animal models is one of the established ways to study the mechanisms of liver diseases and possible therapeutic applications. Several genetic disorders which involve hepatocytes have been modeled in rodents and large animals (Supplementary Table 1). Although these models of human congenital and acquired diseases are invaluable, they provide a limited representation of human pathophysiology. Animal models do not always faithfully mimic human diseases, particularly those for human contiguous gene syndromes. For example, mice carrying the same genetic deficiencies as Fanconi's anemia patients do not develop spontaneous bone marrow failure which is the hallmark of the human disease [19]. Huntington's disease patients show dyskinesia (involuntary movements), whereas mice do not [106]. Moreover, many genetic diseases within the liver do not have hot spots nor do they arise from mutations within a single gene. For example, the gene for α-1 antitrypsin has been mapped on the long arm of chromosome 14 and, to date, although as many as 100 alleles have been found for α-1 antitrypsin deficiency, only a small number of them are associated with liver disease [32]. In addition, it is difficult to undertake genetic studies of human diseases in animal models when the genes involved are unknown [4].

Researchers have tried to overcome these problems by using human cell cultures along with animal models as essential complements to human disease studies. Primary human cells have a limited life span in culture. As a result, most human cell lines in wide use today carry genetic and epigenetic artifacts which arise from their accommodations to tissue cultures and are derived either from malignant tissues or have been genetically modified in order to drive immortal growth [37]. Indeed, many human cell types have never faithfully been adapted for growth *in vitro*. Human embryos which have been shown to carry genetic diseases by virtue of preimplantation genetic diagnoses can yield ES cell lines that model single-gene disorders [142]. For example, human ES cell lines have been generated for disorders such as cystic fibrosis [80], Huntington's disease [80], and Fragile X syndrome [30]. However, the vast majority of diseases that show more complex genetic patterns of inheritance are not represented in this pool.

The ability to create pluripotent stem cell lines from patients exhibiting specific diseases may facilitate the construction of a library of iPSCs. As such, disease-specific human-iPS cells provide an unprecedented opportunity to recapitulate and investigate human pathologies *in vitro*. Several groups have successfully derived a wide range of iPS cells from patients with diseases (for review see [112]). Therefore, it is possible to generate iPS cells from patients who have inherited liver diseases. These cells can be used as instruments to study the pathogenesis, disease mechanism(s) and possible cures for inherited liver disorders. However, the demonstration of disease-related phenotypes and the ability to model pathogenesis and treatment of disease remain key challenges in this field. It should be noted that many diseases might be non-autonomous with the involvement of more than one cell type in the disease process. One possible solution would be the simultaneous culture of several cell types. Another problem is the time frame which disease symptoms are manifested. For example, amyotrophic lateral sclerosis (ALS) or Parkinson's disease (PD) symptoms may take years to develop.

Role of human-iPS cells in drug development and toxicity evaluation

According to a report by the US Food and Drug Administration, "a 10-percent improvement in predicting failures before clinical trials could save $100 million in development costs per drug." [1]. Therefore, there is increasing demand for new *in vitro* models to improve drug development by narrowing selected target drugs or eliminating toxic and non-effective drugs during the first stages of development. A cell-based assay should be simple, fast, reproducible, and cost effective with a consistent supply. Traditionally, whole embryo cultures and cells for drug research and analysis have been derived from animal or human tissues; either as primary cultures in which batch-to-batch variability is a common problem or as immortalized lines which often harbor uncharacterized genetic abnormalities [124]. The mouse ES cell test [14,99] is also one of the most recently developed tests used to assess the embryotoxic potentials of test chemicals, however, it may not entirely mimic human pharmacological risk assessment due to differences in xenobiotic biotransformation pathways and capacities. Human ES cells can be utilized as *in vitro* models for drug development and toxicology analyses, as well [42,55]. Today approximately 70% of the top 20 pharmaceutical companies utilize stem cells in their research and among these, 64% use human ES cells or their derivatives [22,43,45,55]. Although of value, human ES cells and their derivatives do not encompass all the variances within a population or all ethnicities. The generation of iPS cells from individuals will more faithfully represent the highly polymorphic variants in metabolic genes of human populations and provide the pharmaceutical industry with a unique opportunity to revolutionize toxicological assays [17,113,130]. In support of this, human iPS cell-derived cardiomyocytes have recently been used to study the effect of cardioactive drugs [135,151]. Moreover, Lee et al. have used neural disease familial dysautonomia (FD)-iPS cells for validating the potency of candidate drugs in reversing aberrant splicing and ameliorating neuronal differentiation and migration of FD [68].

The liver is the primary organ involved in drug metabolism and therefore one of the most common tissues affected by drug toxicity [45]. Although there are no current studies, iPS cell-derived hepatocytes could soon replace hepatocytes or hepatoma cell lines in drug toxicity screening assays [39,45]. Hepatocytes generated from iPS cells that have been derived from individuals with different cytochrome p450 polymorphisms would be of immense value for predicting potential liver toxicities of new drugs in patients [148]. Additionally, liver disease-specific iPS derived hepatocytes can be utilized to discover the effects of new drugs on specific disorders before proceeding to animal studies and clinical trials.

Human iPS cell-derived hepatocytes in a bio-artificial liver design

Extracorporeal bio-artificial liver (BAL) systems aim to bridge patients until a suitable donor organ becomes available for whole organ or cell transplantation, or liver regeneration. BAL systems consist of viable hepatocytes in different perfusion bioreactor conformations. The patient's blood perfuses through one of these systems, theoretically compensating for the liver's vital functions.

Different BAL systems are currently undergoing clinical trials [34,81,83,108,141]. Although these devices have bridged a number of patients to transplantation, [25,108,141] their efficiency should be enhanced. Among the considerations that are necessary for the development of BAL devices (for review see [100]), the cellular component plays a critical role. To date, the different cell types that have been used in various BALs include primary porcine or human hepatocytes [101], cell lines [105], fetal liver cells [87], and ES cell-derived cells [123]. Primary hepatocytes often retain their differentiated functions for a short duration *in vitro*. In addition, immunogenic reactions resulting from the xenogenecity of porcine hepatocyte products and the possibility of xenozoonotic retroviral infection of patients with porcine endogenous retrovirus (PERV) [144] are major drawbacks to using these cells. Primary human hepatocytes, on the other hand, are not available in sufficient amounts needed for clinical BAL usage. Continuous cell lines, as the other cell source, often lose functions that cells possess *in vivo* [97].

Human ES cells and iPS cells, however, show great promise as cell sources for BAL devices. Self-renewal and the high potential to differentiate into all cell types, including hepatocytes, make them good candidates. Optimization of the current differentiation protocols can allow these cells to substitute for presently used cell sources.

Human-iPS cells and tissue engineering: founders of personalized regenerative medicine

Tissue engineering approaches facilitate the pathway from laboratory to clinic. The tissue engineering technique, which mimics *in vivo* conditions, leads to maximum cellular function *in vitro* [75] and eases the scale-up cultures which are important for transplantation strategies or extracorporeal bio-artificial livers.

Under monolayer culture conditions hepatocytes lose their liver specific functions within a few days [21]. Therefore, researchers have studied various extracellular matrix (ECM) compositions, scaffolds and bioreactor designs, such as galactosylated polymers [20,149], spheroid cultures [137] and perfusion bioreactors [24,117] to enhance primary hepatocyte function *in vitro* [91,131]. Differentiation of human ES and iPS cells into hepatocytes in spheroid cultures [86] or in perfusion bioreactors and culturing ES/iPS cell-derived HLCs onto galactosylated polymers could one approach to enhance the yield and functionality of differentiated cells. Additionally, tissue engineering may assist in deriving more mature HLCs from ES and iPS cells within rationally tailored three-dimensional microenvironments [79] which simulate the natural ECM and maintain mature, functional hepatocytes [33]. In recent years, studies have demonstrated the effects of different ECMs or bioreactor configurations on the hepatic differentiation of ES cells [7,54,86], however additional research should be undertaken to determine the optimum conditions necessary for maturation of ES cell-derived hepatocytes.

The combination of tissue engineering with iPS cells as a personalized and easily expandable cell type shows great potential for the treatment of multiple liver diseases. By using this approach, hepatic induction of iPS cells would be the first step in personalized treatment for end stage liver diseases either through cell therapy, BAL treatment, or by personalized drug screening; all of which have been discussed in detail in this review.

Human iPS cell transplantation for the treatment of liver disease

Hepatocyte transplantation has recently been used as an alternative to orthotopic liver transplantation (OLT) in patients with liver-based congenital metabolic disorders such as: alpha-1 antitrypsin deficiency [129], urea cycle defects [49,128], Crigler–Najjar I [5,35,59], glycogenosis type Ia [89], Refsum disease (heredopathia atactica polyneuritiformis), coagulation factor deficiency [119], argininosuccinate-lyase deficiency [126], and factor VII deficiency [26]. Promising results and partial correction of disease symptoms have been obtained from most of these studies [5,26,35,59,89,119,126]. However, the metabolic improvement does not persist and therefore it is necessary to repeat hepatocyte infusions [126,150].

The routine clinical deployment of cell transplantation is further complicated by the scarcity of transplantable allogeneic hepatocytes, their variable engraftment rates and the difficulty in monitoring allograft rejection. Moreover, *in vitro* expansion of mature hepatocytes is not feasible because long term cultivation of hepatocytes results in a reduction in hepatocyte metabolism.

Although limited clinical success has been reported with banking cryopreserved hepatocytes [109,127], this may not alleviate cell shortages since human hepatocytes are easily damaged during the freeze-thaw procedure. Hence, there is a need for additional cell sources such as stem or progenitor cells that can be expanded and differentiated into hepatocytes. Many studies have shown the therapeutic potential of various stem cells (for review see [29] and Supplementary Table 2). The iPS cells are merging as the most promising source for the derivation of truly isogenic grafts. Differentiated derivatives of iPS cells that ameliorate a range of diseases in animal models have been reported. For instance, human iPS cell-derived neural progenitors transplanted into the brains of rats with PD generated functional dopamine neurons [15] and human iPS cell-derived cardiomyocytes have demonstrated *in vivo* functional integration in animals with infarcted hearts [90]. Moreover, mouse iPS cells have been used as sources for transplants to restore auditory spiral ganglion neurons [96]. Transplantation of mouse iPS cell-derived endothelial cells and endothelial progenitor cells into the livers of irradiated hemophilia A mice have increased survival rates and plasma factor VIII [146]. To date, there is no report on the transplantation of iPS cell-derived hepatocytes into animal models, but successful differentiation of human-iPS cells into hepatocytes [118,122,130] has paved the way for the future application of patient-specific iPS cells to be utilized as cell therapies for liver diseases.

Human iPS cell therapies for hereditary metabolic liver diseases

Cell therapy of hereditary liver diseases (Supplementary Table 3) with patient-specific iPS cells would require ex vivo gene correction before (somatic cell) or after reprogramming (Fig. 1). There are reports of successful gene therapies with different cell types, including ES cells and hepatocytes, that can overcome genetic disorders such as familial hypercholesterolemia, Gligler–Najjar, hypercholestasis and albumin deficiency [53,63,66]. In a liver-directed gene therapy study of patients with homozygous familial hypercholesterolemia, Grossman et al. [38] have demon-

strated the feasibility of engrafting limited numbers of retroviral-transduced hepatocytes. In this study, the results have shown a lack of toxicity and persistent gene expression lasting at least four months following gene therapy. Successful reports of human hepatocyte genetic correction, using lentiviral vectors, followed by transplantation in the Gunn rat [94] [10,92] and nonhuman primates [82] have been published. These findings confirm the potential feasibility of gene therapy by using lentiviral vectors to treat liver-based inborn errors of metabolism, which are a prerequisite to clinical applications (for review see [93]).

Patient-specific iPS cells are considered a promising alternative for an ex vivo gene therapy approach. With this strategy, mouse iPS cells have been successfully derived from a mouse model of sickle cell anemia. The defective gene was replaced by homologous recombination at the beta-globin locus prior to the generation of hematopoietic stem cells. Treated iPS cells subsequently differentiated into hematopoietic precursors which have been successfully used in the sickle cell mouse [41]. Raya et al. corrected the genetic defect existent in somatic cells of Fanconi's anemia patients and generated iPS cells that have the potential to differentiate into hematopoietic progenitors of phenotypically normal disease-free myeloid and erythroid lineages [104]. These data offer proof of concept that iPS cell technology can be used to generate patient-specific cells with the potential value for cell therapy applications and curing diseases.

Induced liver progenitor cells/hepatocytes

To generate iPS cells, somatic cells must first be completely dedifferentiated into pluripotent stem cells and then subsequently redifferentiated into the adult cell type of interest; a time-consuming procedure that risks teratoma formation of pluripotent stem cells. If the generation of either somatic stem or progenitor cells, or fully differentiated cells directly from fibroblasts or other types of somatic cells could be achieved, it might not be necessary to produce iPS cells and therefore the risk of teratoma formation would be eliminated. The reprogramming of mouse and human fibroblasts into iPS cells with a combination of reprogramming factors has raised the question of whether transcription factors could directly induce other defined somatic cell fates. A remarkable breakthrough that addressed this question was achieved when the transient activation of three transcription factors (Ngn3, Pdx1, and Mafa) induced the direct reprogramming of adult mouse pancreatic exocrine cells into insulin-producing endocrine cells with robust (20%) efficiency [159]. Motoyama et al. showed reprogramming of adult hepatocytes into insulin-producing cells by nucleofection of non-viral bisictronic vectors including Pdx1 and Ngn3 [88]. However, in these and most other studies, the reprogramming occurred only between closely related cell lineages. Recently, Veirbuchen et al. set out to determine whether specific transcription factors could directly reprogram fibroblasts into functional neurons [143]. They began with a set of 19 candidate transcription factors involved in neuronal development or function. Eventually a combination of only three transcription factors (Ascl1, Brn2, and Myt1l) that rapidly and efficiently converted mouse embryonic and postnatal fibroblasts into functional neurons *in vitro* was discovered.

Although transdifferentiation may eventually replace current technologies for generating iPS cells and ES cells, future studies will be necessary to determine whether induced differentiated cells could represent an alternative method to generate patient-specific hepatocytes.

Challenges in the application of human-iPS cells for therapy

Although iPS technology offers multiple treatment opportunities, substantial technical advances are necessary before clinical applications can be considered.

Collection and establishment of iPS cells under GMP guidelines

The starting cell material should be obtained and processed in conditions that are acceptable under good manufacturing practice (GMP) guidelines from rather young donors in order to avoid accumulation of environmental DNA damage. A recent report indicates that banked cord blood samples might offer a solution to this problem and provide "naive" cells as starting material for the future generation of clinical grade iPS cells [36,40]. Furthermore, the maintenance, expansion, and differentiation of iPS cells will require GMP compatible cell culture conditions which need to avoid cells, chemicals and proteins that are from animal sources. Recent reports have shown that iPS cells can be generated in serum and feeder-free conditions and maintained in chemically defined and xenobiotic-free media and supplements [132,138].

However, whether there are physiological differences between human-iPS cells established and grown under standard conditions versus feeder- and serum-free conditions remains unknown.

Generation of iPS cells without genetic modifications

One major challenge is to design methods that involve minimal modification to the genome and generate safer iPS cells for cell therapy (for review see [112]). Virus-mediated delivery of reprogramming factors could result in potential harmful genomic alterations. Several groups have reported the derivation of iPS cells by integration of the four reprogramming factors with the use of plasmids [56] and lentiviruses [120] followed by removing transgene sequences from the host genome using Cre-lox mediated excision (Fig. 1). The combination of a single-vector system and piggyBac transposon has been utilized for reprogramming mouse embryonic fibroblasts which resulted in the seamless elimination of vector and transgene sequences from iPS cells by transposase re-expression [56,145] (Fig. 1).

Removal of the reprogramming vector increased the similarity of iPS cells to ES cells [120] and markedly improved the developmental potential and differentiation capacity of iPS cells [121]. iPS cells were also generated without viral integration by exploiting non-integrating viruses or plasmids to introduce reprogramming factors [98,125,152].

The protein transduction approach has been suggested as an alternative to a nucleic acid-based approach for the reprogramming of somatic cells into iPS cells without genetic manipulation. It has been demonstrated that protein transduction domains, also called transduction peptides or cell-penetrating peptides (CPP), allow the cargo protein to enter the cell when fused to them. The generation of stable iPS cells from mouse fibroblasts has been reported by protein transduction of Oct4, Sox2, Klf4, and c-Myc fused with a CPP in combination with a histone deacetylase inhibitor, valproic acid [158]. However, human-iPS cells have

been generated by exposing human neonatal fibroblasts to cell extracts from HEK293 cell lines that expressed high levels of the four reprogramming factors in the absence of additional chemicals [60] (Fig. 1). In contrast to the prior study, the efficiency of iPS cell generation is low in the protein transduction approach and requires further optimization. The application of small molecules to increase reprogramming efficiency may also facilitate reprogramming with a virus-free approach [52,115] (Fig. 1). Using a high-throughput screening of exogenous factors and small molecules, Jaenisch and colleagues identified a small molecule, kenpaullone, that can replace Klf4 in the formation of mouse iPS cells [76,113,67]. The screening of small molecules that can contribute to the generation of iPS cells may eventually lead to the development of fully chemically defined conditions for the production of iPS cells.

The application of protein transduction and small molecular approaches possibly represent a significant advance in generating iPS cells, effectively eliminating any risk of modifying the target cell genome by exogenous genetic sequences which have been associated with all previous iPS cell methods, and consequently offer a method for generating safer human-iPS cells. Moreover, removal of the reprogramming vector has increased the similarity of iPS cells to ES cells [120] thus markedly improving their developmental potential and differentiation capacity [121]. Although promising, the efficiency of iPS cell generation is low in both methods and requires further optimization. In particular, the concentrations of the individual factors need to be calibrated to approximate normal endogenous levels. Additionally, strategies for exploiting endogenous gene expression in certain cell types has also allowed for easier reprogramming and/or fewer required exogenous genes [60,61,115].

Target cell purification before clinical application

Assuming that ES and iPS cell-derived progenitors of mature cells or the final differentiated cells used for transplantation are successfully derived in a quantity and quality appropriate for transplantation; it will still be necessary to remove undifferentiated iPS cells and other cell types that have the potential to form tumors *in vivo* (for review see [62,64,73]).

The most direct strategy is to purify cells using fluorescence activated cell sorting (FACS). The application of this method relies on the expression of a cell-type-specific reporter (e.g. promoter driving expression of a fluorescent protein) or by cell-surface molecules recognized by antibodies such as the anti-cell membrane hepatocyte marker, ASGPR [9]. The cells could be sorted via progenitor cell specific markers (positive sorting) or stem cell specific markers (negative sorting). Consequently, it is highly desirable to identify suitable markers for the purification of human cells for clinical applications. In addition, antibody production and transfection reagents will require standardization to comply with FDA regulations. The cell-sorting procedures will also need to be carried out with dedicated flow cytometers that are free from exposure to xenobiotics. Introduction of a stem cell specific suicide gene to eliminate stem cells is another strategy to purify a given cell type [62,64,73].

Increasing the efficiency of reprogramming

Within three years of publication of the first iPS cells in 2006 [134], much progress has been made towards their generation and applications. However, since the generation of the first iPS cells, attempts have been made to increase the efficiency of fully-reprogrammed iPS cells for therapeutic applications.

These include the application of various sets of reprogramming factors [70,77], reprogramming different somatic cell types [2,31,140], application of chemicals/small molecules to increase efficiency, replacing reprogramming factors [51,88,114], and the use of hypoxia conditions [65]. Nevertheless, the generation of iPS cells has, so far, been an inefficient process (<2%) resulting in a heterogeneous population of cells which makes the identification of fully-reprogrammed iPS cells challenging. More recently, several groups have shown that the inactivation of p53 remarkably increased the efficiency of iPS cellular generation [48,58,78,140]. However, the inactivation of p53 may result in low quality iPS cells, thus causing genomic abnormalities. Given the rapid advancements within the iPS cell field, it is most likely that highly efficient, safe iPS cells will be routinely generated in the near future.

Patient-specific iPS cells: custom- versus ready-made iPS cells

A potential benefit would be the ability to generate patient-specific iPS cells that can be transplanted without the concern of rejection or the need for immunosuppressive drugs. However, custom-made iPS cells may not be available any time soon. Even if the capability to generate safe iPS cells becomes available, the generation and expansion of iPS cells would take a couple of months. Moreover, several more months will be needed to differentiate iPS cells into the required cell types and to subsequently expand them. Subsequently, several steps should be taken to ensure the cells are safe for transplantation and do not form tumors.

All in all, the generation of custom-made iPS cells will be a very costly process and therefore only available to a subset of the population. Additionally the process is presently too slow for the treatment of disorders such as spinal cord injuries which would need prompt treatment. Therefore, ready-made iPS cells have been proposed as a solution. Japanese scientists are preparing a national library of therapy-ready iPS cell lines. It has been estimated that 50 well-chosen cell lines could provide close immunological matches for 90% of the Japanese population.

Therefore, patients who require urgent treatment could use the best immunological match, whereas people with chronic disorders might chose to have an iPS cell line that is patient specific, provided they could afford it [23].

Generation of fully-reprogrammed iPS cells

It has been shown that some of the iPS clones are only partially reprogrammed into an ES cell state [18,74]. Shutdown of the exogenously expressed transcription factors which can be detected early during reprogramming is one of the most specific single markers of reprogramming success. Lowry et al. have reported in their research, that some of the human iPS clones generated were only partially reprogrammed to an ES cell state whereas other clones appeared faithfully reprogrammed, as measured by their gene expression program and their inability to form embryoid bodies [74]. Recently, Chen et al. identified three distinct colony types which morphologically resembled ES cells but differed in molecular phenotype and differentiation potential [18]. They demonstrated that incompletely reprogrammed colo-

nies exhibited some features of reprogramming; including down-regulation of fibroblast markers, expression of pluripotency markers, changes in the histone modification state, formation of ES cell–like morphology, and the ability to differentiate into teratoma-like tumors. According to Chen et al., SSEA-4, alkaline phosphatase, hTERT or GDF-3 were insufficient to reliably distinguish a fully reprogrammed from a partially reprogrammed state whereas proviral silencing and expression of TRA-1-60, DNMT3B and REX1 were suggested as markers to distinguish between fully and partially reprogrammed iPS cells. Clearly more work should be undertaken for the rigorous characterization and standardization of putative iPS cells [18].

Are iPSCs and ESCs functionally equivalent?

An important outstanding question is the extent to which iPS cells and ES cells are functionally equivalent. The first mouse iPS cells generated by Yamanaka and Takahashi were similar to ES cells in the expression of certain ES cell marker genes, morphology, proliferation, and the formation of teratomas but these cells failed to produce adult chimeric mice and showed a different global gene expression [134]. Germline transmission with mouse iPS cells has been reported [11,157]. However, several studies have shown that although human-iPS cells are quite similar to human ES cells, they are clearly not identical. Yamanaka and his colleagues reported at least 1267 genes that display a greater than five-fold difference in expression levels in iPS cells when compared with human ES cells [133]. Chan et al. applied genome-wide methods to compare mouse and human-iPS cells with ES cells' subkaryotypic genome alteration, mRNA, small noncoding RNA expression and histone modification profiling [18]. They showed that iPS cells represented a unique type of pluripotent cell as defined by gene expression and should be considered as a unique subtype of pluripotent cell.

Recently, it has been shown that removal of the reprogramming vector increased the similarity of iPS cells to ES cells [120] and markedly improved the developmental potential and differentiation capacity of iPS cells [121]. The detailed implications of these differences between iPS cells and ES cells and their functional role in self-renewal or differentiation are unknown and need extensive investigation.

Cell source for reprogramming

Another important issue facing researchers concerns the varied origins of iPS cells which may affect their propensities to differentiate and safety. Certain cell types may be better suited for complete reprogramming with reduced risks of teratoma formation [6,85]. It has been assumed that the production of pancreatic-β cells and hepatocytes from somatic cell-derived iPS cells of an endodermal origin, such as gastric epithelial cells or hepatocytes, may be more effective; however, direct evidence for this assumption is lacking. Recently, Miura et al. have reported that hepatocyte and gastric epithelial-iPS cell clones fail to generate neurospheres while fibroblast-iPS cells are efficient neurosphere producers [85]. Notably, iPS cells derived from epithelial cells and tissues such as the liver, gastric mucosa [6], and skin [2] have less retroviral integration than iPS cells derived from fibroblasts. Those iPS cells which have been derived from mouse hepatocytes or human keratinocytes show fewer retroviral integration sites when compared with iPS cells derived from fibroblasts. More recently, the generation of iPS cells from human hepatocytes was reported [71], however, more studies should examine whether hepatocytes from these iPS cells offer any advantages over differentiated cells from human ES cells or from iPS cells of other origins.

Concluding remarks

Human-iPS cells bypass the ethical and immunological concerns associated with human ES cells. They represent an unlimited resource for *in vitro* modeling and the development of patient-specific medicines suitable for treating a number of deficiency states, including human liver disease. That being said, human iPS cell technology is still in its infancy and a number of hurdles need to be overcome before cell therapies become a reality. These include: (i) generation of iPS cells without viral integration, (ii) increasing the efficiency of iPS cell production such that enough cells will be generated for iPS cell screening and quality evaluation, (iii) differentiation of iPS cells into desired cell types that are comparable to their *in vivo* counterparts, (iv) isolation of differentiated cells with sufficient purity, and (v) the proper homing and function of iPS cells post-transplantation.

Given the rapid pace of developments within the iPS cell field, it is likely that the generation of safe and effective iPS cells for use in cell therapy will be achieved in the near future.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript. The authors who have taken part in this study do not have a relationship with the manufacturers of the drugs involved either in the past or present and did not receive funding from the manufacturers to carry out their research.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2010.05.009.

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