Review

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The potential of induced pluripotent stem cell derived hepatocytes

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Summary

Orthotopic liver transplantation remains the only curative treatment for liver disease. However, the number of patients who die while on the waiting list (15%) has increased in recent years as a result of severe organ shortages; furthermore the incidence of liver disease is increasing worldwide. Clinical trials involving hepatocyte transplantation have provided encouraging results. However, transplanted cell function appears to often decline after several months, necessitating liver transplantation. The precise aetiology of the loss of cell function is not clear, but poor engraftment and immune-mediated loss appear to be important factors. Also, primary human hepatocytes (PHH) are not readily available, de-differentiate, and die rapidly in culture. Hepatocytes are available from other sources, such as tumour-derived human hepatocyte cell lines and immortalised human hepatocyte cell lines or porcine hepatocytes. However, all these cells suffer from various limitations such as reduced or differences in functions or risk of zoonotic infections. Due to their significant potential, one possible inexhaustible source of hepatocytes is through the directed differentiation of human induced pluripotent stem cells (hiPSCs). This review will discuss the potential applications and existing limitations of hiPSC-derived hepatocytes in regenerative medicine, drug screening, *in vitro* disease modelling and bioartificial livers.

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Introduction

The demand for a stable source of functional hepatocytes is continuously growing, in addition to the requirement for viable organs for transplantation [1]. The former is due to the increased use of hepatocytes in various applications including drug screening, disease modelling and cell based therapies.

From the clinical perspective, transplantation of hepatocytes may represent an alternative to orthotopic liver transplantation (OLT) for specific life-threatening liver diseases. The aim of hepatocyte transplantation (HT) in such diseases, where the liver is normal, is to partially replace the missing function without the need to replace the whole organ. In this circumstance, the transplanted hepatocytes have both the time and the opportunity to engraft. The types of liver diseases can be broadly grouped into three categories:

(i) *Chronic liver diseases due to metabolic genetic disorders*. Metabolic diseases account for 26% of the indications for paediatric orthotopic liver transplantation in Europe (ELTR: www.eltr.org/). The successful replacement of deficient liver functions by transplantation of healthy hepatocytes has been reported in patients with Crigler-Najjar syndrome due to UGT1 enzyme deficiency, familial hypercholesterolemia due to low density lipoprotein receptor (LDLR) deficiency, and with urea cycle enzyme deficiencies [1]; the main cause to date is children with urea cycle defects. These diseases represent the major clinical application of cell therapy. It has been recently reported that inherited metabolic disorders could also be the

most common group of disorders that cause acute liver failure (ALF) in paediatrics. However, the use of HT in these cases has not yet been discussed [2].

(ii) *ALF that does not damage normal tissue architecture but is associated with direct injury and loss of hepatocytes.* HT in patients with ALF aims to restore liver function for a period of time, bridging to OLT or until the native liver regenerates. To provide sufficient function, the number of cells required for the treatment of ALF needs to be higher than for metabolic disorders. The severity of liver dysfunction requires that the transplanted hepatocytes function immediately, but it remains unclear if hepatocyte engraftment can occur on a clinically relevant time scale. Although restoration of liver function with full recovery of patients has been reported, in most published cases HT did not affect the clinical outcome of these patients. Encapsulation of hepatocytes in alginate microbeads which allows the cells to be transplanted intraperitoneally with the advantage of avoiding immunosuppression or extracorporeal artificial liver devices could offer a "bridge" allowing endogenous liver to recover spontaneously [1,3].

(iii) *Chronic liver failure with accompanying cirrhosis characterized by expansion of extracellular matrix, loss of fenestrae, widespread tissue damage and scar-based remodelling.* HT has also been used in patients with chronic liver disease with variable outcomes probably due to the presence of fibrosis, which makes it difficult for cells to cross the sinusoidal barrier and engraft. To date ectopic sites of cell transplantation are being considered [4].

The current benchmark cell type for all the applications envisioned for hepatocytes is freshly isolated primary human hepatocytes (PHHs). Isolation of hepatocytes competes with organs for OLT. Therefore, current sources of tissue for hepatocyte isolation are mainly adult organs that are unsuitable for OLT, or "healthy" livers, that have often already undergone aggressive therapies, or tissue surrounding excised tumours, and are often of a marginal quality. These cells are in limited supply, suffer from batch to batch variability and exhibit declining function in culture. In an effort to overcome the limited supply of PHHs, various groups have investigated the ability to cryopreserve PHH. Despite progression in these processes, cryopreservation still has detrimental effects on the viability and metabolic function of hepatocytes [5,6]. PHHs also lose their ability to proliferate when isolated from the normal *in vivo* microenvironment.

The problems with PHH quality and availability are not limited to cell therapy applications. Indeed, rapid loss of functionality in culture and batch to batch viability pose significant challenges for their use in drug screening and disease modelling, as long term analysis and reproducibility are mandatory for the generation of accurate datasets. Absence of proliferation and reduced functions of thawed hepatocytes hinder their potential for cell based therapies as large amounts of hepatocytes are required per treatment ($1–3 \times 10^8$ cells/kg body weight). The cells should also be readily available when needed for emergency treatment of ALF, and for planned and repeated treatment of liver-based metabolic disorders. The other existing sources of hepatocytes are tumour-derived human hepatocyte cell lines, immortalised human hepatocyte cell lines, adult stem/progenitor cells, foetal progenitors [7]. Each of these sources also suffer from one or a combination of various limitations such as reduced metabolic function, short term survival in culture, limited availability and proliferation ability [5,6,8], which hampers their use in the different applications. The HepaRG cell line represents an improvement over previous cell line models in terms of hepatocyte functions, particularly in CYP function and nuclear receptor pathways [9]. However, this cell line is derived from a tumour, resulting in reduced sensitivity to toxic insult. As for all hepatoma cell lines, it is also limited to a single genotype available for toxicity investigations, and thus is not representative of a broad patient population. Xenogeneic primary hepatocytes, in addition to the zoonotic risk by porcine endogenous retroviruses (PERVs) [10], also differ phenotypically from PHHs, resulting in different susceptibility to pathogens, drug metabolism and transporter activity, and are therefore regarded as unsuitable for many *in vitro* applications.

A large amount of research is being carried out focusing on the generation of a stable source of viable and functional hepatocytes. One possible inexhaustible source of hepatocytes is human induced pluripotent stem cells (hiPSCs) which have the capacity to indefinitely self-renew and to differentiate into all cell types, excluding extra-embryonic tissues. Compared to human embryonic stem cells (hESCs), hiPSCs have less ethical concerns and can be generated from unlimited sources with varying genetic backgrounds [11]. Therefore, hiPSC-derived hepatocytes (hiPSC-HEPs) hold significant potential as promising tools for drug discovery, cell therapy, *in vitro* disease modelling and bioengineered livers (Fig. 1). In addition to describing the use of hiPSC-HEPs in the mentioned applications, the review will also cover the current developments in the direct differentiation of hiPSCs into functional hepatocytes. The limits of using

Abbreviations: (h)iPSC(s), (human) induced pluripotent stem cell(s); hESC(s), human embryonic stem cell(s); hiPSC-HEP(s), hiPSC-derived hepatocyte(s); A1AT, α1-Antitrypsin; WD, Wilson's disease; FH, familial hypercholesterolemia; FTA, familial transthyretin amyloidosis; GSD1a, glycogen storage disease type 1a; ER, endoplasmic reticulum; LDLR, Low Density Lipoprotein Receptor; Dil-LDL, 3, 3′-dioctadecylindocarbocyanine-low density lipoprotein; HCV, hepatitis C virus; GMP, good manufacturing practices; BMP4, bone morphogenic protein 4; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; HNF4α, hepatocyte nuclear factor 4α; CYP, cytochrome p450; CHIR99021, GSK-3 Inhibitor; hPSC-HEP(s), human pluripotent stem cell derived hepatocyte (s); HLA, human leukocyte antigen; TALENs, transcription activator-like effector nucleases; CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated endonuclease 9; uPA, urokinase-type plasminogen activator; 2D or 3D cultures, 2 or 3 dimensional cultures; LSEC(s), liver sinusoidal endothelial cell(s); BAL, bio-artificial liver; iPSC-HE, hiPSC-derived hepatic endoderm; HUVECs, human umbilical vein endothelial cells; iPSC-LB, iPSC-derived liver buds; iPSC-MH, "monolayer hepatocytes" conventionally 2D differentiated from iPSCs by contrast with iPSC-LB; ECM, extracellular matrix; UPD, uniparental disomy; CGH-array, comparative genomic hybridization-array; SNP, single nucleotide polymorphism; CNV(s), Copy Number Variation(s); PCSK9, Proprotein Convertase Subtilin/Kexin 9.

Key point

Hepatocyte transplantation now appears as a credible and promising bridge before, or even as an alternative to orthotopic liver transplantation in many life-threatening liver diseases but is hampered by cell shortage.

Review