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Stem cell-derived hepatocytes as a predictive model for drug-induced liver injury: are we there yet?

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Amongst the different types of adverse drug reactions, drug-induced liver injury is the most prominent cause of patient morbidity and mortality. However, the current available hepatic model systems developed for evaluating safety have limited utility and relevance as they do not fully recapitulate a fully functional hepatocyte, and do not sufficiently represent the genetic polymorphisms present in the population. The rapidly advancing research in stem cells raises the possibility of using human pluripotent stem cells in bridging this gap. The generation of human induced pluripotent stem cells via reprogramming of mature human somatic cells may also allow for disease modelling *in vitro* for the purposes of assessing drug safety and toxicology. This would also allow for better understanding of disease processes and thus facilitate in the potential identification of novel therapeutic targets. This review will focus on the current state of effort to derive hepatocytes from human pluripotent stem cells for potential use in hepatotoxicity evaluation and aims to provide an insight as to where the future of the field may lie.

Introduction

Adverse drug reactions (ADRs) continue to feature as a major problem to the clinician, the pharmaceutical industry and the regulatory authorities [1]. In the UK, 15% of hospital in-patients have been reported to suffer from a form of ADR during admission, with 20% of these patients readmitted again within 12 months of discharge [2, 3]. These admissions resulting from ADRs were also estimated to cost the NHS £466 million annually in a prospective observational study performed in 2001–2002 [4]. It is also the leading cause of drug attrition and confers a deep financial burden on the pharmaceutical industry [5].

Amongst the different types of ADRs, drug-induced liver injury (DILI) is the most prominent cause of patient morbidity and mortality [6–8]. This is attributed to the liver's role in drug metabolism particularly in circumstances when xenobiotics cannot be sufficiently cleared, for example in overdoses [9, 10]. Various hepatocyte models have thus been developed for use in safety pharmacology and toxicology research to understand the mechanisms of DILI and to screen new chemical entities (NCEs) for their potential to cause adverse reactions [11, 12]. Freshly isolated hepatocytes, cryopreserved hepatocytes, immortalized cancer cell lines, liver tissue preparations (slices, microsomes and S9 fractions) and animal models broadly categorize the numerous hepatocyte models available for studies into the pathophysiology of DILI. However, the utility and relevance of these models are also limited. The gold standard *in vitro* model for the study of DILI in humans is primary culture of freshly isolated human hepatocytes. However, the use of human primary hepatocytes (hPHs) is impeded by their limited availability,

inter-donor differences, variable viability following isolation and rapid dedifferentiation of the hepatocyte phenotype in culture, particularly in the loss of cytochrome P450 (CYP) enzyme expression [11, 13, 14]. The limited life span and phenotypic instability also limits the utility of the hPH model to short term studies only and compromises their use in mechanistic studies of DILI which often occurs following prolonged exposure to drugs [11, 14, 15]. Immortalized cancer cell lines have been used to overcome these problems as they have an infinite life span and are readily available. However, they suffer from a deficit in metabolic activity [11]. Transfection methods to enable overexpression of CYP enzymes in these cells have been adopted, but this approach is still limited to the expression of one CYP isoform per cell line and therefore does not fully recapitulate the metabolic capacity of a fully functional hepatocyte [11, 16–20]. Furthermore, all the currently available hepatocyte models do not sufficiently represent the genetic polymorphisms present in the population that are now acknowledged to play an important role in ADRs [21–23]. Although the use of animal models is a more amenable approach for *in vivo* studies, experimentation on animals raises ethical concerns, while interspecies differences limit the translation of data into the clinic [24–26]. Therefore, there is still a clear need to improve current hepatocyte models, and to adopt new advances in experimental techniques to develop new models that will enable better prediction and understanding of the mechanisms causing DILI.

With the rapidly advancing stem cell technology, it is hoped that progress will be made in bridging this gap in toxicology research through the use of human embryonic stem cells (hESCs). The pluripotent nature and the ability of the embryonic stem cells to proliferate indefinitely are the two main attractions in using ESCs not only for safety pharmacology and toxicology research, but also in regenerative medicine, tissue engineering and cell therapy [27–31]. Directed differentiation of hESCs to somatic cells with mature phenotypes in the laboratory could potentially provide a readily available source of metabolically competent cells such as mature hepatocytes with comparable functional status to freshly isolated hepatocytes for use in safety pharmacology and toxicology applications. By doing so, the problems of using the gold standard freshly-isolated hPHs such as their limited availability, inter-donor differences and variable viability following isolation, can theoretically be solved by the use of a standard protocol-driven derivation of HLCs with batch-to-batch consistency and purity.

More recently, pluripotent stem cells (PSCs) have also been produced by reprogramming of mature somatic cells and are termed as induced pluripotent stem cells (iPSCs) [32]. This approach negates the controversies surrounding the use of embryonic tissue and potentially allows for *in vitro* modelling of normal and variant phenotypes for safety pharmacology and toxicology evaluations. iPSCs were first generated by cellular reprogramming of murine fibroblasts using a retroviral vector that expressed transcription factors noted to be abundant in embryonic stem cells [32]. Since then, other groups have reported a variety of techniques using various human somatic cells to induce pluripotency, albeit with different efficiencies. These methods include viral-free approaches to deliver the pluripotency gene set expressing the essential transcription factors into target somatic cells using either episomal vectors, piggyBac transposons or minicircle vectors [33–37]. Reprogramming somatic cells via delivery of the reprogramming factors in their protein or messenger ribonucleic acid (RNA) form have also been reported [38–40]. Small molecules have also been used with all or some of the classical reprogramming factors in a bid to improve the efficiency of induction [41–44]. More recently, microRNAs (miRNAs) that are shown to be abundant in ESCs and known to play important roles during cellular reprogramming were used instead of the classical pluripotency factors to produce hiPSCs [45, 46].

In view of the potential of human pluripotent stem cells (hPSCs) in providing an alternative model for safety pharmacology and toxicology applications, many pharmaceutical and biotechnology companies in recent years have invested or developed joint collaborations with academia, to develop *in vitro* systems based on hPSCs [47, 48]. This review will focus on the current state of efforts to derive hPSCs for potential use in hepatotoxicity evaluation.

Derivation of hepatocyte-like cells (HLCs) from human pluripotent stem cells

hESC-derived HLCs

In general, studies reporting on 'hepatocytes' derived from hESCs have focussed on generating a closer representation of a mature hPH phenotype. However, as no reports to date have confirmed complete recapitulation of a freshly isolated hPH, the term HLCs has been used to describe them.

Many groups have attempted to improve the differentiation of hESCs to HLCs *in vitro* by mimicking the developmental pathway of the liver during embryogenesis. The aim is to derive mature hepatocytes from pluripotent hESCs using differentiation protocols encompassing the three main stages of hepatic development: definitive endoderm differentiation, hepatocyte progenitor specification and hepatocyte maturation [49]. Methods employed to induce differentiation of hESCs towards HLCs include the formation of embryoid bodies by aggregation of ESCs to mimic the gastrulation stage during embryogenesis before subsequent induction of hepatocyte development and addition of exogenous differentiation factors at appropriate stages of hepatic development as characterized by their gene expression profile (Table 1) [50–67]. However, refinement of the differentiation protocol to

Table 1

Summary of recent studies with reports of HLC-derivation from human pluripotent stem cells

Reference	Stem cell (cell line)	Differentiation method	Differentiation factors	Differentiation efficiency		
				% ALB +ve HLCs	% AAT +ve HLCs	Method of assessment
Cai *et al.*, 2007 [50]	hESC (H1, H9)	Monolayer, EB formation	AF V, AA, ITS, BMP2, FGF4, HGF, OSM, DEX	70	ND	ICC
Ek *et al.*, 2007 [51]	hESC (SA002, SA002.5, SA167)	Monolayer	Proprietary differentiation medium, FGF2	ND	ND	–
Söderdahl *et al.*, 2007 [52]	hESC (SA001, SA002, SA002.5, AS034, SA121, and SA167)	Monolayer	Proprietary differentiation medium, bFGF	ND	ND	–
Hay *et al.*, 2008 [53]	hESC (H1, H9)	Monolayer	AA, Wnt3a	90	ND	ICC
Shiraki *et al.*, 2008 [54]	hESC (Khes-1)	Co-culture with M15 cell line	AA, BMP4, bFGF, HGF, DMSO, DEX, Ly294002	9	ND	ICC
Agarwal *et al.*,2008 [55]	hESC (WA01, WA09)	Monolayer	AA, FGF4, HGF, BSA, OSM, DEX	67.4	84.7	ICC
Moore *et al.*, 2009 [56]	hESC (H1)	Monolayer, EB formation	AA, Wnt3a, HGF, OSM, DEX	72.8	ND	ICC
Basma *et al.*, 2009 [57]	hESC (H1)	Monolayer, EB formation	AA, FGF2, HGF, DMSO, DEX	55.5	ND	ICC
Song *et al.*, 2009 [58]	hESC (H1), hiPSC	Monolayer	AF V, AA, ITS, BMP2, FGF4, OSM, DEX, KGF, B27	60	ND	ICC
Duan *et al.*, 2010 [59]	hESC (H9)	Monolayer	AA, sodium butyrate, BMP2, BMP4, FGF4, HGF DMSO, B27	75–90	64–75	ICC, FACS, qRT-PCR, GFP reporter gene
Synnergren *et al.*, 2010 [60]	hESC (SA002, SA167, SA461)	Monolayer	AA, ITS, FGF1, FGF2, BMP2, BMP4, HGF, OSM, DEX	ND	ND	–
Touboul *et al.*, 2010 [61]	hESC (H9)	Monolayer	AA, BMP4,FGF2, FGF4, FGF10, HGF, EGF, retinoic acid, SB431542, Ly294002	ND	ND	–
Brolén *et al.*, 2010 [62]	hESC (SA001, SA002, SA002.5, SA167)	Monolayer	AA, BMP2, BMP4, FGF1, FGF2, HGF, OSM, DEX, Wnt3A	ND	ND	–
Ghodsizadeh *et al.*, 2010 [82]	hiPSC	EB formation	AA, FGF2, HGF, DMSO, DEX	50	ND	FACS
Liu *et al.*, 2010 [63]	hESC (WA01, WA09), hiPSC (*hPH-derived)	Monolayer	AA, FGF4, HGF, OSM, DEX	ND	ND	–
Si-Tayeb *et al.*, 2010 [65]	hESC (H9), hiPSC	Monolayer	AA, BMP4, FGF2, OSM, B27	80	ND	FACS
Sullivan *et al.*, 2010 [81]	hiPSC	Monolayer	AA, HGF, Wnt3A, DMSO, OSM, hydrocortisone, tryptose phosphate broth, B27	70–90	ND	ICC
Rashid *et al.*, 2010 [83]	hiPSC	Monolayer	AA, BMP4, FGF2, HGF, OSM, Ly294002, CHIR99021 (GSK-3 inhibitor)	83	ND	FACS
Zhang *et al.*, 2011 [66]	hESC (H9), hiPSC	Monolayer, EB formation	AA, BMP2, FGF4, HGF, KGF, OSM, DEX	60–80	20	ICC, FACS
Bone *et al.*, 2011 [64]	hESC (Shef1, Shef3)	Monolayer	FGF4, HGF, OSM, DEX, 1 m (GSK-3 inhibitor)	ND	ND	–
Chen *et al.*, 2012 [67]	hESC (H9), hiPSC	Monolayer	AA, ITS, HGF, Wnt3A, OSM, DMSO, DEX	ND	ND	–

AA, activin A; AF V, albumin fraction V; bFGF, human recombinant basic FGF; BMP, bone morphogenic protein; BSA, bovine serum albumin; DEX, dexamethasone; DMSO, dimethyl sulfoxide; EGF, epidermal growth factor; FACS, fluorescence-activated cell sorting; FGF, fibroblast growth factor; GFP, green fluorescent protein; GSK, glycogen synthase kinase; HGF, hepatocyte growth factor; ICC, immunocytochemistry; ITS, insulin-transferrin-selenium; KGF, keratinocyte growth factor; ND, not determined; OSM, oncostatin M; qRT-PCR, quantitative real time polymerase chain reaction; Wnt3a, wingless-type MMTV integration site family, member 3a.

generate HLCs with a phenotype matching hPHs continues. For example, a recent report has suggested that a greater differentiation efficiency could be gained from earlier use of the hepatocyte growth factor at the stage of definitive endoderm differentiation, rather than during the hepatocyte maturation stage as currently employed [67]. To date however, the perfect differentiation protocol has remained elusive. This is also compounded by the fact that there is currently no standardization of the methods used to characterize these HLCs and in assessing their differentiation potential, though helpful recommendations for minimal criteria to allow comparison of protocols have been proposed [68].

Currently, the measure of success (differentiation efficiency) in the derivation of 'mature HLCs' broadly consists of the purity of the derived HLC population with typical epithelial morphology and gene expression profiling for liver-associated markers such as albumin (ALB), and α_1-antitrypsin (AAT) (Table 1). Although gene expression profiling is useful, it is the dynamic functional capabilities of these differentiated HLCs when compared with hPHs that will determine their suitability for use in safety pharmacology and toxicology. These functional assessments include hepatic enzyme activity, albumin secretion, glycogen storage, uptake of indocyanine green (ICG) and uptake of low density lipoprotein (Table 2).

In the liver, drug metabolism is largely governed by phase I and II hepatic enzymes, with DILI widely accepted to be associated with the formation of reactive metabolites following metabolism by the CYP family, the most common phase I enzyme group. Therefore, one of the key tests for the functional relevance of HLCs for drug screening purposes would be the demonstration of inducible CYP activity at levels similar to freshly isolated hPHs. To the best of our knowledge, no studies using hESCs have successfully derived HLCs with adequate CYP activity in response to known reference compounds (Table 2). Although many studies of hESC-derived HLCs report on the expression of CYP either by detection of their messenger RNA or their protein, their functional activity has only been assessed in a handful of studies [50, 51, 53, 56, 57, 59, 61–63, 66, 69]. More importantly, only half of these studies have inducible CYP activity compared with the gold standard comparator of hPHs [51, 53, 57, 59, 69]. Interesting but less informative comparisons have also been made with undifferentiated hESCs or hepatocellular cancer cell lines which are known to have limited CYP activity [50, 62].

However, even if the gold standard comparators of hPHs are used, major differences in the experimental factors make comparison of results between studies difficult. For example, in the studies reporting on the activity of the CYP3A isoform, the levels have been shown to vary considerably from 0–90% of the hPHs used as comparators [51, 57, 59, 66, 69]. This vast range of reported CYP activity is likely to be reflective of the differences in experimental factors such as the multiple hESC lines used as starting cell source, the differences in the multi-stage differentiation protocols employed, the variety of methodology used to measure CYP activity and most pertinently, the variable quality of the reference hPHs being used in different studies [70, 71]. It is also important to note that full characterization of the hPH comparators used in all of these studies was not reported, making it difficult to judge the quality of the hPHs and their metabolic capacity.

As mentioned earlier, inducible CYP activity in hESC-derived HLCs has been assessed using a variety of techniques. For example, fluorescence and luminescence-based assays have been used to measure CYP3A4 activity following induction with reference inducer compounds such as rifampicin and midazolam [51, 69]. Activities of other CYP isoforms with corresponding gene expressions have also been tested using defined chemical substrates, with results similarly suggestive of detectable but variable CYP activity [50, 51, 56]. High performance liquid chromatography with tandem mass spectrometry (LC-MS-MS) has also been applied to measure the activity of four well-established human CYP isoforms (CYP1A2, CYP2C9, CYP3A4 and CYP2D6), and to conduct metabolite profiling based on the known metabolic pathways of bufuralol, a non-selective β-adrenoceptor blocking agent [59]. Using this approach, the results suggested comparable CYP activity of hESC-derived HLCs with reference hPHs. Furthermore, four new metabolic pathways of bufuralol were identified in addition to the three previously reported. These new revelations also indirectly suggest the effectiveness of the differentiation protocol employed in this study in obtaining a phenotype comparable with hPH.

In contrast to efforts to assess the activity of phase I enzymes in hESC-derived HLCs, only one study has reported the presence and activity of phase II enzymes [52]. In this study, glutathione-S-transferases (GSTs) were found to have overall comparable activity with that of the reference hPHs, though further examination of subunits has shown a more differential expression, with GSTM-1 showing the least. The quality of the reference hPHs used was again not reported and therefore their metabolic capacity uncertain.

The large variability, reported between different laboratories of the activity of these key enzymes associated with drug metabolism in hESC-derived HLCs, implies that the application of these cells for safety pharmacology and toxicology assessment is still premature. Perhaps more importantly, problems such as the lack of agreed endpoints of hepatic differentiation and maturation as well as the lack of standardized comparators for differentiated hESC-derived HLCs, need to be addressed urgently [70]. Although hPHs are the gold standard comparators, their metabolic capability can differ markedly between different preparations [72]. Hence, standardized and validated criteria to define the quality of the reference hPHs are also needed. Comparisons between the various differentiation protocols could then be addressed, with the aim of

Table 2

Summary of recent studies reporting on the functional capabilities of human pluripotent stem cell-derived HLCs

Reference	Phase I and II enzyme activity			Albumin secretion in media		Other functional activity shown
	Enzyme	% of hPH comparator (assay method)	Other comparators used (assay method)	% of hPH comparator	Other comparators used	
Cai *et al.*, 2007 [50]	CYP2B6	ND	hESC (fluorescence)	ND	Huh-7 hepatoma cell line	Glycogen storage, ICG uptake, LDL uptake
Ek *et al.*, 2007 [51]	CYP1A1 CYP3A4	0 (fluorescence) 0 (fluorescence)	– –	ND	ND	–
Söderdahl *et al.*, 2007 [52]	GST	80 (fluorescence)	HepG2 hepatoma cell line (fluorescence)	ND	ND	Glycogen storage
Hay *et al.*, 2008 [53]	CYP1A2	4 (LC-MS-MS)	hESC (LC-MS-MS)	ND	HLCs assayed with no comparator	Glycogen storage
Shiraki *et al.*, 2008 [54]	ND	–	–	ND	–	Glycogen storage
Agarwal *et al.*, 2008 [55]	ND	–	–	ND	hESC	Glycogen storage, ICG uptake
Moore *et al.*, 2009 [56]	CYP1A2	ND	hESC-derived HLCs cultured in media with different components (fluorescence)	ND	hESC-derived HLCs cultured in media with different components	ICG uptake
Basma *et al.*, 2009 [57]	CYP1A CYP3A	30 (fluorescence) 90 (LC-MS-MS)	–	75	–	–
Song *et al.*, 2009 [58]	CYP2B6	ND	hiPSC-derived HLCs compared with hESC-derived HLCs (fluorescence)	ND	hiPSC-derived HLCs compared with hESC-derived HLCs	Glycogen storage
Duan *et al.*, 2010 [59]	CYP1A2 CYP2C9 CYP2D6 CYP3A4	100 (LC-MS-MS) 60 (LC-MS-MS) 95 (LC-MS-MS) 90 (LC-MS-MS)	– – – –	ND	HLCs assayed with no comparator	ICG uptake
Touboul *et al.*, 2010 [61]	CYP3A	ND	HLCs assayed with no comparator (bioluminescence)	ND	Thawed foetal hepatocytes	Glycogen storage, ICG uptake, LDL uptake
Brolén *et al.*, 2010 [62]	CYP1A, CYP2C, CYP3A	ND	Spontaneously differentiated hESC-derived HLCs, HepG2 (LC-MS-MS)	ND	–	Glycogen storage, ICG uptake
Ghodsizadeh *et al.*, 2010 [82]	CYP2B6	ND	hiPSC (fluorescence)	ND	HLCs assayed with no comparator	Glycogen storage, ICG uptake, LDL uptake
Liu *et al.*, 2010 [63]	CYP1A2, CYP3A4	ND	HLCs assayed with no comparator (bioluminescence)	ND	ND	Glycogen storage
Si-Tayeb *et al.*, 2010 [65]	ND	–	–	ND	hiPSC-derived HLCs compared with hESC-derived HLCs	Glycogen storage, ICG uptake, LDL uptake
Sullivan *et al.*, 2010 [81]	CYP1A2 CYP3A4	–	HLCs assayed with no comparator (bioluminescence)	ND	ND	–
Rashid *et al.*, 2010 [83]	CYP3A4	ND	hiPSC (bioluminescence)	ND	hiPSC	Glycogen storage, LDL uptake
Zhang *et al.*, 2011 [66]	CYP3A4	0.32 (bioluminescence)	hESC-derived HLCs (bioluminescence)	60	–	Glycogen storage
Bone *et al.*, 2011 [64]	ND	–	–	22.6	–	–
Yildirimman *et al.*, 2011 [69]	CYP1A2 CYP3A4 CYP2B6 CYP2C9 CYP2C19	50 (LC-MS-MS) 50 (LC-MS-MS) 10 (LC-MS-MS) 50 (LC-MS-MS) 50 (LC-MS-MS)	–	ND	–	–
Chen *et al.*, 2012 [67]	CYP3A4	100 (bioluminescence)	hiPSC (bioluminescence)	ND	–	Glycogen storage, LDL uptake

ELISA, enzyme-linked immunosorbent assay; GST, glutathione transferase; ICG, indocyanine green; LC-MS-MS, liquid chromatography with tandem mass spectrometry; LDL, low density lipoprotein; ND, not determined.

developing one that is efficient, reproducible and sufficiently robust for drug safety and toxicology screening.

hiPSC-derived HLCs

Although there are advantages in using hiPSCs compared with using hESCs as a starting cell source for differentiation into HLCs (Table 3), there are still limitations in efficient generation of hiPSCs. Established methods of inducing pluripotency have so far failed to negate the problem of low reprogramming efficiency and concerns persist regarding potential genomic insertions of exogenous sequences with the current standard technique of using viral vectors [45]. Identifying solutions to these limitations is important, especially with regards to the application of these HLCs for high throughput screening of compounds, which requires high yields of genetically uncompromised cells with high purity to generate reliable and sustainable results.

A new technique with the hope of improving on the reprogramming efficiency, works by the expression of defined microRNAs (miRNAs) to induce pluripotency in mature human somatic cells [46]. miRNAs belong to a recently discovered class of small non-coding RNAs that play important post transcriptional regulatory roles in cellular and developmental events [73]. They act as master regulators by binding to a specific sequence motif of a target messenger RNA to induce their degradation or translational repression [74]. The use of the miRNA302/367 cluster, which plays an important role in global DNA demethylation and chromosomal modification, has been shown to induce pluripotency in human fibroblasts with a two order of magnitude increase in efficiency when compared with 'classical' transcription factor-based cellular reprogramming [32, 46, 75]. Whilst these results demonstrated huge improvements in reprogramming efficiency and may allow for high throughput generation of hiPSCs, this technique still uses a viral vector for delivery of the miRNA cluster. A similar approach harnessing the potential of miRNAs, uses direct transfection of mature double-stranded miRNAs without utilizing any viral vector [45]. hiPSCs were produced from human adipose stromal cells and dermal fibroblasts by repeated transfection of miRNAs that are found to be expressed more than two-fold in murine ESCs and iPSCs compared with murine adipose stromal cells. However, the authors also reported low efficiency levels similar to transcription factor-based cellular reprogramming originally described by Takahashi & Yamanaka [32, 45]. This technique may have the potential to deliver clinical and therapeutic benefits as it uses a relatively simple protocol to induce pluripotency without compromising genomic integrity, but has yet to be replicated by other groups. It also remains to be seen if the efficiency can be improved further with a view to producing hiPSC-derived HLCs for use in high throughput screening of NCEs.

The precise mechanisms by which pluripotency is induced in mature somatic cells by miRNAs remains incompletely understood. However, increasing evidence has suggested that specific miRNAs inhibit mRNAs associated with epigenetic regulation to cause global DNA demethylation and chromosomal modification, resulting in DNA methylation patterns in iPSCs which are very similar but non-identical to ESCs [75–79]. Interestingly, they also display distinct methylation patterns similar to their parental cells from which they were derived, suggesting a retained epigenetic memory in iPSCs, possibly due to incomplete reprogramming occurring during transformation from a differentiated somatic state to the undifferentiated iPSC status [78]. Paradoxically, this raises the possibility of harnessing this epigenetic memory to improve the efficiency and differentiation potential using these iPSCs, to rederive cells with a phenotype comparable with their parental cells [78]. This possibility was first highlighted by a study which used hiPSCs reprogrammed from pancreatic β cells to rederive insulin-producing cells [80]. Using these hiPSCs as the starting cell source, greater dif-

Table 3

Comparison of advantages and disadvantages between HLCs derived from hESCs and hiPSCs

hESC-derived hepatocyte-like cells (HLCs)	hiPSC-derived hepatocyte-like cells (HLCs)
Advantages	**Advantages**
• More experience in the literature reporting on the functional characteristics of hESC-derived HLCs and development of multi-stage differentiation protocols	• Potential to model DILI *in vitro* for mechanistic studies • Potential to encapsulate the phenotypic variation of phase I and II enzymes present in the population by establishing a library of HLCs derived from different individuals representing the global and ethnic genotypic variation • Potential application in robust high throughput screening for DILI of new chemical entities • Human embryos not required
Disadvantages	**Disadvantages**
• Use of hESCs subject to ethical debate • Limited genotypic variation with all the available hESC lines	• Low reprogramming efficiency to hiPSCs from parental somatic cells • Concerns with regards to the impact of genomic insertions from viral vectors used in the majority of methods for reprogramming parental cells

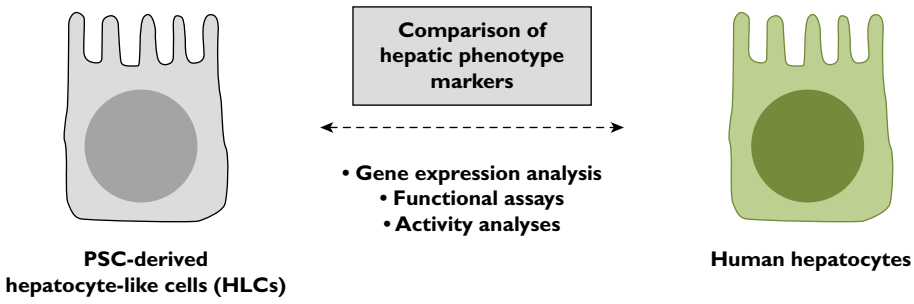

Figure 1

Full characterization of hPSC-derived HLCs against the gold standard hPHs is essential, particularly when these cells are to be used for modelling DILI *in vitro*

ferentiation efficiency was shown compared with using hESCs or hiPSCs derived from other somatic cell types. This approach was also similarly applied to create HLCs from hiPSCs derived from human hepatocytes though in this study, the authors did not detect any greater differentiation efficiency compared with using hESC lines [63].

Similar to their hESC-derived counterparts, HLCs derived from hiPSCs not surprisingly have also been reported to have a variable gene expression profile, functional characteristics and differentiation efficiencies (Tables 1 and 2) [58, 63, 65–67, 81–84]. When compared with hESC-derived HLCs, transcriptomic analyses between hESC- and hiPSC-derived HLCs, have shown differences in the gene expression of CYP isoforms [85]. hESC-derived HLCs demonstrated increased expression of CYP19A1, CYP1A1 and CYP11A1, whilst hiPSC-derived HLCs had enriched CYP46A1 and CYP26A1. Although comparative studies such as this are useful particularly when deciding if hiPSCs can be considered suitable alternatives to hESCs to derive HLCs for use in safety pharmacology, findings generated from a particular laboratory may not be generalizable due to different protocols employed, varied laboratory environments, and inconsistencies in the definitions of endpoints, as discussed in the previous section [71, 86]. As with hESC-derived HLCs, it is important that for any given differentiation protocol using hiPSCs, a thorough gene expression and functional characterization of the 'mature' HLCs should be carried out, and compared against the gold standard of hPH, which should itself also be fully characterized (Figure 1).

A major limitation associated with using hESC-derived HLCs for potential application in safety pharmacology and toxicology is their inability to encapsulate fully the phenotypic variation of key metabolic enzymes present in the population. This could in theory be overcome through the use of hiPSCs to derive HLCs. hiPSC-derived HLCs from different individuals could in theory produce a library of HLCs representing the phenotypic and genotypic variation of the global population. For example, hiPSC-derived HLCs from individuals with varied CYP polymorphisms would be invaluable for drug screening. hiPSC-derived HLCs from individuals who suffered idiosyncratic DILI could also be used as an *in vitro* model for mechanistic studies and to detect as yet unknown defective or variant metabolic pathways (Figure 2). The potential of hiPSC-derived HLCs to model metabolic disorders *in vitro* also allows for a better understanding of disease processes and thus facilitates the potential identification of novel therapeutic targets. It has been shown that it is possible to produce hiPSCs from dermal fibroblasts of patients with a metabolic disorder and subsequently derive HLCs with a retained disease phenotype. This has been shown in AAT deficiency, familial hypercholesterolaemia, glycogen storage disease, tyrosinaemia, progressive familial hereditary cholestasis and Crigler-Najjar syndrome [82, 83]. Recent studies have also shown the therapeutic potential of using hiPSC-HLCs with corrected point mutations for restoration of function, as demonstrated in AAT deficiency and Wilson's disease [66, 84].

Conclusions

Recent progress in the understanding and generation of hPSCs has enabled significant progress to be made in attempts to develop a novel model system based on differentiated HLCs to study and screen for DILI. The use of hiPSCs to derive HLCs additionally offers the unique capacity to model various diseased phenotypes *in vitro* for mechanistic studies and identification of novel therapeutics targets, as well as the potential to study the effect of genetic polymorphism, a key factor in predicting an individual's susceptibility to ADRs and DILI. Currently, the use of the gold standard hPHs for such applications has been limited due to unpredictable availability, variable quality, phenotypic instability in culture and ethical issues surrounding procurement. However, with an optimal differentiation protocol, it is hoped that hPSCs will be able to provide an unlimited supply of HLCs for drug safety and toxicology applications. However, progress in deriving a mature HLC phenotype comparable with freshly isolated hepatocytes has been slow and HLCs obtained from these experiments have only demonstrated a phenotype which

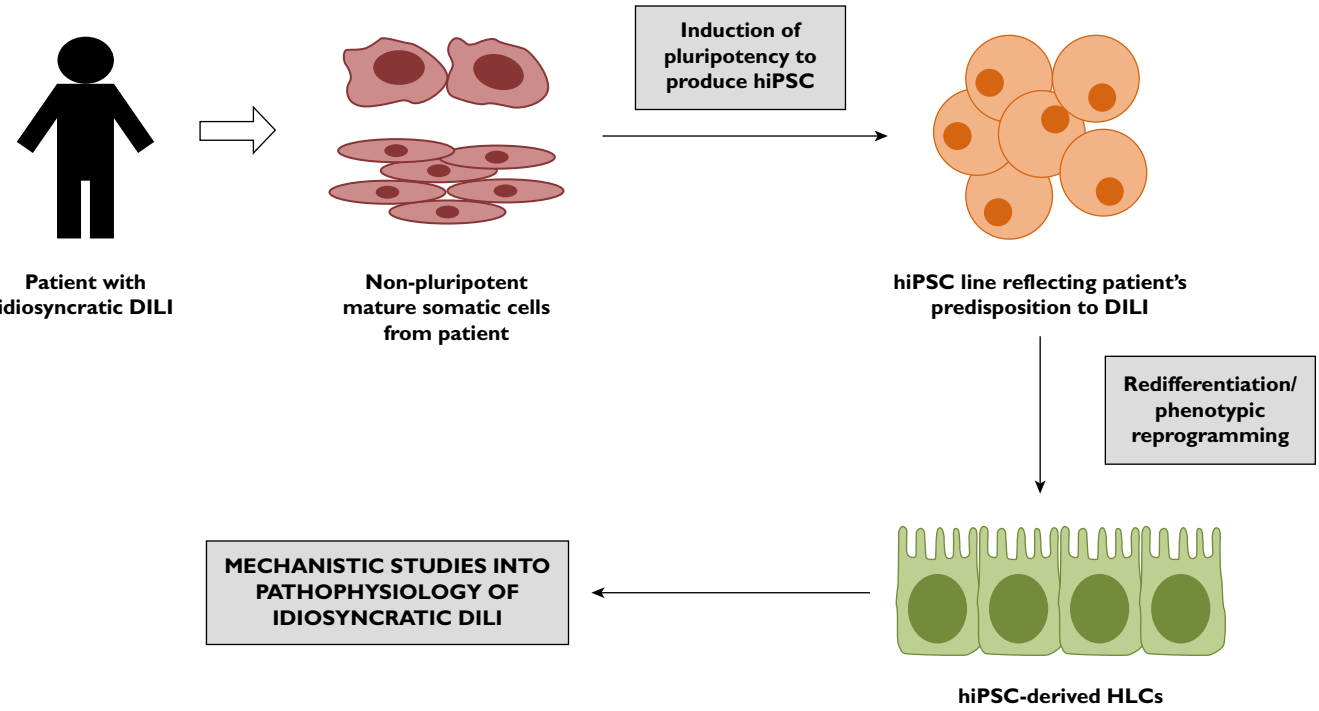

Figure 2
Application of hiPSC-derived HLCs for mechanistic understanding of idiosyncratic DILI *in vitro*

at best resembles more of a foetal liver [70]. Comparison of results between research groups has also been hampered by the lack of agreed and validated endpoints of HLC maturation and the lack of standardized characterization of hPHs as reference comparators.

It must also be borne in mind that even with the development of HLCs capable of recapitulating the function of a primary hepatocyte, various practical limitations still need to be addressed. Firstly, dedifferentiation of the HLCs in culture as observed in primary hepatocytes is likely to be an issue. With regards to using either hESC- and hiPSC-derived HLCs for modelling DILI, few studies have attempted long term culture of HLCs. This is an area of difficulty for both hPHs and HLCs and requires attention as many cases of DILI result from chronic exposure to drugs. Of the relatively few studies which have attempted to culture HLCs for a longer time period, the results are inconclusive. There is some evidence that HLCs *in vitro* gradually undergo maturation and display increased AAT expression, though the levels remain less than that of hPHs [87]. Similar results were also found with ALB and CYP3A4 gene expression in HLCs cultured for 50 days [54]. However, functional assessments of HLCs cultured for longer periods *in vitro* has never been reported to our knowledge, and therefore concerns of dedifferentiation of the HLC phenotype similar to the pattern observed with hPHs remains [70]. The predicted limited life span and phenotypic instability of the HLCs will limit their use to short term mechanistic studies of DILI, which paradoxically often occurs following prolonged exposure to drugs [11, 70]. Therefore, further understanding is still required of the differentiation patterns of HLCs *in vitro*. Lastly, despite the significant technological progress in miniaturization, scale-up of HLC production is mandatory if they are to be successfully deployed for high throughput screening of drug candidates by the pharmaceutical industry.

The development of the optimal protocol using either hESCs or hiPSCs to derive HLCs continues and ongoing effort should be greatly encouraged, considering the benefits this resource can potentially offer for modelling DILI *in vitro*.

Competing Interests

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