Generation of Hepatocyte-Like Cells from Human Pluripotent Stem Cells

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Abbreviations

AFP	Alpha-fetoprotein
BMP	Bone morphogenetic protein
CYP450	Cytochrome P450
ESCs	Embryonic stem cells
FGF	Fibroblast growth factor
HGF	Hepatocyte growth factor
HNF4a	Hepatocyte nuclear factor 4 alpha
iPSCs	Induced pluripotent stem cells
LDL	Low density lipoprotein
OSM	Oncostatin M
TGFb	Transforming growth factor beta
TTR	Transthyretin

Introduction

The liver is a vital organ that is responsible for a broad array of functions such as the production of bile, biotransformation, detoxification, and the synthesis of a myriad of secreted serum factors including Albumin, Alpha-1-antitrypsin, and several blood clotting factors. The liver also has various metabolic activities including, gluconeogenesis, glycogen synthesis, hormone production, urea production, and regulation of cholesterol and lipid flux. This diversity of activities associated with the liver equates with the complexity of pathologies caused by liver dysfunction and infection [1]. The hepatocyte is the major functional cell type of the liver and cultured primary hepatocytes have been used extensively for the study of metabolic liver disease. Unfortunately, under normal culture conditions, plated primary hepatocytes dedifferentiate and rapidly lose many of their characteristic metabolic functions, which limit their usefulness Repopulation of the liver with exogenous hepatocytes could be used to treat a subset of inborn errors of hepatic metabolism and provide an alternative to orthotopic liver transplantation [2]. Although hepatocyte transplant therapies could offer significant advantages over liver transplants, a number of challenges must be overcome before such therapies become routine [2]. Such challenges include the need to access large numbers, $>1 \times 10e^9$ cells per transplant [3], of highly differentiated functional hepatocytes that ideally are genetically matched to the patient. The need for an abundant supply of high fidelity primary human hepatocytes that can be used for both research and therapeutics is therefore substantial.

A potentially inexhaustible source of hepatocytes could be provided by human pluripotent stem cells, such as embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). In addition, iPSCs derived from patients with metabolic liver disease may offer culture models to study the molecular mechanisms underlying hepatocyte dysfunction in these patients. Furthermore, if hepatocytes differentiated from iPSCs can rescue animal models of liver disease, not only would this provide proof-of-principle supporting the therapeutic use of pluripotent stem cells, but could provide patient-specific animal models to study drug toxicity, efficacy, and metabolism.

Differentiation and Characterization of Hepatocyte-Like Cells Derived from Pluripotent Stem Cells

An overwhelming number of protocols have been published that describe the generation of hepatocyte-like cells from human pluripotent stem cells. We have recently provided through open-access publishing a step-by-step procedure that has been used successfully by many labs to produce hepatocytes from both human ESCs and iPSCs (http://www.stembook.org/node/721) (Fig. 1).

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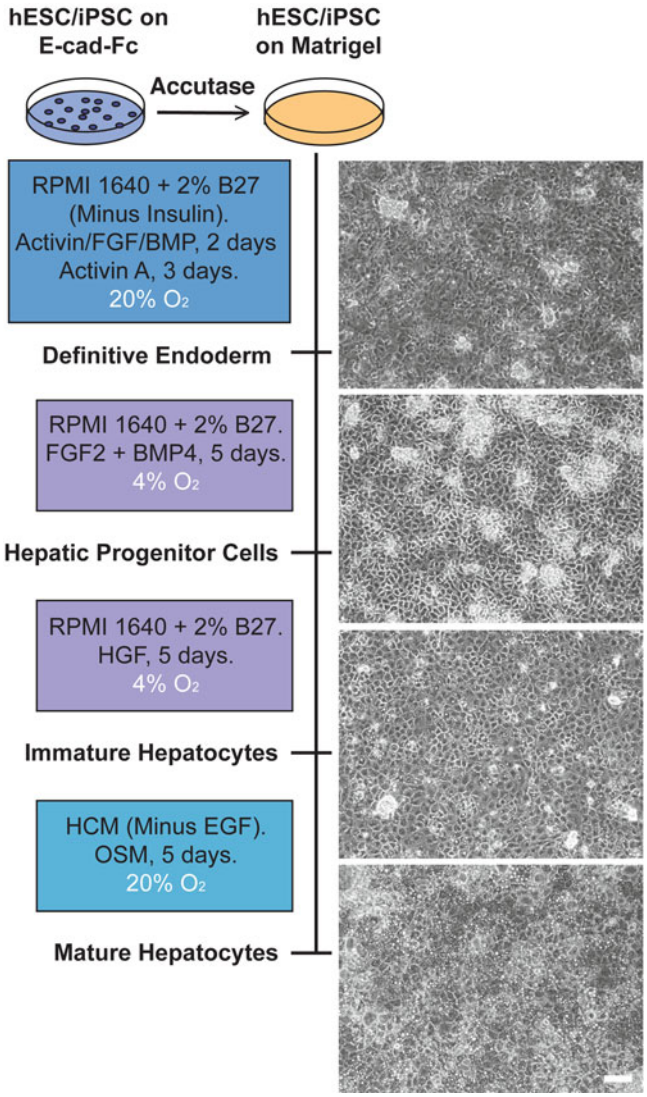

Fig. 1 Overview of the protocol used to differentiate human pluripotent stem cells into hepatocyte-like cells. Pluripotent cells are maintained on an E-Cad-IgG Fc substrate before being passaged to Matrigel-coated dishes for differentiation. Cells are exposed to growth factors to differentiate the cells in a stepwise fashion that recapitulates hepatogenesis. Images on the *right* show phase contrast micrographs of the cells during each stage of the differentiation (scale bar = 100 μm) (figure modified from Cai et al. 2012. Protocol for directed differentiation of human pluripotent stem cells toward a hepatocyte fate. 2012. Stembook. Harvard Stem Cell Institute. http://www.stembook.org/node/720)

Differentiation by Embryoid Body Formation

The first studies attempting to produce hepatocytes from human ESCs were based on protocols developed using mouse ESCs, which were classically differentiated by growing the cells in small clusters as suspension aggregates on non-adherent dishes [4, 5]. The resulting clusters are called embryoid bodies because they generate cell types from all three germ layers in a process that recapitulates aspects of gastrulation. Lavon et al. showed that formation of embryoid bodies without any exogenous factors could produce a population of cells of which ~6 % expressed albumin, and culturing the cells with media conditioned by mouse primary hepatocytes increased the number of albumin-expressing cells dramatically [6]. These results suggested that exogenous factors could direct differentiation of human pluripotent cells to a specific cell fate and as a consequence many protocols now use growth factors to enhance differentiation after generating an embryoid body intermediate [7–19]. The choice of growth factors used in these various protocols differ; however, most were selected because of their known effects during hepatogenesis and include bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), oncostatin M (OSM) and/or dexamethasone. Given the substantial number of variations in the cocktails of growth factors added following embryoid body formation and the observation that no single protocol is uniquely efficient, it appears that there is flexibility in inducing hepatocyte-like cells using embryoid body-based approaches. While cells generated by these procedures all express at least a subset of hepatocyte markers it is important to realize the heterogeneous nature of embryoid body-mediated differentiation. Although, contaminating non-hepatic cell populations may well contribute factors that positively affect hepatocyte differentiation, the heterogeneity associated with embryoid body formation can be problematic if pure populations of hepatocytes are needed for downstream applications. In this regard, procedures have been developed to facilitate the purification of hepatocyte-like cells from mixed cell populations that include using hepatocyte transcriptional regulatory elements to drive expression of reporter constructs or selectable markers or the isolation of hepatocytes by FACS [6, 9, 20, 21].

Directed Differentiation by Cell Signaling Factors

Because pluripotent stem cells are reminiscent of early progenitor cell types, such as those found in the inner cell mass or epiblast, it seems logical that human iPSCs or ESCs could be directed toward a specific cell fate in culture by recapitulating the environmental cues normally encountered during embryonic development. The study of liver development has revealed several signaling factors that have integral roles in regulating hepatogenesis and several recent reviews have described advances in the field in detail [22–24]. The parenchymal components of the liver originate from the ventral portion of the anterior endoderm after the endoderm is produced during gastrulation in response to Nodal signaling [24]. At around embryonic day (E) 8.0 in the mouse (2–4 somites), morphogenesis of the foregut positions the presumptive hepatic endoderm next to the developing heart

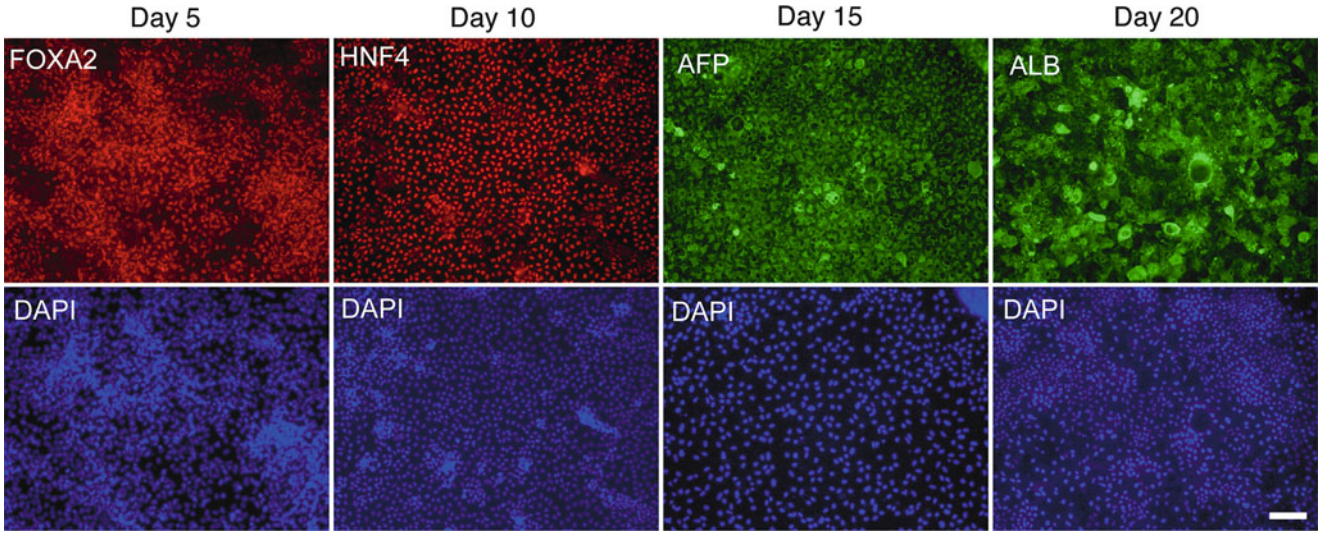

Fig. 2 Characteristic proteins expressed at each stage of differentiation. Immunocytochemistry was used to identify definitive endoderm cells expressing FOXA2 expression after 5 days of differentiation. After 10 days, specified hepatic cells express HNF4a. Hepatoblasts express alpha-fetoprotein after 15 days, and finally, after 20 days of differentiation, albumin expression can be detected in the cells (scale bar—100 μm)

and in close proximity to the mesoderm that will form the mesothelial linings of the intraperitoneal cavity. BMPs and FGFs, that appear to originate from the mesoderm, instruct the endoderm to follow a hepatic fate. By E8.5, an anatomical expansion of the ventral endoderm, called the liver bud, can be identified and shown to express several characteristic hepatic mRNAs including albumin, alpha-fetoprotein (AFP), Ttr and Hepatocyte nuclear factor 4 alpha (HNF4a). At E9.5, the hepatic progenitor cells delaminate and invade the surrounding mesenchyme. The liver undergoes a tremendous amount of growth from this point until E15.0. Signals from the mesenchyme, including FGFs, BMPs, HGF, and WNTs stimulate hepatoblast migration and proliferation [25] and by mid-gestation stages of development the liver becomes the major site of hematopoiesis within the fetus. The hematopoietic cells within the liver secrete the cytokine OSM, which is required for maturation of hepatocytes [26].

A significant advance in using human pluripotent stem cells to generate endoderm-derived lineages was provided by D'Amour et al. [27]. Using hESCs, the authors demonstrated that definitive endoderm could be induced with impressive efficiency by treating the cells with high concentrations of Activin A, a Nodal mimic, under conditions that inhibited PI-3 kinase activity [27, 28]. Under these conditions 80–90 % of the cells express proteins, such as CXCR4, FOXA2, SOX17, and FGF17, that together define endodermal character (Fig. 2) [20, 28, 29]. Marker analyses suggested that the formation of the endoderm from hESCs was preceded by the transient production of mesendoderm, which reflects the process through which definitive endoderm is normally formed in the embryo [27, 28]. Profiling mRNA expression also suggested that the endoderm specifically exhibited foregut characteristics, expressing GATA4, HHEX, and CER1 all of which are enriched in the ventral foregut endoderm in pre- to early-somite stage embryos [30–32]. The ventral foregut nature of this specified endoderm is important because key endodermal organs, including the liver and pancreas are derived from this specific portion of the endoderm, suggesting that endoderm produced by the D'Amour approach could be ideal for further differentiation to produce hepatocytes and pancreatic islet cell types [33]. Although the majority of directed differentiation protocols rely on induction using Activin A, some modifications have been described that may increase the efficiency of endoderm production such as inclusion of WNT3A, BMP4, or HGF along with Activin A treatment; however, the increases in efficiency that are reported appear to be relatively modest [34–36]. More recently, modifications to the addition of Activin A, including inhibition of TGF-β and BMP activity or inclusion of WNT3A and FGF4 after initial formation of the definitive endoderm, can also impact the character of the endodermal cells causing them to express anterior or posterior markers, respectively [37, 38]. This may have important consequences for the generation of other endodermal-derived cell lineages such as those that generate the lung or gastrointestinal tract.

In most cases the efficient generation of endoderm from the pluripotent stem cells is crucial for the successful production of hepatocyte-like cells. Once the definitive endoderm is produced most protocols rely on the removal of Activin A and the subsequent addition of FGF2 and BMP4 to induce the endoderm to adopt a hepatic fate. The choice of adding FGF and BMP was informed by studies in the mouse predominantly by the Zaret laboratory, who demonstrated, using ex vivo embryo culture models, that addition of these

factors was sufficient to induce hepatic specification in isolated ventral endoderm [39, 40]. The efficiency of induction of the endoderm can again be measured by examining the identification of markers that are known to be expressed in the early liver bud including several liver transcription factors such as HNF4a, HNF1b, TBX3 and HHEX all of which have known roles in controlling early development of the hepatic progenitor cells (Fig. 2) [41–46]. Again the efficiency through which the endoderm is converted to a hepatic fate is generally very high, with the more robust protocols generating upwards of 80 % of cells expressing early hepatic progenitor markers.

A variety of factors have been used to induce the hepatic progenitor cells to further differentiate including addition of BMPs, a variety FGFs, HGF, and Dexamethasone to complex media commonly containing Insulin, Transferrin, and Selenium [21, 47–53]. The consequence of these factors is to produce cells that express proteins that are commonly enriched in the fetal hepatocytes including AFP, fibrinogen alpha chain, angiotensinogen, and transferrin (Fig. 2) [29]. As with previous stages, the conversion of the hepatic progenitor cells to immature hepatocytes is upwards of 80 % when using the most efficient protocols.

Finally, several groups add OSM at various steps of the differentiation process to induce maturation of the immature hepatocytes [12, 34, 36, 54–68]. The choice of adding OSM again was the result of original studies by the Miyajima laboratory that examined regulation of mouse liver development by cytokines [26, 69, 70]. This work led to a model in which factors from hepatic mesenchymal cells including HGF and Integrin signaling pathways converge with OSM, which is secreted from hematopoietic cells that are abundant within the fetal liver, to drive the fetal hepatocytes toward a mature phenotype. While the role of OSM during hepatocyte maturation in the mouse is supported by several genetic experiments, many of the protocols used to differentiate hepatocytes from human ESC/iPSCs circumvent the addition of the OSM suggesting that under some conditions this factor is dispensable.

In general, these protocols lead to populations of cells that have many features associated with hepatocyte function. The differentiated cells express a large number of proteins that reflect key activities of the liver, including albumin, tyrosine aminotransferase, several apolipoproteins, orosomucoids, coagulation factor VII, and the asialoglycoprotein receptor (Fig. 2) [8, 29, 55, 63]. In addition to secreting albumin, the resulting cells have also been shown to be capable of synthesizing glycogen, internalizing and secreting vLDL/LDL, metabolizing indocyanine green, producing urea, storing lipid, and in a subset of studies the cells were capable of engrafting into the parenchyma of the mouse liver, at least in short-term assays [8, 9, 34, 36, 53–55, 63, 71, 72].

While many protocols can achieve populations of cells that are highly enriched in liver-specific markers, all protocols have one major pitfall in common: no protocol has generated cells that are functionally equivalent to primary human hepatocytes. Most protocols produce cells that retain expression of fetal proteins that are normally silenced in adult liver cells such as AFP and commonly lack expression of a subset of proteins necessary for full hepatocyte function. Global gene expression studies that compare expression profiles of human ESC/iPSC-derived hepatocytes to adult or fetal liver samples reveal a substantial overlap in mRNA distribution [29, 63]. However, quantitative studies using qRT-PCR show that the levels of many genes normally expressed in adult livers or primary hepatocytes are significantly lower in hepatocytes derived from pluripotent stem cells [51, 58, 59, 63, 64, 73, 74]. Unfortunately, the majority of protocols describing the differentiation of hepatocytes from human ESCs or iPSCs fail to compare mRNA levels to those found in fresh primary hepatocytes or liver samples and instead report the relative increases in mRNA that occur during the differentiation process. The failure to compare mRNA levels to accepted standards makes it difficult to judge the quality of cells produced by different protocols and leads to considerable confusion in interpreting differentiation efficiency. Among the genes whose mRNA levels are commonly significantly lower in stem cell-derived hepatocytes compared to adult hepatocytes are those encoding phase I, II, and III enzymes, which have critical roles in detoxification [49, 63, 73]. The reduced levels of these enzymes is important because hepatocytes are the principal site for the metabolism of xenobiotics and pharmaceuticals and so human ESC/iPSC-derived hepatocytes could be extremely useful for drug toxicity testing if the cells recapitulated expression of detoxification enzymes, such as CYP3A4. A number of groups have tried to improve the fidelity of the metabolic response of iPSC-derived hepatocytes by introducing exogenous factors known to regulate expression of phase I, II, and III enzymes. Takayama et al. demonstrated that by sequentially increasing the expression of transcription factors during differentiation using adenoviruses, they could increase the levels of CYP3A4, CYP2D6, and CYP7A1 [68]. In a separate study, increasing expression of the constitutive androstane receptor (CAR) also resulted in increased expression of detoxification and metabolic mRNAs, including several encoding Cytochrome P450 (CYP450) proteins [57].

While forced expression of exogenous transcription factors provide insight into how to overcome the lack of maturity of stem cell-derived hepatocytes, less invasive methods will likely be necessary for such cells to be useful in a therapeutic setting. It could be argued that the culture of hepatocytes in a 2D environment is too simplistic and to produce hepatocytes that more closely resemble those in the liver and it will be necessary to generate a culture environment that more closely recapitulates the structure of the liver. Indeed, modifying the extracellular matrix and using sandwich

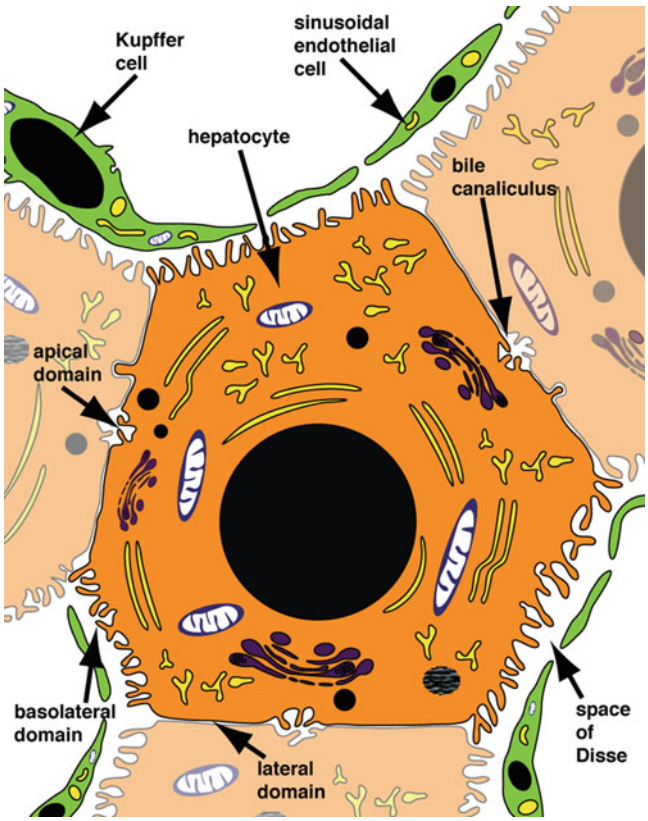

Fig. 3 Illustration showing the relationship between hepatocytes and the sinusoidal endothelium. Hepatocytes are polarized epithelial cells with apical domains that generate the bile canaliculi, lateral domains that face neighboring hepatocytes, and basolateral domains that face the sinusoids. The basolateral surface directly contacts the peri-sinusoidal space (also called the space of Disse) which contains matrix proteins. The close association with Kupffer cells, sinusoidal endothelial cells, and extracellular matrices are believed to influence hepatocyte function and maturity (illustration by Cameron B. Duncan, interpreted from Bloom and Fawcett, 1994. A textbook of histology, 12th edition. New York, NY, Chapman & Hall)

cultures has been shown to improve the quality of primary hepatocytes [75, 76]. Although hepatocytes are responsible for the majority of liver functions, hepatocytes closely interact with several other cell types found in the liver. Biliary epithelial cells, also known as cholangiocytes, form the bile ducts, sinusoidal endothelial cells and Kupffer cells, which are resident hepatic macrophages, form the hepatic capillaries, and stellate cells, which resemble pericytes found within the peri-sinusoidal space, collaborate to form the basic architectural unit of the liver known as the lobule [77]. The architecture of the hepatic lobule is integral to the liver's function (Fig. 3). The basolateral side of the hepatocyte is lined by sinusoidal endothelial cells, which facilitates absorption of toxins and metabolites by the hepatocytes and the secretion of serum factors into the blood stream. Adjacent hepatocytes are connected by tight junctions, generating a canaliculus that transports bile acids and salts to the bile duct. Within the lobule the hepatocytes are supported by a fine extracellular matrix consisting primarily of collagen type III with small amounts of collagen type I. Since complex interactions between several cell types and matrix components in the liver are likely to influence the activity of the hepatocytes, several laboratories have attempted to use culture conditions that more closely resemble the in vivo environment such as coculture with supportive cell types including human fetal liver stromal cells (hFLSCs) and Swiss 3T3 cells [13, 78]. A number of groups have also used 3D formats including culture in dynamic 3D perfusion bioreactors [79]. A combination of tissue engineering, 3D culture, and matrix optimization, may well be important because several studies have shown that such variables can affect the maturity of cultured primary hepatocytes [80–83].

Although the differentiation protocols discussed above have produced cells that promote hepatocyte maturation, the resulting cells still fall short of the activity associated with fresh human hepatocytes. A limitation of the hepatocytes derived from human ESCs and iPSCs is the failure of the cells to extensively repopulate a damaged liver in the long term. In contrast, extensive, upwards of 80 %, repopulation of the hepatic parenchyma is routinely achieved when using primary human hepatocytes in several different animal models of liver damage [84–88]. A number of groups have demonstrated that human ESC/iPSC-derived hepatocytes can integrate into the hepatic parenchyma in short-term analyses [8, 9, 18, 34, 36, 53–55, 63, 72, 89, 90]. In the limited cases where long-term engraftment has been claimed, the level of human albumin that can be detected in the serum appears to be vanishingly low [72]. When primary human hepatocytes are transplanted into the FRG mouse, if 3 % of the liver contains human hepatocytes, ~1 mg/mL of human albumin can be detected in the serum of the transplanted mouse and this level rises to 15 mg/mL when repopulation approach 90 % [84, 86]. In the study by Liu et al., which examined mice after 8-weeks of engraftment, the maximum level of albumin detected in the serum was ~40 ng/mL [72]. These results would suggest that the human cells are either very poorly differentiated or that the levels of engraftment are much lower than has been estimated using antibody staining techniques, which can be prone to artifact.

Why human ESC/iPSC-derived hepatocytes fail to repopulate damaged mouse livers with high efficiency is unclear. It seems unlikely that human pluripotent stem cells are inherently unable to generate functional hepatocytes because several studies have demonstrated that mouse iPSCs are capable of forming fully functional livers in vivo [63, 91, 92]. It would seem more plausible that the differentiation procedure requires improvement, animal models need to be optimized, and efficient transplant techniques need to be employed to enhance the implantation and survival of the exogenous cells [93].

Using Pluripotent Stem Cells to Model Liver Disease and Hepatocyte Differentiation

Although current differentiation protocols generate hepatocytes that retain fetal characteristics, it is important to acknowledge that pluripotent stem cell-derived hepatocytes also display many of the activities normally associated with the adult liver [17, 29, 48, 49, 63, 65, 94]. The ability to generate cells with the majority of hepatocyte function intact, particularly from iPSCs, raises the possibility of using such cells to study inborn errors of hepatic metabolism and a number of groups have generated iPSCs from patients with inherited liver disease [11, 62, 95, 96]. The Vallier laboratory demonstrated that hepatocyte-like cells derived from patients with a variety of metabolic disorders displayed pathologies characteristic of their respective liver deficiencies [62]. For example, cells derived from patients with alpha 1-antitrypsin deficiency accumulated misfolded alpha 1-antitrypsin protein in the endoplasmic reticulum and cells derived from patients with glycogen storage disease had significantly elevated glycogen accumulation. In addition, the group demonstrated that it is possible to genetically repair the causative mutations in alpha 1-antitrypsin deficient iPSCs using a combination of zinc finger nucleases (ZFNs) and piggyBac transposon mediated gene targeting [97].

Many heritable liver metabolic disorders are highly complex where the dysfunctional response of the hepatocyte to a given mutation involves multiple pathways. An example of such a disease is familial hypercholesterolemia that is primarily caused by mutations in the low density lipoprotein receptor (LDLR). In familial hypercholesterolemia patients, the hepatocytes not only fail to correctly internalize and clear low density lipoprotein (LDL)-cholesterol, but also secrete extraordinarily high levels of VLDL/LDL into the serum [98, 99]. To determine the feasibility of modeling such complex metabolic disorders our laboratory recently generated iPSCs from a familial hypercholesterolemia patient with well characterized mutations in the LDLR [100]. Hepatocytes derived from these iPSCs were found to reproduce key aspects of the pathophysiology associated with familial hypercholesterolemia including a failure to internalize LDL-cholesterol, a dramatic increase in LDL secretion, and an inability to respond to lovastatin treatment [95]. Such results are encouraging because they suggest that patient iPSCs could be useful for determining the contribution of specific allelic variations to control lipid metabolism.

The use of iPSC-derived hepatocytes is not limited to the study of metabolic disease, since such cells could potentially be used to study infectious liver disease including hepatitis virus infections and malaria. Two groups have independently demonstrated that iPSC-derived hepatocytes are capable of supporting the entire lifecycle of hepatitis C virus [101, 102]. Such findings open up the possibility of using iPSC-derived hepatocytes from individual patients to examine the role of host genetics in modifying viral replication. Treating HCV infected iPSC-derived hepatocytes with antiviral drugs block viral replication, which suggests that iPSC-derived hepatocytes could also provide a platform to identify novel pharmaceuticals that could be useful in blocking HCV infection. If iPSC-derived hepatocytes are capable of supporting the lifecycle of other liver pathogens, such cells are likely to make a significant contribution to our understanding and treatment of infectious liver disease.

Pluripotent Stem Cells as a Model for Human Hepatocyte Differentiation

The process through which factors that control cell differentiation are identified has historically been extremely laborious, relying heavily upon the generation of transgenic and knockout mice. The use of mice has been necessary because a robust model to study hepatocyte differentiation in culture has been lacking. One can deplete target mRNAs in hepatoma cells quite easily; unfortunately, the data generated through this approach have relatively little relevance to the differentiation of hepatocytes in vivo. This is because (1) hepatoma cells are pathologically abnormal and have lost many hepatic functions as well as normal control of gene expression, and (2) hepatoma cells at best represent a snapshot of a specific developmental stage, or more accurately an abnormal dedifferentiated state caused, in part, by loss of appropriate transcription factor networks and genomic rearrangements. The static state of hepatoma cells is a serious limitation because the developmental process is dynamic, with many factors being essential at specific developmental stages. Metaphorically, using hepatoma cells as a model to study differentiation is akin to attempting to understand how to construct a skyscraper by examining only the fifth floor! However, the observation that the stepwise differentiation of pluripotent stem cells toward hepatocytes appears to mimic the process that occurs during hepatogenesis suggests that human ESCs or iPSCs could offer a model system that would facilitate the study of the fundamental molecular mechanisms that control hepatocyte differentiation. Our laboratory is exploring the usefulness of this system to determine the role of specific transcription factors in controlling the specification of hESC-derived hepatocytes. Oligonucleotide array analyses throughout the differentiation procedure have established mRNA profiles that are characteristic of each stage of differentiation [29]. In addition, these analyses identified mRNAs encoding proteins with potential roles in regulating differentiation of hepatocytes from the hESCs. HNF4a is one such protein that is initially expressed coincident with specification of hepatoblasts from hESC-derived

endoderm [29, 63]. We generated hESCs that expressed an shRNA that efficiently depleted HNF4a following differentiation and found that when HNF4a was depleted the differentiating cells were incapable of adopting a hepatic fate. These findings determined that human pluripotent stem cells can be used to efficiently probe the molecular basis of hepatocyte differentiation and that, in comparison to using the mouse as a developmental model, the use of human ESCs was extremely efficient.

Summary

Substantial progress has been made in the effort to generate high quality functional hepatocytes from human pluripotent stem cells. Although many protocols have been described, most attempt to mimic the native signaling events that occur during hepatogenesis. Although the efficiency of the many different procedures varies significantly, in the better protocols upwards of 80 % of the cells express hepatocyte mRNAs and proteins and display several activities associated with liver function. Although the cells produced by these procedures are extremely useful in providing models of liver disease and cell differentiation, these hepatocyte-like cells are not identical to freshly isolated hepatocytes. Current protocols are unlikely to produce cells that would be useful in drug toxicity testing nor could they supply cells to be used in cell transplant therapy for the treatment of liver disease [103]. With this in mind, the effort to improve the quality of hepatocytes derived from human pluripotent stem cells continues aggressively and new procedures that use 3D culture of mixed cell populations are particularly promising. In addition, several groups are attempting to repopulate livers of animals with human pluripotent stem cell-derived hepatocytes including mice, rats and pigs with genetic lesions that facilitate humanization of the hepatic parenchyma [8, 84, 104, 105]. Similar approaches have allowed the maturation of pancreatic endocrine progenitor cells in diabetic mouse models [106, 107]. If successful, such transplantation models could provide a limitless supply of patient-specific, highly differentiated hepatocytes.

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