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Current Status of Human Adipose–Derived Stem Cells: Differentiation into Hepatocyte-Like Cells

Feras Al Battah[1,2,*], Joery De Kock[1], Tamara Vanhaecke[1], and Vera Rogiers[1]

[1]Department of Toxicology, Center for Pharmaceutical Research, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [2]Department of Biology and Biotechnology, Faculty of Science, Arab American University (AAUJ), Jenin, Palestine

E-mail: falbatta@vub.ac.be; jdekock@vub.ac.be; tamara.vanhaecke@vub.ac.be; vrogiers@vub.ac.be

The shortage of human organ donors and the low cell quality of available liver tissues represent major obstacles for the clinical application of orthotropic liver transplantation and hepatocyte transplantation, respectively. Therefore, worldwide research groups are investigating alternative extrahepatic cell sources. Recent *in vitro* studies have demonstrated that mesenchymal stem cells (MSCs) from various sources, including human bone marrow, adipose tissue, and umbilical cord, can be differentiated into hepatocyte-like cells when appropriate conditions are used. In particular, interest exists for human adipose–derived stems cells (hASCs) as an attractive cell source for generating hepatocyte-like cells. The hASCs are multipotent MSCs that reside in adipose tissue, with the ability to self-renew and differentiate into multiple cell lineages. Moreover, these cells can secrete multiple growth factors and cytokines that exert beneficial effects on organ or tissue injury. In this review, we will not only present recent data regarding hASC biology, their isolation, and differentiation capability towards hepatocytes, but also the potential application of hASC-derived hepatocytes to study drug toxicity. Additionally, this review will discuss the therapeutic potential of hASCs as undifferentiated cells in liver regeneration.

KEYWORDS: human adipose–derived stem cells, liver regeneration, adipose tissue, hepatic differentiation, hepatocyte, hepatotoxicity, *in vitro*

INTRODUCTION

At present, orthotropic liver transplantation (OLT) remains the only therapeutic option for patients with end-stage liver disease, including (non) alcoholic fatty liver disease and chronic viral hepatitis[1]. However, its clinical use is limited due to the shortage of organ donors[2]. Hepatocyte transplantation has emerged as a promising alternative approach to OLT for the treatment of some liver-based metabolic disorders (e.g., Crigler Najjar) and/or acute liver failure (e.g., acute intoxication)[1]. Low cell quality, however, represents its main limitation[2]. Consequently, alternative extrahepatic human cell sources and,

in particular, stem cells are being extensively investigated. Experimental evidence has indicated that adult stem cells, more specifically mesenchymal stem cells (MSCs), can be induced into cells exhibiting hepatic properties[3], pointing to the possibility that adult stem cell–based therapies might provide alternative therapeutic approaches towards the treatment of liver diseases.

MSCs are postnatal multipotent stem cells of mesodermal origin that have the capacity to differentiate into cells of mesenchymal lineage, including bone, fat, and cartilage[4,5]. MSCs display plasticity beyond their conventional mesodermal lineage. They have been induced to generate neural cells and hepatocytes *in vitro*[6,7]. In the last decade, studies by Zuk et al. indicated that human adipose tissue contains MSC populations, so-called human adipose–derived stem cells (hASCs)[8,9]. Based on their biological properties, including growth kinetics, cell surface phenotype, gene expression, and differentiation potential, these cells have properties that are similar to those of bone marrow–derived MSCs (BM-MSCs)[10,11,12]. However, compared to BM-MSCs, hASCs have the relative advantage of abundance[13]. Apart from their differentiation capability, several recent reports have shown that the ASCs have a supportive role in organ regeneration[14,15,16,17]. In 2005, Seo et al. reported that hASCs cultured in media supplemented with growth factors and cytokines yielded a cell population that expressed a number of hepatocyte-specific functions, such as albumin production and urea synthesis[3]. Several studies followed, demonstrating the ability of hASCs to differentiate into hepatocyte-like cells. The identification of cell populations capable of generating hepatocytes has gained immense interest. In this context, adipose tissue could not only represent a promising cell source for cell-based therapy of liver diseases, but could also be considered as a potential cell source for *in vitro* models used in drug safety testing during the preclinical phase of drug development (Fig. 1). The aim of this review is to discuss recent data regarding hASC biology, including cell isolation, differentiation capability towards hepatocytes, and future application of hASC-derived hepatocytes in drug toxicity studies, as well as their role as undifferentiated cells in supporting liver regeneration.

INTRAHEPATIC SOURCES OF CELLS FOR LIVER REGENERATION

The adult liver has a unique and remarkable regenerative capacity. It depends on the presence of mature, differentiated, liver parenchymal cells (hepatocytes) and liver stem/progenitor cells. Hepatocytes constitute the first line of response to acute injury, such as partial hepatectomy, while liver progenitor cells function as a reservoir[18]. Hepatocytes are normally quiescent cells and, upon acute liver injury, display a high proliferative capacity to restore the liver mass. Indeed, transplantation studies in mice have shown that these cells are capable of more than 70 cell division cycles[19]. In humans and in animal models of chronic liver disease, intrahepatic liver stem/progenitor cell populations located within the canals of Hering, known as oval cells in rodents or hepatic progenitor cells (HPCs) in humans, proliferate whenever hepatocyte proliferation is blocked[20,21]. These bipotent, hepatic, progenitor cells are positive for cytokeratin 19 and α-fetoprotein, and in order to restore liver mass, can give rise to both hepatocytes and cholangiocytes**.** However, practical use of HPCs has a number of major limitations, including the isolation of homogeneous cell populations and large-scale expansion for application[22,23].

EXTRAHEPATIC SOURCES OF STEM CELLS

Recently, attention was given to the generation of human hepatocytes from extrahepatic human stem cells. Stem cells are roughly classified into two major categories in accordance with their origin and their capacity of differentiation. The first consists of pluripotent embryonic stem cells (ESCs), which are derived from embryos at the blastocyst stage. The second category is composed of adult (postnatal) stem cells. These are multipotent cells that are found *in vivo* in differentiated organs or tissues, within distinct regions*,* the so-called niches, providing a specialized microenvironment for the regulation and maintenance of these stem cells[24,25,26]. Numerous experimental studies have clearly demonstrated that
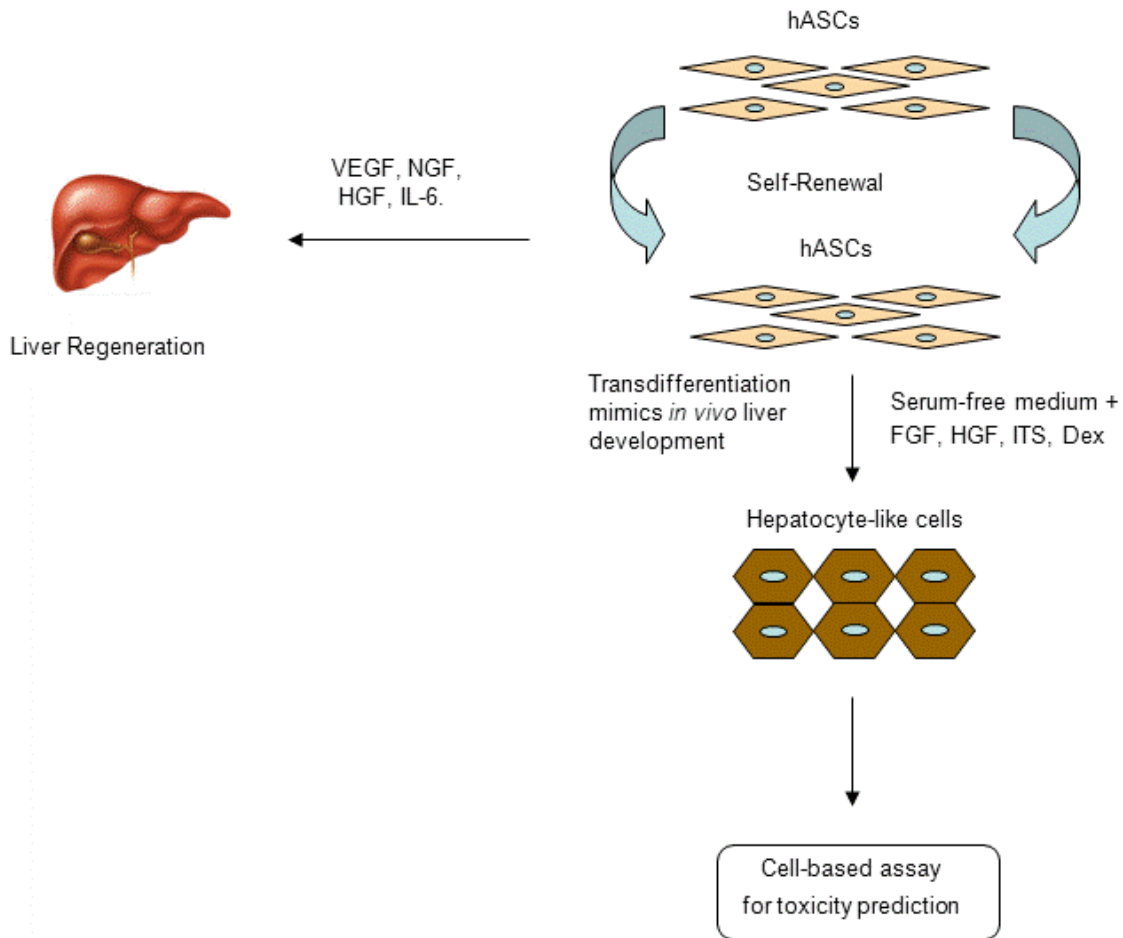
FIGURE 1. ASCs are self-renewing, making them a scale-up cell source. They can also differentiate along the hepatic lineage when cultured in serum-free medium supplemented with hepatic-specific induction factors. ASC-derived hepatocyte-like cells might provide the possibility to be used in toxicity prediction assays. In addition, undifferentiated ASCs secrete growth factors and cytokines that are involved in liver regeneration. Abbreviations: Dex: dexamethasone; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; IL-6: interleukin 6; NGF: neural growth factor; ITS: insulin-transferrin-sodium selenite; VEGF: vascular endothelial growth factor

extrahepatic stem cells, including embryonic and adult (nonhepatic) stem cells, are able to differentiate towards the hepatic lineage[27]. In addition, the recent approach of reprogramming human differentiated cells into pluripotent cells, known as induced pluripotent stem cells (iPSCs), has opened a new era to obtain iPSC-derived hepatic cells[28,29,30,31,32].

## ADULT STEM CELLS FROM ADIPOSE TISSUE

Nomenclature

Different nomenclatures have been suggested to describe ASCs, e.g., adipose stem/stromal cells (ASCs), processed lipoaspirate cells, adipose-derived stromal cells, human multipotent adipose-derived stem cells, adipose mesenchymal stem cells, and preadipocytes. In order to avoid confusion, the International Fat

Applied Technology Society adopted the term adipose-derived stem cells (ASCs) to describe the multipotent, plastic-adherent, cell type isolated from adipose tissue[33,34] .

Isolation, Culture, and Proliferation of hASCs

Adult human adipose tissue originates from the embryonic mesoderm and represents, for regenerative medicine, an abundant and less invasive source of MSCs than bone marrow[8,35]. hASCs can be easily isolated from routine liposuction (lipoaspirate) and reconstructive surgery (lipectomy) waste materials. In brief, the lipoaspirate or minced fat tissue is extensively washed with phosphate buffered saline containing 5% penicillin/streptomycin. The adipose tissue is digested with collagenase to break down the stroma, then filtered and centrifuged. After centrifugation, the resulting pellet is termed the stromal vascular fraction (SVF). Under proper conditions, cells within the SVF subsequently adhere to plastic tissue culture dishes and exhibit a fibroblast-like appearance (Fig. 2A)[8,36]. The SVF is a heterogeneous population of stromal cells, including ASCs, fibroblasts, endothelial cells, pericytes, smooth muscle cells, and circulating cell types, such as immune cells and hematopoietic stem cells. The adherent multipotent ASCs are capable of differentiating to several cell lineages[8,9,37,38,39,40,41,42,43].
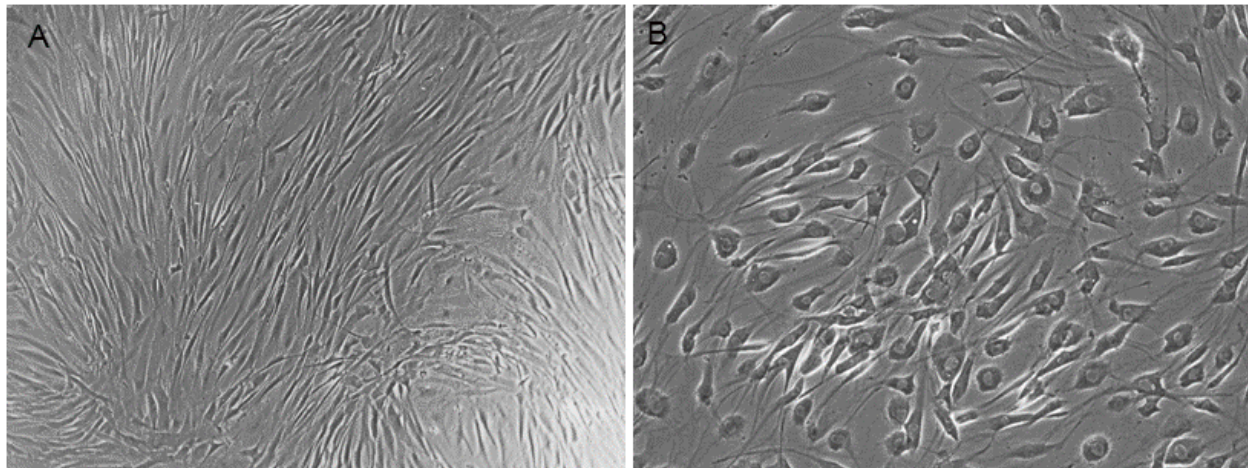
FIGURE 2. Morphology of undifferentiated hASCs (A) and when cultured in hepatic induction medium (B). Undifferentiated hASCs display a fibroblast-like shape, while during hepatic differentiation, they acquire a polygonal or round shape. Phase contrast magnification ×100.

In principle, the selection of hASCs out of SVF is based on their physical adherence to plastic tissue culture dishes. Due to the lack of specific cell surface markers, a number of strategies have been employed to define this ASC population, including the use of immunomagnetic beads coated with specific antibodies, such as CD34, CD105, and CD271[44,45], fluorescence activated cell sorting[46], and the SP (side population) approach based on aldehyde dehydrogenase activity[47,48].

hASCs are cultured and expanded in media, such as minimal essential media (MEM), Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute-1640 medium (RPMI-1640), and Dulbecco's modified Eagle's medium/Ham's nutrient mixture F-12 (DMEM/F12) supplemented with 10% fetal bovine serum (FBS)[49]. Serum supplementation is pivotal because it provides the cells with vital nutrients, attachment factors, and growth factors[50]. Today, efforts are being made to work under serum-free conditions, in particular, the use of serum-replacement factors, e.g., human platelet lysate, has become a promising technique[51].

The proliferation assay showed that ASCs obtained from 20 donors, and cultured under standard conditions (10% FBS), exhibited an average population doubling time of 60 h[8]. Generally, ASCs display a cell doubling time within 2–4 days[11,47]. Additionally, ASCs can be cultured extensively (up to 30 passages), but as any normal somatic cell, they have a finite life span *in vitro* and undergo replicative senescence upon prolonged culture[11,52,53].

Characterization of hASCs

Characteristics of hASCs include positive staining for osteopontin, osteonectin, Muc18 (cluster of differentiation [CD] 146); receptors for extracellular matrix (ECM) proteins, i.e., interstitial cell adhesion molecules (ICAM-1, CD54), ALCAM (CD166), tetraspan (CD9), integrin β_1 (CD29), integrin α_4 (CD49d), endoglin (SH2, CD105), Thy1 (CD90), CD13, CD73, CD44; and negative staining for hematopoietic markers, such as CD45, and CD34, and endothelial markers, such as CD31[33,54,55].

hASCs also exhibit immunomodulatory properties because they lack expression of the major histocompatibility complex class II. ASCs inhibit activation and proliferation of immune cells *in vitro*[56]. Their impact on immune cells is due to both cell-to-cell contact and the release of soluble factors, such as hepatocyte growth factor (HGF), leukemia inhibitory factor, and prostaglandin E2[56,57,58]. Although the mechanisms of ASC-induced immunosuppression are unclear, this property could be valuable for the potential use of ASCs in immunosuppressive and regenerative medicine[56].

PROTOCOLS, MARKERS, AND FUNCTIONAL ANALYSIS OF HEPATOCYTE-LIKE CELLS FROM HASCS

The majority of studies on stem cell–derived hepatocytes are based on the use of ESCs, liver stem/progenitor cells, and bone marrow–derived cells[27]. However, emerging evidence has shown that hASCs represent an interesting alternative cell source[3,43,59,60,61,62]. Multilineage plasticity of hASCs is well established, but with respect to endodermal differentiation, particularly towards the hepatic lineage, only a limited number of reports have been published with a number of highly promising results. The methodology used is summarized in Table 1.

Seo et al.[3] demonstrated for the first time that hASCs can differentiate into hepatocyte-like cells by a one-step protocol using HGF, oncostatin M (OSM), and dimethyl sulfoxide (DMSO). The differentiated cells show functional characteristics of parenchymal liver cells, including uptake of low-density lipoprotein (LDL) and urea production. In addition, they found that differentiated hASCs can *in vivo* repopulate the livers of severe combined immunodeficient (SCID) mice suffering from acute liver injury induced by carbon tetrachloride (CCl$_4$) injections.

Another interesting work is the one reported by Talens-Visconti et al.[59] that comparatively demonstrated that adult human MSCs, derived from bone marrow and adipose tissue, exhibit similar expression patterns of surface protein markers and a comparable hepatic differentiation potential. Evidence was also provided that differentiated hASCs express drug metabolizing enzymes at the mRNA level, e.g., cytochrome P450 2E1 (CYP2E1) and CYP3A4**.**

Banas et al.[60] further investigated the hepatic differentiation of the CD105$^+$ hASCs. Over a period of 3 weeks, they observed morphological changes during hepatic induction. Specifically, the hASCs changed from flat, elongated, spindle-shaped cells to rounded epithelial cells with tight cell-to-cell interactions, and bile canaliculi structures became visible. Hepatic maturation was followed for another 2 weeks, during which the number of cells exhibiting the hepatic phenotype increased substantially as demonstrated by LDL uptake. More importantly, transplantation of CD105$^+$ ASC-derived hepatocyte-like cells into mice showed their incorporation into the liver parenchyma. An additional report from that group showed that hASCs can be differentiated *in vitro* towards hepatocyte-like cells by a short induction and that the latter can improve liver functions when transplanted *in vivo*[61].

TABLE 1
Overview of ASCs Isolation, Expansion, and Hepatic Differentiation

Isolation Procedure	Expansion Medium	Hepatic Differentiation Medium	Ref.
Collagenase type I followed by one-step centrifugation	60% DMEM-LG/5% FBS, 40% MCDB-201, 1x ITS, 10^{-9} M Dex, 10^{-4} M ascorbic acid 2-phosphate, 10 ng/ml EGF, 100 U penicillin, 1000 U streptomycin	DMSO (0.1%), HGF (10 ng/ml), OSM (10 ng/ml)	[3]
Collagenase type I followed by serial centrifugations	DMEM-LG, 15% human serum, 50 µg/ml gentamicine	EGF (20 ng/ml), bFGF (10 ng/ml), HGF (20 ng/ml), nicotinamide (4.9 mmol/l), OSM (20 ng/ml), Dex (1 µmol/l), ITS (10 µl/ml), BSA (1.25 mg/ml), linoleic acid (190 µmol/l)	[59]
Collagenase type I followed by positive selection of CD105$^+$	DMEM, 10% FBS	Transferrin (5 µg/ml), hydrocortisone-21-hemisuccinate (10^{-6} M), BSA (0.5 mg/ml), ascorbic acid (2 mM), EGF (20 ng/ml), insulin (5 µg/ml), Dex (10^{-8} M), HGF (150 ng/ml), FGF1 (300 ng/ml), FGF4 (25 ng/ml), OSM (30 ng/ml), Dex (2×10^{-5} mol/l)	[60]
Collagenase type I followed by positive selection of CD105$^+$	DMEM, 10% FBS	Activin A (20 ng/ml), FGF4 (20 ng/ml), transferrin (5 µg/ml), hydrocortisone-21-hemisuccinate (10^{-6} mol/l), BSA (0.5 mg/ml), ascorbic acid (2 mmol/l), EGF (20 ng/ml), insulin (5 µg/ml), Dex (10^{-8} M), HGF (150 ng/ml), FGF1 (100 ng/ml), FGF4 (25 ng/ml), OSM (30 ng/ml), Dex (2×10^{-5} mol/l), 1x ITS, nicotinamide (0.05 mmol/l), DMSO (0.1%)	[61]
Collagenase followed by Percoll density gradient centrifugation	60% DMEM, 40% MCDB, 5 mg/ml apotransferrin, 5 ng/ml selenous acid, 5 mg/ml linoleic acid, 5 mg/ml bovine insulin, 100 mM ascorbic acid 2-phosphate, 1 nM Dex, 10 ng/ml PDGF, 10 ng/ml EGF, 100 U/ml penicillin, 10 mg/ml streptomycin, 15% FCS	5′Azacytidine (20 µM), human hepatocyte maintenance medium, FCS (2%), HGF (40 ng/ml), EGF (20 ng/ml)	[43]
Collagenase type II followed by Lymphoprep density gradient centrifugation	60% DMEM-LG, 40% MCDB-201, 1x ITS, 1 nM Dex, 100 mM ascorbic acid 2-phosphate, 10 ng/mL EGF, 5% FBS	Dex (1 nM), ascorbic acid (100 µM), EGF (10 ng/ml), bFGF (10 ng/ml), HGF (10 ng/ml), OSM (10 ng/ml), DMSO (0.1%)	[62]

Abbreviations: bFGF: basic fibroblast growth factor; BSA: bovine serum albumin, Dex: dexamethasone; DMSO: dimethyl sulfoxide; EGF: epidermal growth factor; FBS: fetal bovine serum; FCS: fetal calf serum; FGF1: fibroblast growth factor 1; ITS: insulin-transferrin-sodium selenite; LG: low glucose; OSM: oncostatin M; PDGF: platelet-derived growth factor.

Aurich et al.[43] used induction media supplemented with HGF and epidermal growth factor (EGF) to obtain hepatocyte-like cells from hASCs. Prior to hepatic induction, they treated the hASCs with the epigenetically modifying substance 5′azacytidine for 24 h. After 21 days of induction, they found that differentiated cells could store glycogen, synthesize urea, and metabolize 7-ethoxyresorufin to resorufin, indicative of CYP1A enzyme activity. hASCs were transplanted into the liver of immunodeficient Pfp/Rag2-/- mice with and without prior *in vitro* hepatic differentiation. The results demonstrated that

differentiated cells expressed albumin and hepatocyte paraffin 1. Moreover, predifferentiated hASCs showed better cell engraftment in comparison to those that were undifferentiated. After 10 weeks, more than 10% of all hepatocytes in the host liver were replaced by hepatocyte-like cells derived from hASCs.

By using a floating culture technique, hepatocyte-like cell clusters could be obtained from hASCs[62]. After culture in medium supplemented with hepatic induction factors, hepatocyte-like cell clusters were generated, exhibiting functional properties of hepatocytes as characterized by gene expression analysis and functional assays. Transplantation of these cell clusters into nonobese diabetic-SCID mouse with chronic liver injury resulted in a significant improvement of serum albumin and total bilirubin levels[62].

Our group recently investigated the hepatic potential of BM-MSCs by administrating hepatic inductive factors in a sequential way, reflecting the embryonic liver development *in vivo*[63]. This approach resulted in the efficient production of human hepatocyte-like cells (approximately 25%) that displayed hepatic functions, including albumin secretion, urea production, and CYP1A1 activity. To demonstrate the feasibility of this approach on other MSC types, we applied the same sequential protocol on hASCs and this resulted in hepatocyte-like cells that expressed hepatic-associated markers as characterized by immunocytochemistry at the protein level (unpublished data).

MOLECULAR MECHANISMS UNDERLYING THE PLASTICITY OF HASCS TOWARDS A HEPATOCYTE-LIKE PHENOTYPE

At present, the molecular mechanisms underlying the plasticity of hASCs towards a hepatocyte-like phenotype remain poorly understood. Yamamoto and colleagues examined the gene expression profiles of hASC-derived hepatocytes in order to identify the genes responsible for hepatic differentiation using several microarray methods. The resulting sets of differentially expressed genes were comprehensively analyzed in order to identify the pathways expressed in hASC-derived hepatocytes. Microarray analysis revealed that the gene expression pattern of hASC-derived hepatocytes was similar to that of adult human hepatocytes and liver. Further analysis showed that enriched categories of genes and signaling pathways, such as complementary activation and the blood clotting cascade in the hASC-derived hepatocytes, were relevant to liver-specific functions. Notably, decreases in Twist and Snail expression indicated that mesenchymal-to-epithelial transition (MET) occurred during differentiation of hASCs into hepatocytes[64].

More recently, Saulnier et al.[65] compared the expression profile of the hASCs before and after *in vitro* hepatogenic treatment by means of a high-throughput molecular analysis. They identified several targets that depict the numerous biological functions exerted by the liver, including protein metabolism, innate immune response regulation, and biodegradation of toxic compounds. Furthermore, microarray analysis highlighted down-regulation of transcripts associated with the mesenchymal lineage, such as N-cadherin-2 and vimentin, while epithelial-related genes were overexpressed, such as the vascular cell adhesion molecule. Altogether, these reports suggest that cellular plasticity observed in hASCs is dependent on MET.

THERAPEUTIC POTENTIALITY OF HASCS IN ANIMAL MODELS OF LIVER DISEASES

Besides their high differentiation potential, several recent studies have shown that MSCs, in particular BM-MSCs, have the ability to support the proliferation and functionality of hepatocytes either via direct cell-cell contact or production of bioactive factors, such as cytokines[66]. This ability has been investigated as well *in vitro* as *in vivo*. Both cocultivation of hepatocytes with BM-MSCs or adding conditioned medium of BM-MSCs to hepatocytes demonstrated that MSCs were able to sustain albumin and ammonia production at higher levels than was the case in control cells. Interleukin (IL)-6 was one of

the factors involved[67]. Furthermore, when BM-MSCs were administrated to a D-galactosamine–induced fulminant hepatic failure rat model, using either conditioned medium or cell lysates, the mortality among these animals was significantly reduced[68].

However, *in vitro* exposure of hepatocytes to hASCs has not yet been investigated. To date, studies on animal models reported the beneficial effects of hASCs in promoting liver regeneration. Transplantation of hASCs into SCID mice with acute liver failure caused by CCl$_4$ injection revealed that undifferentiated hASCs were able to engraft into the liver and improve its function. Investigators postulated that the beneficial effects of hASCs could be due to MSC ability to secrete bioactive molecules that modulate the local microenvironment, and contribute to hepatocyte proliferation and function. Interestingly, it was found that undifferentiated hASCs *in vitro* produce a large number and volume of bioactive factors, such as HGF, vascular endothelial growth factors, nerve growth factor, and IL-6[15].

Although these findings have potential, the approach of using hASCs or MSCs in general is in its infancy and plenty of issues still remain to be addressed before application is justified in clinical settings. ASCs are a heterogeneous cell population and this represents a main limit to their use. A standardized protocol for isolating a specific cell population is lacking. This heterogeneity in isolation methods generates variable results that make the interpretation of data very difficult[69]. The behavior of ASCs within the liver could also be questioned, as *in vivo* studies demonstrated that transplanted MSCs may contribute to scar-forming myofibroblasts in the liver and thus enhance the fibrotic process[70]. Moreover, risk of tumor formation after transplantation it is not yet well studied. It has been shown that hASCs undergo malignant transformation when prolonged passaging over 4 months takes place[71]. Despite many unresolved issues in this field, at present, one clinical trial using autologous hASCs for liver regeneration has been registered (http://clinicaltrials.gov).

POTENTIAL APPLICATION OF hASC-DERIVED HEPATOCYTE-LIKE CELLS IN TOXICOPHARMACOLOGICAL SCREENING ASSAYS

Besides the presence of other cell types, the adult human liver is predominantly composed of parenchymal cells, so-called hepatocytes. They comprise approximately 80% of the total liver mass[72]. Hepatocytes perform various, essential liver functions. They are polarized epithelial cells. Through the apical surface, they secrete bile and detoxification products by transport proteins into bile canaliculi. Through the basolateral surfaces, they take up substances from the blood and secrete serum factors back[73]. Moreover, their unique structure, along with various enzymes expressed in hepatocytes, show that they are involved in protein synthesis, transformation of carbohydrates, synthesis of cholesterol, bile salts, and phospholipids. Also, detoxification and toxification (activation) of exogenous substances mainly occurs in hepatocytes[74]. These features make hepatocytes an attractive tool, not only for studying xenobiotic biotransformation, but also as target cells for toxic reactions[75,76]. Indeed, while the ultimate effect of biotransformation is to facilitate removal of the xenobiotic from the body, it can also result in formation of reactive metabolites, which directly or indirectly may cause hepatic injury[77,78,79,80]. This impairment of cellular function can culminate in cell death by apoptosis or necrosis, and in hepatotoxicity or so-called drug-induced liver injury (DILI)[77,78,81]. Animal models and *in vitro* studies with respect to DILI reveal different mechanisms that might be relevant to the development of liver damage. In human hepatocytes, DILI can be initiated through organelle dysfunction. Mitochondrial dysfunction is a good example, including disruption of mitochondrial energy production and/or release of proapoptotic proteins into the cytoplasm that end up in hepatocyte necrosis or apoptosis and cytolytic hepatitis[82]. Additionally, other potential mechanisms may be involved: parent drugs and their reactive metabolites might also specifically inhibit other hepatocellular functions, such as the apical bile salt export pump (ABCB11)[77]. In this case, the subsequent intercellular accumulation of its substrates (e.g., conjugated bile salts) may result in the development of cholestatic liver cell injury, also known as cholestasis[77,83].

As described above, DILI can have detrimental effects on hepatocytes and may lead to liver failure. Therefore, DILI has emerged as a major challenge for the pharmaceutical industry. It is a leading cause of postmarketing drug failure, as well as at the preclinical and clinical phases of drug development[77,82]. Thus, identification of potential liver toxicity in the preclinical phase could be a critical step for pharmaceutical companies in order to reduce drug attrition and to bring drugs on the market with fewer side effects. Liver-based *in vitro* models could represent key experimental systems here, in particular when they are human derived.

During the development of pharmaceuticals, the potential toxicity of the lead molecules is carefully assessed. Usually, experimental animals are used. This approach has, besides ethical considerations, a number of limitations, including the fact that they are time consuming, expensive, and animals frequently do not fully reflect the human response[84]. For these reasons, the development of *in vitro* cell models, preferably human based, is receiving growing interest, particularly in the preclinical phase of drug development.

Several *in vitro* human hepatic models have been developed in the past few decades, including perfused liver, liver slices, primary liver cells, liver cell lines, transgenic cell lines, subcellular fractions, and isolated enzyme systems[85,86]. In particular, cultured primary hepatocytes, either derived from rodents[87] or humans, have been shown to be useful in toxicity studies, as they maintain a number of *in vivo* liver functions[78,84,85,88]. During the last years, however, the availability of human primary hepatocytes became more limited by the scarcity of donor organs because of transplantation possibilities. Furthermore, once brought in culture, they dedifferentiate and lose their phenotypic functionality[78,89]. Therefore, a significant demand exists to obtain fully functional human hepatocytes from alternative sources. In that respect, stem cells could represent a potential source to provide an unlimited number of human hepatocytes, as they retain proliferation capacity *in vitro* and, under appropriate conditions, may differentiate into a variety of functional cell types, including hepatocytes[27,31,90,91].

Both pluripotent stem cells (ESCs and iPSCs) and somatic stem cells show good potential and ability to acquire liver metabolic functions with respect to drug metabolism after *in vitro* hepatogenic differentiation[92,93,94,95,96]. The use in toxicology of hepatocyte-like cells derived from whatever the source of human stem cells implies that they should be metabolically competent and express phase I and phase II xenobiotic metabolizing enzymes, as well as transporter proteins at levels consistent with those measured in the liver or at least in primary hepatocyte cultures[97]. Given the importance of CYP activity for drug inactivation, prodrug activation, or the generation of toxic metabolites, assessment of these enzymes in hASC-derived hepatocyte-like cells is critical and, as yet, incomplete. Whereas some studies of hASC-derived hepatocyte-like cells have detected CYP transcripts or proteins such as CYP1A1, 1B1, 2E1, 2C9, 3A4, 3A7 and 7A, actual enzyme concentrations have not been quantified[43,59,60,62]. In two reports, CYP activity comparable to that of adult human hepatocytes has been described. Aurich et al. detected CYP1A expression as measured by ethoxyresorufin-O-deethylase activity in hepatocyte-like cells[43], whereas Okura et al. reported CYP3A4 activity as detected by hydroxylation of 7-benzyloxy-4-trifluoromethyo-coumarin[62]. The data obtained from these studies suggest that hASC-derived hepatocytes exhibit a level of functionality, but as indicated above, in order to determine the feasibility of using the hASC-derived hepatic cells in studies of drug-induced hepatotoxicity, these differentiated cells really need to develop a complete biotransformation system, not only by expressing phase I enzymes, but also phase II enzymes and phase III transporters in order to be considered metabolically competent.

CONCLUSION

Accessibility, abundance, and immunosuppressive properties of hASCs have attracted attention for the use of these cells in regenerative medicine, in particular for liver diseases. Subsequently, obtaining mature hepatocytes from adipose-derived stem cells has become a priority research area. As mentioned in this review, *in vitro* studies have shown that, under proper stimulation, hASCs are capable of differentiating into endodermal cells. They can acquire hepatic characteristics including polygonal morphology (Fig.

2B), albumin secretion, and expression of several CYP enzymes. Moreover, transplantation of undifferentiated hASCs seems to ameliorate liver injury and to improve liver functions. These properties suggest that transplantation of hASCs as fully or partly differentiated cells could have a positive effect on liver regeneration. Before clinical applications can be considered, more research work is needed to understand and elucidate the main mechanisms whereby hASCs improve liver functions *in vivo*.

While some efforts were undertaken to prove the presence and functionality of phase I enzymes in hASC-derived hepatocyte-like cells, the expression and, in particular, activity of phase II and phase III transporter proteins should also be addressed. This is necessary to evaluate to what extent hASC-derived hepatocyte-like cells can be used as a human *in vitro* cell model to predict hepatotoxicity in the early phases of drug development.

ACKNOWLEDGMENTS

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