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Review Article

Pluripotent Stem Cell-derived Strategies to Treat Acute Liver Failure: Current Status and Future Directions

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Abstract

Liver disease has long been a heavy health and economic burden worldwide. Once the disease is out of control and progresses to end-stage or acute organ failure, orthotopic liver transplantation (OLT) is the only therapeutic alternative, and it requires appropriate donors and aggressive administration of immunosuppressive drugs. Therefore, hepatocyte transplantation (HT) and bioartificial livers (BALs) have been proposed as effective treatments for acute liver failure (ALF) in clinics. Although human primary hepatocytes (PHs) are an ideal cell source to support these methods, the large demand and superior viability of PH is needed, which restrains its wide usage. Thus, a finding alternative to meet the quantity and quality of hepatocytes is urgent. In this context, human pluripotent stem cells (PSC), which have unlimited proliferative and differential potential, derived hepatocytes are a promising renewable cell source. Recent studies of the differentiation of PSC into hepatocytes has provided evidence that supports their clinical application. In this review, we discuss the recent status and future directions of the potential use of PSC-derived hepatocytes in treating ALF. We also discuss opportunities and challenges of how to promote such strategies in the common applications in clinical treatments.

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Abbreviations: ALF, Acute liver failure; BAL, Bioartificial liver; DE, Definitive endoderm; HB, Hepatoblast; hESC, Human embryonic stem cell; HLC, Hepatic like cell; HT, Hepatocytes Transplantation; iPSC, induced pluripotent stem cell; OLT, Orthotopic liver transplantation; PH, Primary hepatocytes; PSC, Pluripotent stem cell.

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Introduction

Liver diseases, including acute liver failure (ALF) are a public health challenge worldwide, because of death caused by liver dysfunction.[1–3] ALF is a severe condition with significant morbidity and mortality even for the patients without pre-existing liver disease. The causes of ALF vary geographically with viral infections of the liver, primarily hepatitis B, C, and E in developing countries and drug overdose-induced ALF, usually paracetamol (acetaminophen), in developed countries such as USA and parts of Europe.[4–8] Because of the severity of ALF, there are few ways to prevent or cure patients other than orthotopic liver transplantation (OLT), which is now the only treatment that is considered effective to avoid the life-threatening complications caused by ALF.[9–11] However, OLT is limited by the scarcity of available donor livers, complicated surgery procedures, and high financial burden.[12] Therefore, other than OLT and drug supplements for the maintenance of basic vital signs, there is a need for effective therapeutic treatments for ALF.

In recent years, hepatocytes transplantation (HT) and bioartificial liver (BAL) system have emerged as effective methods for the compensatory treatments of ALF related liver dysfunction.[13–16] These two methods potentially build up the fundamental niche for host liver regeneration and decelerate the disease progression, which creates a bridging time for OLT. As reported, effective HT involves reconstitution of as much as 2.5% functional liver tissue in treating acute-on-chronic liver failure (ACLF).[17] Consistent with that, primary hepatocytes (PHs) are considered the ideal cell source for such treatments. Unfortunately, it remains a bottleneck to meet the demand of large quantity and clinical quality of PH from limited viable organ donation. To solve these problems, studies have focused on developing strategies using human pluripotent stem cell (PSC)-derived hepatic-like cells (HLCs), including hepato-blasts and hepatocytes. The differentiation of PSCs into clinical-grade HLCs has been studied.[18–20] The aim of this review is to summarize the current opinions regarding the therapeutic effectiveness of PSC-derived HLC for ALF treatment and to discuss recent progresses in preclinical and clinical treatments and challenges, which need to be improved in using PSC-derived HLC (Fig. 1).

Characteristics of ALF

ALF is characterized by severe injury of liver cells that has

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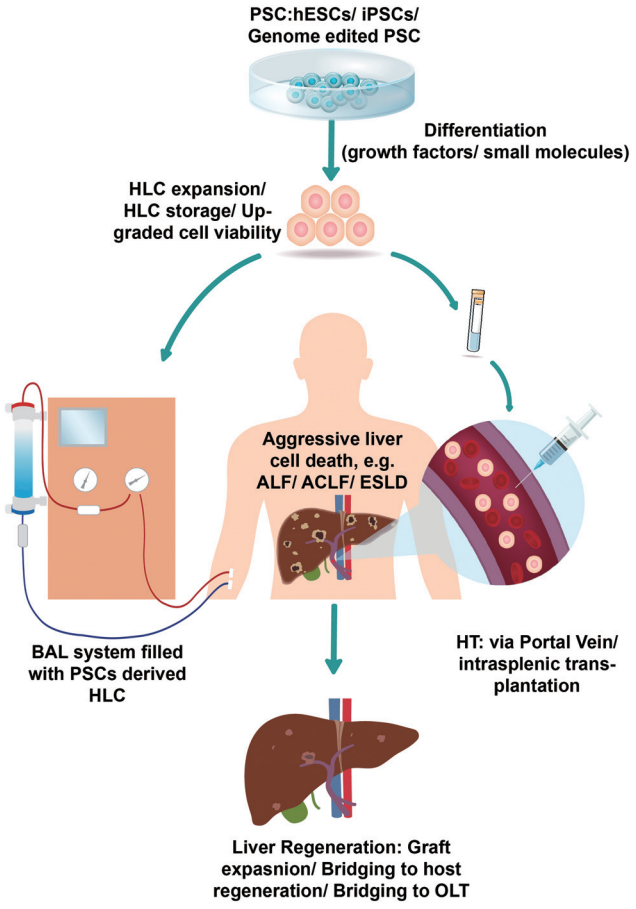

Fig. 1. Producing pluripotent stem cell (PSC)-derived hepatic-like cells (HLCs) for use in bioartifical liver support and hepatocyte transplantation applications. The advantage of using PSC derived of HLC is their unlimited proliferation potential, which addresses both the shortage of viable donor livers and primary hepatocytes. By differentiating PSC (hESCs or iPSCs) or genome edited PSC into HLC, we can obtain HLCs of the required quantity and quality for BAL and HT in severe liver disease (e.g., ALF, ACLF, and ESLD). After BAL or HT treatment, the ideal outcome is either graft expansion and the regeneration of the host liver or bridging to OLT. ALF, acute liver failure; ACLF, acute on chronic liver failure; BAL, bioartificial liver; ESLD, end-stage liver disease; hESC, human embryonic stem cell; HLC, hepatic-like cell; HT, hepatocyte transplantation; iPSC, induced pluripotent stem cell; OLT, orthotopic liver transplantation; PSC, pluripotent stem cell.

a rapid onset and leads to a frequent fatal outcome, with up to 30% mortality.[21] Paracetamol overdose and autoimmunity caused liver injuries are the most frequent causes in developed countries. HBV infection is the primary cause of ALF in developing countries.[2] Paracetamol toxicity, which induces mitochondrial oxidant stress-related cell death and sterile inflammatory responses in hepatocytes, accounts for more than 46% of the ALF cases in the USA.[22] At the early stage of paracetamol-induced liver injury, treatment with N-acetyl-cysteine or 4-methylpyrazole (fomepizole) can effectively control the progress.[23] However, at later stages, drugs are no longer effective to slow disease progression, which leaves OLT as the last option to save such patients. HBV infection has plagued China for a long time, and is involved in 84% of hepatocellular carcinoma and 77% of liver cirrhosis patients annually.[6] Control of HBV is fundamental to preventing ALF. Anti-HBV drugs focus on how to slow the replication of viral DNA, but completely eliminating HBV DNA is hard to achieve, and is the main reason of HBV relapse and progression.[24,25] Once the HBV replication is out of control, there's a large chance to cause ALF. The pathology and autopsy of ALF patients often shows widespread hepatic apoptosis and necrosis with few viable hepatocytes remaining, which leads to the failure of liver regeneration. To save ALF patients, the question to answer is how to buy time for patients to carry out liver regeneration.

Treatment of ALF must deal with systemic complications including the release of pro-inflammatory cytokines, multiple organ failure, and a hypotensive environment. Hepatic encephalopathy frequently appears because they hepatocyte death results in aberrant liver function and toxins that travel to the brain and affect the brain function. Although L-ornithine-L-aspartate and ornithine phenylacetate inhibit ammonia synthesis to relieve symptoms, OLT is current, y the last chance for ALF patients currently. Development of novel treatments of ALF patients is currently urgent.

Current knowledge of the treatments for ALF

In addition to the basic symptomatic supporting treatments to stabilize the vital signs, cell therapy-based supplement for liver regeneration and bioartificial liver (BAL) support system have been developed as effective tools for ALF patients. Both of these methods require a large quantity of viable hepatocytes.

BAL system

Before the emergence of BAL, abiotic artificial liver therapy, including plasmapheresis, hemoperfusion absorption, and venous hemodiafiltration, were used as clinical treatments with limited success.[26,27] The molecular adsorbent recirculating system and Prometheus system are widely used non-bioartificial liver systems with benefits for ALF patients.[28,29] However, as it relies on exogenous detoxification, is not able to provide an environment needed for hepatic regeneration as it is complicated to mimic all the functions of host hepatocytes. BAL systems include functional hepatocytes in a bioreactor that simulates the function of a normal human liver. To a large extent, it can not only remove the toxic substances but also provide functions such as synthesis and metabolism, which temporarily replace the function of the damaged liver in order to survive from the fatal onsets of ALF.[16,30] The indispensable factor within the BAL system are the functional hepatocytes. The quality of functional hepatocytes, the ease of obtaining them and safety are decisive in determining whether the BAL can play an important role in clinical treatment.

Prior to this, the main sources of functional hepatocytes were primary liver cells, porcine liver cells, human liver cancer cell lines like HepG2, HepaRG, and immortalized human liver cell lines like L-02. Human PH are the best for use in BALs, but organ sources are limited, and it is difficult to obtain a sufficient number of human PH for BALs. Porcine liver cells are used because of their functions, abundant source, and the easy accesses. For example, the AMC artificial liver system using porcine liver cells successfully helped 12 patients with ALF to gain time for OLT. One patient no longer needed because of the effectiveness of therapy.[31,32] The HepaAssist system, which uses porcine liver cells, is the only BAL system that has been a investigated in a multicenter randomized controlled clinical trial in the USA. Although it has achieved encouraging therapeutic effects in phase III clinical trials, it has not yet obtained Federal Drug Administration approval. It is underlying safety concerns including heterogeneous immune rejection and animal-derived virus infections have made it difficult to obtain regula-

Table 1. Clinical use of hepatocyte transplantation to treat acute liver failure (ALF)

Time (year)	Number of recipients	Delivery route	Outcomes	Reference
Drug-induced ALF				
1999	2	Portal vein	2 Deaths: days 4 and 35	41
2000	3	intrasplenic	3 Deaths: 6 h, days 14, and 20	42
2006	6	Intrasplenic and portal vein	3 Deaths: days 1, 3, 18; 2 OLT: days 2 and 10; 1: Full recovery	43
Hepatitis virus-induced ALF				
2000	1	Intrasplenic	1 Full recovery	45
2000	2	Intrasplenic and portal vein	2 Deaths: 18 h and day 52	42
2006	2	Portal vein	2 Deaths: days 2 and 7	43
2010	1	Portal vein	1 Death: day 11	44
Acute-on-chronic liver failure				
2014	7	Intrasplenic	3: Full recovery; 3 Death: 2.5–12 months; 1: OLT	17

tory approval.[33] The superiority of human liver cancer cell lines and immortalized human liver cell lines is that they can proliferate indefinitely *in vitro*. However, their functions are greatly compromised and there is a potential tumorigenic risk, which limits their application prospects. For example, the Vital Therapies artificial liver system, which uses C3A liver cancer cells, failed a phase III clinical trial because of poor therapeutic effects, even though the effectiveness in animal experiments was good.[34,35] Therefore, to obtain a large quantity and clinical-grade quality of functional hepatocytes is the major hindrance for BAL.

Nowadays, in the research of regenerative medicine, PSC has received much attention due to the potential to be differentiated into functional hepatocytes as the source of seed cells in the BAL system. Precise differentiation of human embryonic stem cell (hESCs) or induced pluripotent stem cell (iPSCs) into HLC has been achieved and improved tremendously. In addition, with the appearance of 3D culturing system, hepatic organoid formation brings out more mature HLC, which owns comprehensive functions.[36,37] Moreover, Lijian Hui of Shanghai also successfully transdifferentiated human fibroblasts into human hepatocytes (hiHep), and overexpressed SV40 Large T through gene editing, thus obtaining the ability to be expanded *in vitro*, providing a potential cell source for BAL.[38] This technology also successfully conducted a clinical trial of a bioartificial liver in 2016, and achieved good therapeutic effects, which greatly improved the confidence to promote hiHep into the clinic applications. In addition, bioreactors, as the key devices in BAL system, are able to provide a favorable proliferative and metabolic platform for a large-scale liver cell culture and storage.[39] For example, a fluidized-bed bioreactor with alginate-based spherical beads is able to scale up 10^{11} liver cells culture and retains their hepatic functions.[40] Yet the challenge is to extend such design to clinical applications.

Hepatocyte transplantation (HT)

The concept of HT therapy was first described by scientists in the early 1970s. After more than 20 years of development, HT therapy was translated from animal experiments to clinical trials, and was shown to be effective in ALF, or acute-on-chronic liver failure (Table 1).[17,41–45] HT has several key therapeutic advantages. (1) It is less invasive OLT surgery and can be performed multiple times. (2). The patient's liver is preserved and retains its ability to regenerate itself. (3) With the development of gene editing and stem cell technology, HT can be coupled with targeted genome modifications, realizing individualized and precise treatment.[15,46] These advantages are not available in OLT or BAL support systems. So far, many liver diseases have undergone clinical trials of HT treatment, laying the foundation for clinical promotion and application.

How to gain time is a significant issue for ALF patients. For one thing, HT helps patients to regenerate their own livers, providing a proliferative niche for transplanted hepatocytes. While OLT is inevitable, HT plays a role as a transitional bridge connecting patients with an appropriate donor liver. In animal models of drug-induced ALF, HT significantly improves survival. In clinical trials, there have been more than 40 cases of ALF caused by drugs or viral infections treated by HT worldwide.[47,48] Although, they were not multicenter randomized controlled trials and the delivery method, volume of transplanted cells, and cell sources were not standardized, which makes them difficult to compare statistically, most patients responded well to treatment, with prolonged survival time, bridging to OLT, and even fully recovery (Table 1).[17,41] The limited clinical data fully confirms the therapeutic effect of HT, but it needs to be further standardized and unified.

PSC-derived hepatocytes

With both BAL support or HT treatment, the key to success is the quality and quantity of functional liver cells. Human PSCs, including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), have unlimited proliferation ability and the pluripotency to differentiate into any somatic cell type. Therefore, the differentiation of PSCs into HLCs with similar gene expression profiles and functions as human hepatocytes can, to a large extent, solve the problem of limited sources of functional hepatocytes. Recent advances in stem cell research have found methods that have increased the ease of inducing *in vitro* differentiation into HLCs. However, often not more than 10^9–10^{10} the hepatocytes are available for treatment, which is a barrier

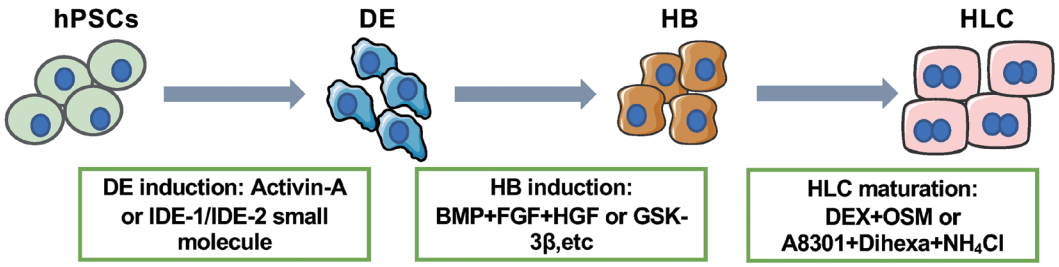

Fig. 2. Introduction of the differentiation of human pluripotent stem cells (hPSCs) into mature hepatocyte-like cells (HLCs). Adapting hPSCs with activin-A is a well-known protocol for definitive endoderm (DE) induction. IDE-1 and IDE-2 are small molecules that can replace activin-A, which is an easier and inexpensive way of induction. The combination of growth factors for hepatoblast (HB) induction is well studied. Hepatic-like cell (HLC) induction and maturation is the last step for a successful differentiation. This step can be induced by dexamethasone (DEX) and oncostatin M (OSM). Small molecules could be developed for use in clinical applications.

between PSC differentiation and clinical application. One of the obstacles is that the efficiency of differentiation is limited, which often accompanied by the risk of incomplete differentiation or incorrect cell fates, resulting in unpredictable safety issues. Additionally, the current hepatocyte culture system has not been well developed, which is hard to maintain the proliferation ability and the functions of cultured hepatocytes at the same time. Therefore, we need to reach a more comprehensive and in-depth understanding of the molecular mechanisms of direct differentiation of PSC into HLC, to establish an efficient and stable differentiation system. We need to find ways to culture and expand hepatocytes *in vitro* to obtain a large number of clinical-grade hepatocytes, which is of great significance for the treatment of ALF by BAL and HT. The paragraphs below review the current status and progress of PSCs used for the treatment of ALF.

Differentiation of PSCs into HLCs

The study of precise differentiation of PSC into HLC *in vitro* is mainly through simulating the development of human liver, which is accomplished by adding growth factors and small molecules that regulate the related signal pathways. Methods described in the available studies can be used to induce the differentiation of PSC into definitive endoderm (DE), hepatoblasts (HB), and mature hepatic cells, i.e. HLCs. Although the specific induction schemes adopted by different research groups are not the same, the basic method is: (1) induction of DE cells by activin-A; (2) Transformation of DE to HB by treatment with FGF, BMP, and HGF; and (3) use of OSM and dexamethasone (DEX) to induce maturation of HB into HLC (Fig. 2).[49]

The induction of DE is the first step of differentiation and is a key step that determines the final differentiation efficiency. The most frequently used method is the induction of PSC to form DE cells by activin-A. The underlying mechanism is activation of the Nodal signaling pathway, which simulating the early steps of liver development *in vivo*.[50–52] Some studies have reported that inhibiting the PI3K signaling pathway was a prerequisite for the effective use of activin-A for DE induction. Adding PI3K signaling pathway inhibitors improves the efficiency of DE differentiation.[53] Adding a rho kinase (ROCK) inhibitor at that stage reduces cell apoptosis to a certain extent, which improves cell survival and differentiation efficiency. Compared with the complex signaling pathways regulated at the DE stage, the regulation of the differentiation of HB and HLC cells is relatively clear. *In vivo* studies of liver development, *in-vitro* coculture studies and the single-cell sequencing have shown that the transforming growth factor beta (TGF-β), Wnt and NOTCH signaling pathways are the pathways most involved in the induction of DE cells by growth factors such as BMP, FGF, and HGF. This step avoids the establishment of an incorrect cell fate (e.g., bile duct or pancreas cells) and improves the purification of HLC at the final stage.[54]

Differentiation induced by growth factors is recognized as an efficient method of obtaining functional HLCs, but growth factors are expensive and difficult to store, which limits their use for large-scale production of HLCs. In addition, most growth factors are protein products containing animal components that may cause adverse reactions associated with clinical use. In that context, a combination of small molecules can be used to replace the growth factors and obtain functional HLC with high efficiency. Properties of the small molecules include the ability to freely penetrate cell membranes, stable structures, no immunogenicity, low cost, and wide variety. The use of small-molecule compounds is expected to become a safer and more effective method of inducing clinical-grade HLCs. Recent reports by multiple research groups have described the use of small molecules to induce differentiation into HLCs. IDE1 and IDE2 are small molecules that can efficiently induce PSC to form DE, act much as activin-A by simulating the Nodal signaling pathway.[55] In the HB stage, glycogen synthase kinase (GSK)-3β is used to simulate the Wnt pathway to guide DE to a hepatic fate and not bile duct fate.[56,57] Recently, Asuma *et al*.[20] reported the use of small molecules to differentiate hESCs into HLC. A comparison of HLCs induced by small molecules and those derived from growth factors showed a considerable number of functions, such as albumen (ALB) secretion, CYP450 activity which metabolizes drugs and enzymes. In addition, Pan *et al*.[58] introduced an improved combination of small molecules for robust HLC induction. The use of small molecules activity has promising prospects, but further research is needed to develop more stable and efficient combinations of small molecules to increase effectiveness and safety for adapting to clinical use.

Functional HLCs can be obtained by direct differentiation of PSCs. There are also reports of transdifferentiating somatic cells to obtain functional HLCs. Hui, L *et al*.[38] reported that after human fibroblasts overexpressing the transcription factors FOXA3, HNF1α and HNF4α can be transdifferentiated into HLCs and perform a series of functions similar to those of PHs. Transdifferentiation provides another way to source of HLC, but it safety needs further verification, as such transcriptional factors are known to participate in the carcinogenesis of hepatocellular carcinoma.

In vitro expansion of HLCs

Obtaining HLCs from PSCs has been validated by multiple

research groups, proving its reproducibility and efficiency. However, owing to the required volume of cells for transplantation for clinical applications, relying on the differentiated HLC is not enough. As a result, how to expand hepatocytes *in vitro* has attracted widespread attention in recent years. Hepatocytes are terminally differentiated cells, which makes them difficult to culturing *in vitro* and maintain their inherent functional properties. Hui Lijian *et al*.[59] reported that a combination of small molecules, adding Wnt3a to hepatocyte medium and removing Rspo1, Noggin, and forskolin increased the fold-expansion of human hepatocytes by 10,000 times. However, they found that the expanded hepatocytes had a bidirectional differentiation potential that placed them between HPCs and mature hepatocytes. It seems to be a complicated task to expand hepatocytes *in vitro*, and the research is focused on the expansion of hepatic progenitor cells like HBs that still have some degree of stemness.

Compared with mature hepatocytes, HBs has a stronger proliferation ability and the potential of rapid differentiation into both hepatocytes and bile duct cells.[60–63] Amplifying PSC-derived HBs is an ideal alternative source of hepatocytes. On the one hand, it is feasible to develop the proliferation potential of HB, and on the other hand, amplified HBs can be frozen to establish a cell bank, acting as seed cells that could be rapidly obtained for functional HLC differentiation. Recent reports have found that multiple small-molecule compounds are suitable for amplifying HB, such as the GSK-3β inhibitor CHIR99021, the TGF-β signaling pathway inhibitor A83-01, and the ROCK inhibitor Y27632. A recent study combined small molecules to simultaneously regulate the BMP/WNT/TGF-β/Hedgehog pathway, which not only maintains the stemness of HBs, but also retains their proliferative capacity. The HBs amplified by the combination had therapeutic effectiveness after transplantation into ALF-model mice.[64,65] Large-scale expansion of HBs, would be a major step in producing the HLCs in the quantity and with the quality required for clinical development and application.

Clinical benefits of PSC-derived cell therapy

Much effort has been made worldwide to promote PSC-derived methods to cure chronic and acute illness. Induced PSC-derived retinal pigment epithelium cells have used clinically to cure patients with macular degeneration, with good outcomes 1 year after transplantation, which supports the use of PSC-derived cells in clinical applications.[66] The use of PSC-derived HLC for ALF, HT, and BAL applications would serve as a promising tool for clinical alternatives. The clinical indications and benefits of PSC-derived cell therapies for treating ALF or end-stage liver disease are summarized below.

Modulating the regeneration niche

A positive outcome requires that HT promotes sufficient regeneration of the host liver. Besides increasing the homing and engraftment of transplanted hepatocytes, modulating the injury niche to include host immune responses such as the macrophage activation and cytokine release,[67,68] is also an important benefit of using PSC-derived HLCs. Unlike PH-derived HLCs, as hypoimmunogenic PSC-derived HLCs would modulate the host immune recruitment to restrain systemic inflammation. For example, phagocytosis mediated by macrophage activation might be limited by the CD47-SIRPα axis if PSC-derived HLCs overexpressing CD47 were transplanted.[69–72] Such clinical applications could be useful in a broader scope of liver disease and not limited to ALF.

Transplantation feasibility and safety

Even if the shortage of donor livers could be solved, OLT is still a challenging procedure with risks including intraoperative bleeding, postsurgical cardiovascular dysfunction, and unavoidable death.[73,74] PSC-derived HT is a safer alternative with infusion that does not require major surgery and the possibility of multiple transplantation procedures.[75] Improvements in cell culture would make PSC-derived HLCs are a good alternative source of hepatocytes compared with PHs. The feasibility of PSC-derived HLCs is not limited by lack of a large quantity of HLCs, which can be cryopreserved to ensure a constantly available cell source for emergency treatment of ALF patients.[76,77]

Individualized treatment

PSC-derived HLCs combined with Crispr/Cas9 genome editing and PSC differentiation would allow generating multiple PSC cell lines that met individual patient requirements or those of the primary illness.[78,79] For instance, the HBV-induced liver disease could theoretically be corrected by transplantation with HBV receptor (NTCP) knock-out or ectopic expression of NTCP variants in HLCs derived from edited PSCs.[80,81] Following transplantation in such patients, HBV could not enter hepatocytes as they lacked the receptor, which would avoiding the recurrence of HBV. Treatment might thus be adjusted depending on the pathophysiology of the primary illness that caused ALF.

Challenges of current PSC based options

Clinical trials of HT and BAL support systems are ongoing, and strive to promote the two therapeutic methods with broad application prospects in clinical treatment. However, the novelty of the methods and the complexity of ALF, are challenging, and can be summarized as follows:

The lack of rigorous clinical trials makes it difficult to achieve a unified and standardized treatment. Most ALF patients indicated for HT and BAL are in a life-threatening stage of disease and require urgent treatment intervention. It is not possible for multiple centers to formulate detailed treatment procedures in time, which makes it difficult to reach a consensus. Standardized treatment indications, treatment procedures, countermeasures for complications, and the introduction of appropriate treatment guidelines are the prerequisites for the adoption of HT and BAL as clinical applications.

The key requirement of these two treatments is the quantity and quality of functional liver cells. No matter which method is used to obtain functional liver cells, an inevitable core problem is the immunogenicity of the cells. At present, adjuvant immunosuppressive agents or pretransplant radiotherapy are used in patients receiving HT, to suppress the patient's immune system and protect the transplanted cells. Once the immune system is suppressed, the patient is exposed to risks of tumorigenesis and infection. Recently, hypoimmunogenic PSC have been developed to overcome the issue of immune rejection. Through knocking out human lymphocyte antigen (HLA) Class I and II molecule accompanied by overexpression of the natural killer (NK) cell specific inhibition receptor (HLA-E) might help to evade host immune surveillance.[82,83] Human embryonic stem cells

overexpressing CTLA4-Ig and PD-L1 are immune-evasive and have shown therapeutic effectiveness in a humanized mouse model of acute liver injury.[84,85] Further research should be carried out to elucidate the underlying mechanism. Its safety should not be neglected as the risk of tumor formation increases without host immune recognition. The development of novel immune tolerance strategies is of great significance for HT therapy.

Improvement of transplanted-cell engraftment and homing needs to be studied. After the liver is damaged, hepatic stellate cells are activated, become fibroblasts, deposit collagen that makes it difficult for transplanted cells to enter damaged regions of the liver. Different routes of delivery have been validated, among which splenic transplantation and hepatic portal vein are typically used in clinical treatments. There are three ways of delivery via the portal vein, ultrasound guided intrahepatic portal vein puncture, transcutaneous splenic vein puncture, and intrahepatic portosystemic shunt via the hepatic venous system.[35] However, the procedures are associated with risks of portal vein hypertension, bleeding, or thrombosis.[86] Alternate routes include the hepatic artery, which has a higher blood flow velocity and lower thrombosis formation risk.[87] More clinical data should be collected to choose the appropriate routes of delivery. Coupling nanomaterials and HT is a novel opinion that would improve the viability, homing, and engraftment of transplanted hepatocytes.[88,89] Micro-encapsulated HLC patches or decellularized liver scaffolds would avoid intravenous or arterial injection.[90–92] Increasing the rate of homing of transplanted cells is a guarantee for the clinical therapeutic effectiveness of HT and needs further validation.

Concluding remarks

In summary, HT and BAL support have bright prospects and application value in the treatment of ALF. PSC-derived HLCs have the potential for wide clinical application, but demonstration of effectiveness and lack of complications are still needed. The use of humanized immune system animal models can provide more accurate immune-response data for HT studies of reducing the immunogenicity of transplanted cells, establishing immune tolerance strategies, and safety. Last but not least, the combining various therapies for ALF treatment is a future trend.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (JL, QW), acquisition of data (JL, ZY), analysis and interpretation of data (JL, ZY), drafting of the manuscript (JL), critical revision (JL, ZY, QW), critical funding (JL, QW), administration (JL, QW), technical or material support (JL), and study supervision (JL, QW).

Data sharing statement

All data are available upon reasonable request.

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