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

Jingfeng Liu1,2,3*, Zhiming Yuan1 and Qingwen Wang2,3*

1Institute of Biomedicine and Biotechnology, Shenzhen Institute of Advanced Technology, Chinese Academy of Science, Shenzhen, Guangdong, China; 2Shenzhen Key Laboratory of Immunity and Inflammatory Diseases, Peking University Shenzhen Hospital, Shenzhen, Guangdong, China; 3Department of Rheumatism and Immunology, Peking University Shenzhen Hospital, Shenzhen, Guangdong, China

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 strains 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.

Keywords: Acute liver failure; Hepatocyte transplantation; Human pluripotent stem cell; Bioartificial liver system.

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 hepatoblasts and hepatocytes. The differentiation of PSC 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 trials 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...
a rapid onset and leads to a frequent fatal outcome, with up to 30% mortality. 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. Paracetamol toxicity, which involves mitochondrial oxidant stress-related cell death and sterile inflammatory responses in hepatocytes, accounts for more than 46% of the ALF cases in the USA. At the early stage of paracetamol-induced liver injury, treatment with N-acetyl-cysteine or 4-methylpyrazole (fomepizole) can effectively control the progress. 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. 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 is hard to achieve, and is the main reason of HBV recurrence and progression. Once the HBV replication is out of control, there’s a huge 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 hepaticocyte death results in aberrant liver function and toxins that travel to the brain and affect the brain function. Although L-ornithine-L-asparagin and ornithine phenylacetate inhibit ammonia synthesis to relieve symptoms, OLT is currently 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. The molecular adsorbent recirculating system and Prometheus system are widely used non-bioartificial liver systems with benefits for ALF patients. 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. 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. The HepaAssist system, which uses porcine liver cells, is the only BAL system that has been 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-
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). 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 to clinical trials, and was shown to be effective in ALF, or acute-on-chronic liver failure (Table 1).

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 to clinical trials, and was shown to be effective in ALF, or acute-on-chronic liver failure (Table 1).
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).

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. 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. 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.

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. 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. Recently, Asuma et al. 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 albumin (ALB) secretion, CYP450 activity which metabolizes drugs and enzymes. In addition, Pan et al. 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. 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.\(^5\) 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.\(^6-8\) 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.\(^6,4\) 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.\(^4\) 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 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. 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, and have shown therapeutic effectiveness in a humanized mouse overexpressing CTLA4-Ig and PD-L1 are immune-evasive. However, the cutaneous splenic vein puncture, and intrahepatic portosystemic shunt via the hepatic venous system. Nevertheless, the procedures are associated with risks of portal vein hypertension, bleeding, or thrombosis. Alternate routes include the hepatic artery, which has a higher blood flow velocity and lower thrombosis formation risk. 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. Micro-encapsulated HLC patches or decellularized liver scaffolds would avoid intravenous or arterial injection. 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.

**Funding**

This research was supported by Shenzhen Key Laboratory of Inflammatory and Immunology Diseases (No. ZDSYS 20200811143756018), China Postdoctoral Science Foundation (No. 2021M693290), the Key Program for Basic Research of Shenzhen Science and Technology Innovation Commission (No. JCYJ20200109140203849) and Guangdong Basic and Applied Basic Research Foundation (No. 2021A151111000).

**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).

**Data sharing statement**

All data are available upon reasonable request.

**References**

[1] Zhao P, Wang C, Liu W, Chen G, Liu X, Wang X, et al. Causes and outcomes of acute liver failure in China. Liver Int 2013;33(11):e809–11. doi:10.1111/j.1478-3275.2013.02961.x.

[2] Gu WY, Xu BY, Zheng X, Chen J, Wang JB, Huang Y, et al. Acute-on-Chronic Liver Failure in China: Rationale for Developing a Patient Registry and Base-line Characteristics. Am J Epidemiol 2018;187(9):1829–39. doi:10.1093/aje/kwy083.

[3] Shen T, Liu Y, Shang J, Xie Q, Li J, Yan M, et al. Incidence and Etiology of Drug-Induced Liver Injury in Mainland China. Gastroenterology 2019;156(8):2230–2241.e11. doi:10.1053/j.gastro.2019.02.002, PMID:3208382.

[4] Xiao J, Wang F, Wong NK, He J, Zhang R, Sun R, et al. Global liver disease burdens and research trends: Analysis from a Chinese perspective. J Hepatol 2019;71(1):212–221. doi:10.1016/j.jhep.2019.03.004, PMID:30871980.

[5] Wang WJ, Xue F, Hu Q, Niu QJ, Gao H. Development of a mouse model of alcoholic liver disease in China: A review. World J Gastroenterol 2019;25(12):1445–1456. doi:10.3748/wjg.v25.i12.1445, PMID:30948900.

[6] Zhang Y, Zhang H, Albright H, Liu X, Wu QJ. Epidemiology of hepatitis B and associated liver diseases in China. Chin Med Sci J 2013;27(4):243–248. doi:10.1016/s1001-9249(13)60009-7, PMID:23294591.

[7] Wu T, Liu J, Shao L, Xin J, Jiang L, Zhou Q, et al. Development of diagnostic criteria and a prognostic score for hepatitis B virus-related acute-on-chronic liver failure. Gut 2018;67(12):2118–2121. doi:10.1136/gutjnl-2017-314641, PMID:28928275.

[8] Zhao RH, Shi Y, Zhao H, Wu W, Sheng JF. Acute-on-chronic liver failure in chronic hepatitis B: an update. Expert Rev Gastroenterol Hepatol 2018;12(4):341–350. doi:10.1080/17474142.2018.1426459, PMID:29334786.

[9] Mazzoni A, Pardi C, Bortoli M, Uncini Mangeloni C, Vanacore R, Urciuoli F, et al. High-volume plasmapheresis: an effective tool in acute liver failure treatment. Int J Artif Organs 2002;25(8):814–815. doi:10.1177/039139880202500810, PMID:12296467.

[10] Larsen FS, Schmidt LE, Bermsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: An open randomised controlled trial. J Hepatol 2016;64(1):69–78. doi:10.1016/j.jhep.2015.08.018, PMID:26225357.

[11] Ljung M, Laguno M, Moreno A, Rimola A, Hospital Clinic Ot In Hiv Working Group. Management of end stage liver disease (ESLD): what is the current role of orthotopic liver transplantation (OLT)? J Hepatol 2006;44(1 Suppl):S140–S145. doi:10.1016/j.jhep.2005.11.028, PMID:16352366.

[12] Arasri SK, Devabhakti H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70(1):151–171. doi:10.1016/j.jhep.2018.09.014, PMID:30266282.

[13] George J. Artificial liver support systems. J Assoc Physicians India 2004;52:719–722. PMID:15044551.

[14] Pareja E, Gomez-Lechon MJ, Cortes M, Bonora-Centelles A, Castell JV, Miro-v. Human hepatocyte transplantation in patients with hepatic failure awaiting a graft. Eur Surg Res 2013;50(3–4):27–281. doi:10.1177/0391398813050003X3, PMID:23796722.

[15] Anderson TH, Zarirap A. Hepatocyte transplantation: past efforts, current technology, and future expansion of therapeutic potential. J Surg Res 2018;226:48–55. doi:10.1016/j.sjsr.2018.01.031, PMID:29661288.

[16] Garcia Martinez J, Bendjelid K. Artificial liver support systems: what is new over the last decade? Ann Intensive Care 2018;8(1):109. doi:10.1186/s13613-018-0453-z, PMID:30443736.

[17] Wang F, Zhou L, Ma X, Ma W, Wang C, Liu X, et al. Monitoring of intraportal hepatocyte transplantation for acute-on-chronic liver failure: a prospective five-year follow-up study. Transplant Proc 2014;46(1):192–198. doi:10.1016/j.transproceed.2013.10.042, PMID:24505705.

[18] Koeifik KVK, van Mierlo KMC, Lodewick TM, Bloemen JG, van der Kroft G, Schaap FG. Bile Salt and FGF19 Signaling in the Early Phase of Human Liver Regeneration. Hepatol Commun 2021;5(8):1400–1411. doi:10.1002/hepa.20573, PMID:34430784.

[19] Si-Tayeb K, Noto FK, Nagaoa M, Li J, Battle MA, Duris C, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 2010;51(1):297–305. doi:10.1002/hep.23354, PMID:19998274.

[20] Asunina FZ, Hatzistergos KE, Dykxhoorn DM, Jakubski S, Edwards J, Thomas ET, et al. Differentiation of hepatocyte-like cells from human pluripotent stem cells using small molecules. Differentiation 2018;101:16–24. doi:10.1016/j.diff.2018.03.002, PMID:29626713.

[21] Stravitz RT, Lee WM. Acute liver failure. Lancet 2019;394(10180):869–881. doi:10.1016/s0140-6736(19)31589-x, PMID:31498810.

[22] Rychlikandran A, Jeneschke C. Acetaminophen Hepatotoxicity. Semin Liver Dis 2019;39(2):221–234. doi:10.1055/s-0039-1679919, PMID:30849782.
Hansel MC, Gramignoli R, Skvorak KJ, Dorko K, Marongiu F, Blake W, Iansante V, Mitry RR, Filippi C, Fitzpatrick E, Dhawan A, Fisher RA, Bu D, Thompson M, Tisnado J, Prasad U, Sterling R, Meyburg J, Hoerster F, Schmidt J, Poeschl J, Hoffmann GF, Schenk JP, Bilir BM, Guinette D, Karrer F, Kumpe DA, Krysl J, Stephens J, Demetriou AA, Brown RS Jr, Busuttil RW, Fair J, McGuire BM, Rosenthal P, Karvellas CJ, Gibney N, Kutsogiannis D, Wendon J, Bain VG. Bench-to-bedside infusion. Transplantation 2000;69(2):303–307. doi:10.1097/00007890-200002000-00024.

Li M, Sun J, Li J, Shi Z, Xu J, Lu B, Yang HC, Chen PJ. The potential and challenges of CRISPR-Cas in eradication of hepatitis B virus covalently closed circular DNA. Virus Res 2018;244:303–310. doi:10.1016/j.virusres.2018.05.015.

Van der NC, Chen Y, Liu Q, Chen H, Xie Y, Yu X, et al. Potential and challenges of CRISPR-Cas in eradication of hepatitis B virus covalently closed circular DNA. Virus Res 2018;244:303–310. doi:10.1016/j.virusres.2018.05.015.

Verheugen AC, Cheung SP. Role of Monocytes and Macrophages in Acute and Acute-on-Chronic Liver Failure. J Hepatol 2014;61(2):439–445. doi:10.1016/j.jhep.2014.03.007.

Watanabe A, Kurimoto Y, Morinaga C, Daimon T, Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, Liao C, Chen W, Zhang M. Translation and functional maturation of human hepatoblasts by chemical strategy. Stem Cell Res Ther 2021;12(1):151. doi:10.1186/s13287-021-02233-9, PMID:33632238.

Xiang Z, Kang L, Liu W, Ma X, Cen J, Sun Z, et al. In Vivo Expansion of Primary Human Hepatocytes with Efficient Liver Repopulation Capacity. Cell Stem Cell 2018;23(6):508–518. doi:10.1016/j.stem.2018.09.014, PMID:30416071.

Yanagida A, Ito K, Chikada H, Nakashuki H, Kamiya A. In vitro expansion and functional maturation of human hepatocytes by chemical strategy. Stem Cell Res Ther 2021;12(1):151. doi:10.1186/s13287-021-02233-9, PMID:33632238.

Yin T, Chen Y, Zhang J, Getachew A, Zhuang Y, et al. Robust expansion and functional maturation of human hepatocytes by chemical strategy. Stem Cell Res Ther 2021;12(1):151. doi:10.1186/s13287-021-02233-9, PMID:33632238.

Yin J, Liu J. Self-renewal of hepatocytes under chemically defined conditions by iterative growth factor and chemical screening. Hepatology 2015;61(1):337–347. doi:10.1002/hep.27421, PMID:25293445.

Zhang M, Sun P, Wang Y, Chen J, Lv L, Wei W, et al. Generation of Self-Renewing Hepatoblasts From Human Embryonic Stem Cells By Chemical Approaches. Stem Cells Transl Med 2015;4(11):1275–1282. doi:10.5966/scm.20150051, PMID:26371343.

Zhang T, Chen Y, Zhang Y, Fong F, Yu Y, Tao J, et al. Synergistic modulation of signaling pathways to expand and maintain the bipotentiality of hepato blasts. Stem Cell Res Ther 2019;10(1):364. doi:10.1186/s13287-019-1463-y, PMID:31791395.

Zhang T, Xue S, Xue J, et al. Direct reprogramming of human hepatocytes into bipotent progenitors using small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Stem Cells Transl Med 2015;4(11):1275–1282. doi:10.5966/scm.20150051, PMID:26371343.

Zhang Z, Song AP, Chen Y, Wang Y, et al. Clinical observation on the treatment of acute liver failure by combined non-biological artificial liver. Exp Ther Med 2016;12(6):3873–3876. doi:10.3892/etm.2016.3887, PMID:28105119.

Karvellas CJ, Gibney N, Kutsogiannis D, Wendon J, Bain VG. Bridging a patient with acute liver failure to liver transplantation by the artificial liver. SEMIN.0b013e328320fd7b, PMID:19337149. doi:10.1002/0471140856.tx1412s62, PMID:25378242.

et al 1995. doi:10.1002/hep.27751993. PMID:9038.

et al 2003. doi:10.1080/17524596.2003.11607331. PMID:14579924.

et al 2020. doi:10.1080/17524596.2020.1193371. PMID:21337197.

et al 2018. doi:10.1002/0471140856.tx1412s62. PMID:25378242.

et al 2006. doi:10.3727/096368909x485058, PMID:20053320.

et al 2017. doi:10.1002/hep.27751993. PMID:9038.

et al 2009. doi:10.1002/hep.27751993. PMID:9038.

et al 2017. doi:10.1002/hep.27751993. PMID:9038.
Liu J. et al: Stem cell-derived technologies in treating ALF

man stem cell transplants. FASEB J 2019;33(1):484–493. doi:10.1096/fj.201800449R, PMID:30004796.

[73] Eyvazian VA, Gordin JS, Yang EH, Aksoy O, Honda HM, Busuttil RW, et al. Incidence, Predictors, and Outcomes of New-Onset Left Ventricular Systolic Dysfunction After Orthotopic Liver Transplantation. J Card Fail 2019;25(3):166–172. doi:10.1016/j.cardfail.2018.10.013, PMID:30412734.

[74] Addeo P, Noblet V, Naegel B, Bachellier P. Large-for-Size Orthotopic Liver Transplantation: A Systematic Review of Definitions, Outcomes, and Solutions. J Gastrointest Surg 2020;24(5):1192–1200. doi:10.1007/s11605-019-04505-5, PMID:31919740.

[75] Tolosa L, Pareja E, Gómez-Lechón MJ. Clinical Application of Pluripotent Stem Cells: An Alternative Cell-Based Therapy for Treating Liver Diseases? Transplantation 2016;100(12):2548–2557. doi:10.1097/tp.0000000000001426, PMID:27495745.

[76] Messina A, Luce E, Hussein M, Dubart-Kupperschmitt A. Pluripotent-Stem-Cell-Derived Hepatic Cells: Hepatocytes and Organoids for Liver Therapy and Regeneration. Cells 2020;9(2):E420. doi:10.3390/cells9020420, PMID:32059501.

[77] Mun SJ, Ryu JS, Lee MO, Son YS, Oh SJ, Cho HS, et al. Generation of expandable human pluripotent stem cell-derived hepatocyte-like liver organoids. J Hepatol 2019;71(5):970–985. doi:10.1016/j.jhep.2019.06.030, PMID:31299272.

[78] Hockemeyer D, Jaenisch R. Induced Pluripotent Stem Cells Meet Genome Editing. Cell Stem Cell 2016;18(5):573–586. doi:10.1016/j.stem.2016.04.013, PMID:27152442.

[79] Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, et al. Targeted Disruption of HLA Genes via CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility. Cell Stem Cell 2019;24(4):566–578.e7. doi:10.1016/j.stem.2019.02.005, PMID:30853558.

[80] Uchida T, Park SB, Inuzuka T, Zhang M, Allen JN, Chayama K, et al. Genetically edited hepatic cells expressing the NTCP-S267F variant are resistant to hepatitis B virus infection. Mol Ther Methods Clin Dev 2021;23:597–605. doi:10.1016/j.omtm.2021.11.002, PMID:32997339.

[81] Jin Y, Wang H, Yi K, Lv S, Hu H, Li M, et al. Applications of Nanobiomaterials in the Therapy and Imaging of Acute Liver Failure. Nanomicro Lett 2020;13(1):25. doi:10.1007/s40820-020-00550-x, PMID:34138224.

[82] Shani T, Hanna JH. Universally non-immunogenic iPSCs. Nat Biomed Eng 2019;3(5):337–338. doi:10.1038/s41551-019-0401-b, PMID:31036889.

[83] Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, et al. Targeted Disruption of HLA Genes via CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility. Cell Stem Cell 2019;24(4):566–578.e7. doi:10.1016/j.stem.2019.02.005, PMID:30853558.

[84] Liu J, Pan T, Chen Y, Liu Y, Yang F, Chen Q, et al. Repair of acute liver damage with immune evasive hESC-derived hepatoblasts. Stem Cell Res Ther 2020;11(1):121–120. doi:10.1016/j.scr.2020.11.010, PMID:33011360.

[85] Rong Z, Wang M, Hu Z, Strader D, Zhu S, Kong H, et al. An effective approach to prevent immune rejection of human ESC-derived allografts. Cell Stem Cell 2014;14(1):121–130. doi:10.1016/j.stem.2014.01.014, PMID:24381175.

[86] Dissegna D, Sponza M, Falleti E, Fabris C, Vit A, Angeli P, et al. Morbidity and mortality after transjugular intrahepatic portosystemic shunt placement in patients with cirrhosis. Eur J Gastroenterol Hepatol 2019;31(5):626–632. doi:10.1097/MEG.0000000000001342, PMID:30550458.

[87] Dwyer BJ, Macmillan MT, Brennan PN, Forbes SJ. Cell therapy for advanced liver diseases: Repair or rebuild. J Hepatol 2021;74(1):185–199. doi:10.1016/j.jhep.2020.09.014, PMID:32976865.

[88] Yu X, Xiao H, Li J, Cai Z, Zhang L, Wang S, et al. Nano-engineered hepatic cells as a scaffold for hepatocellular carcinoma therapy. Nanomedicine 2018;13(12):1819–1829. PMC:3590618.

[89] Jin Y, Wang H, Yi K, Lv S, Hu H, Li M, et al. Applications of Nanobiomaterials in the Therapy and Imaging of Acute Liver Failure. Nanomicro Lett 2020;13(1):25. doi:10.1007/s40820-020-00550-x, PMID:34138224.

[90] Ihara J, Hoshi T, Yoshida S, Itoh M, Yamauchi Y, Komatsu S, et al. Intravenous administration of human hepatocytes expressing the hepcidin transgene as a novel therapy for iron-overload disease. Hepatology 2019;69(2):1425–1436. doi:10.1002/hep.30562, PMID:30223844.

[91] Pasqua M, Pereira U, de Lartigue C, Nicolas J, Vigneron P, Dermigny Q, et al. Preclinical characterization of alginate-poly-L-lysine encapsulated HepaRG for extracorporeal liver supply. Biotechnol Bioeng 2021;118(1):453–464. doi:10.1002/bit.27583, PMID:32997339.

[92] Zhang J, Shan H, Wang H, Hua D, Sha D, Tao Y, Li H. Stem cell therapy and tissue engineering strategies using cell aggregates and decellularized scaffolds for the rescue of liver failure. J Tissue Eng 2021;12:2041731420986711. doi:10.1177/2041731420986711, PMID:35003615.