Cell Transplantation
2019, Vol. 28(9-10) 1116–1122

DOI: 10.1177/0963689719859262
journals.sagepub.com/home/cll

Stem Cell-Related Studies and Stem Cell-Based Therapies in Liver Diseases

Wei Zhou[1,2], Erek D. Nelson[1], Anan A. Abu Rmilah[1], Bruce P. Amiot[1], and Scott L. Nyberg[1]

Abstract

Owing to the increasing worldwide burden of liver diseases, the crucial need for safe and effective interventions for treating end-stage liver failure has been a very productive line of inquiry in the discipline of hepatology for many years. Liver transplantation is recognized as the most effective treatment for end-stage liver disease; however, the shortage of donor organs, high medical costs, and lifelong use of immunosuppressive agents represent major drawbacks and demand exploration for alternative treatments. Stem cell-based therapies have been widely studied in the field of liver diseases and are considered to be among the most promising therapies. Herein, we review recent advances in the application of stem cell-related therapies in liver disease with the aim of providing readers with relevant knowledge in this field and inspiration to spur further inquiry.

Keywords

cellular therapy, hepatology

Introduction

Many acute and chronic liver diseases cannot routinely be cured by current therapies. In some, chronic inflammation and fibrosis give way to cirrhosis and end-stage liver failure with resultant portal hypertension, hepatorenal syndrome, hepatic encephalopathy, coagulation dysfunction, and other complications. The only durable treatment is orthotopic liver transplantation. Nevertheless, a shortage of donor grafts, high cost, and immunosuppression are prohibitive obstacles for many patients, and therefore investigators have been exploring potential alternative therapies. For example, hepatocytes can be used extracorporeally in a bio-artificial liver for partial replacement of liver function in the treatment of acute liver failure[1]. However, the source of hepatocytes is limited as they are prone to lose their viability and function during in vitro culture or after cryopreservation[2]. Stem cells are group of pluripotent "seed" cells which are not fully differentiated and have the potential to differentiate into many different tissues types. Due to their proliferation and differentiation potential, they are seen as promising candidates to provide the building blocks for future therapies. There are many basic science and preclinical studies on the treatment of liver diseases based on the application of stem cells from different sources and stages of differentiation: embryonic stem cell (ESC), mesenchymal stem cell (MSC), and induced pluripotent stem cell (iPSC), as well as creating chimeric organisms from these cells. Herein, we will elaborate on the research progress in each field.

Studies of ESC in Liver Diseases

ESCs are undifferentiated, pluripotent stem cells isolated from the early blastocyst- or morula-stage embryos of mammals and can proliferate indefinitely in vitro. In 1998, Thomson et al. derived a human ESC cell line for the first time[3]. ESCs have the ability to develop into tissues representing all three embryonic germ layers including gonads and gametes. ESCs can be induced to differentiate into hepatocyte-like cells (HLCs) or hepatoblast-like progenitor cells similar to mature hepatocytes in vitro[4]. Cai et al. used an innovative three-step method featuring activin A, fibroblast growth factor 4, and bone morphogenetic protein 2 to differentiate

[1] Mayo Clinic, William J. von Liebig Center for Transplantation and Clinical Regeneration, Rochester, MN, USA
[2] The First Affiliated Hospital of China Medical University, Hepatobiliary Surgery, Shenyang, China

Submitted: May 13, 2019. Revised: May 23, 2019. Accepted: May 29, 2019.

Corresponding Author:
Scott L. Nyberg, Mayo Clinic, William J. von Liebig Center for Transplantation and Clinical Regeneration, Rochester, MN 55905, USA.
Email: Nyberg.Scott@mayo.edu

human ESCs into HLCs in serum-free medium. Initially, about 70% of these cells expressed albumin. After further in vitro maturation, these cells expressed markers, such as tyrosine aminotransferase, of the mature hepatocyte phenotype. Additionally, these cells were detected in vivo integrated into the host liver in vivo[4]. Li et al. cultured untreated mouse ESCs under specific conditions and allowed them to undergo hepatocyte-directed differentiation. It was found that mouse ESCs differentiated into a population containing hepatic precursor cells. Moreover, the hepatic precursor cells could maintain hepatoblast-like characteristics after long-term expansion, and had the ability to repopulate the liver without tumorigenesis after being transplanted into fuylmaracetoacetate hydrolase (FAH)-deficient mice[5].

Basma et al. used fibroblast growth factor 2, human activin A, hepatocyte growth factor, and dexamethasone to culture and differentiate human ESCs, then isolated functional hepatocytes by selecting for expression of surface asialoglycoprotein receptor expression. The results showed that hepatocytes derived from human ESCs expressed liver-specific genes, but did not express genes from other lineages. Similar to primary human hepatocytes, these cells could secrete human liver-specific functional proteins. The serum of rodents injected with hepatocytes derived from human ESCs contained human albumin and alpha 1-antitrypsin. Cell colonies expressing human albumin could be found in the host liver. This study confirmed that human ESCs could differentiate into HLCs which possess the same characteristics of primary human hepatocytes, thereby making them a good candidate for drug development research[6].

Touboul et al. reported a new method of differentiating human ESCs into functional hepatocytes using fully qualified culture conditions. First, human ESCs were directed to differentiate into a homogeneous endodermal cell population using a combination of four transforming growth factors. Then, the endodermal cells were differentiated into hepatic precursor cells. After the third step—further maturation—these cells expressed various markers of mature hepatocytes. Moreover, these cells demonstrated hepatocyte functionality in vitro. These differentiated cells, after labeling with green fluorescent protein, were transplanted into the liver of immunodeficient mice. It was found that these cells integrated into the host liver and expanded. During 8 weeks after implant, human albumin and alpha 1-antitrypsin could be detected in the mice consistently[7].

Möbus et al. found that the inhibition of microRNA-199a-5p could enhance hepatocyte differentiation of human ESCs in vitro. They also revealed that miR-199a-5p inhibition in human ESC-derived HLCs could enhance the engraftment and proliferation of these cells in the livers of immunodeficient mice and gave rise to a 10-fold increase in the secretion of human albumin[8].

In conclusion, ESCs have the potential for robust proliferation and multi-directional differentiation to produce functioning hepatic progenitor cells or HLCs. Nevertheless, the large-scale expansion and differentiation of ESCs into hepatocytes is still far from their clinical application due to unresolved issues regarding their procurement and potential tumorigenesis[9].

Studies of MSC in Liver Diseases

MSCs are multipotent stem cells that exist in many tissues such as bone marrow, umbilical cord, fat, and placenta. MSCs can secrete a series of cytokines and signaling molecules, such as hepatocyte growth factor (HGF), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), epidermal growth factor (EGF), nitric oxide, prostaglandin E2 (PGE2) and indoleamine 2,3-dioxygenase. Moreover, MSCs have low or no expression of MHC-I and MHC-II molecules and are thus suitable for allogeneic transplantation with reduced risk of rejection[10]. MSCs stimulate hepatocyte proliferation, regulate inflammatory responses, and maintain hepatocyte function[11]. They have roles in growth and tissue repair, anti-apoptosis, angiogenesis, and immunosuppression[12]. MSCs are easily obtainable from adult tissues (such as adipose-derived MSCs) and can be used with few ethical issues[13].

Several studies have shown that rodent or human MSCs can differentiate into HLCs in vivo or in vitro[14,15]. Aurich et al. demonstrated that after treatment with specific growth factors in vitro, human MSCs can acquire the morphology and some functions of human hepatocytes. In their study, predominant engraftment in the periportal zone of the liver lobule took place after transplanting these preconditioned MSCs into the livers of immunodeficient mice. The transplanted cells continued to store glycogen, produce albumin and express HepPar1, one of human hepatocyte-specific antigens[14]. Banas et al. demonstrated that adipose tissue derived MSCs (AT-MSCs) can be differentiated into HLCs in vitro in a hepatocyte culture medium containing EGF, HGF, and other factors, and that AT-MSC-derived HLCs exhibited some functions of human hepatocytes, such as albumin production, low-density lipoprotein uptake, and ammonia detoxification. In in vivo experiments, AT-MSC-derived HLCs incorporated into the host liver and improved hepatic function when they were transplanted into the livers of CCl_4-injured mice[15].

Kuo et al. reported that either intrasplenic transplantation or intravenous injection of MSCs or MSC-derived hepatocyte-like cells (MHCs) in a mouse model of fulminant hepatic failure induced by CCl_4 gavage could improve survival. MSCs were superior to MHCs in terms of the improvement in survival, and intravenous injection was regarded as a more effective approach compared with intrasplenic transplantation. At the same time, because of the rapid onset of recovery after MSC transplant and the need for a relatively small dose of cells, it was suggested that functional complementation was more important than direct hepatocyte differentiation in MSC-mediated therapeutic mechanisms. Moreover, MSCs significantly stimulated hepatocyte proliferation after oxidative stress, suggesting a possible paracrine mechanism[16].

The efficacy of MSCs in acute and chronic liver injury has been confirmed in many animal experiments and some preclinical trials. However, most studies have shown that the main role of MSCs in the treatment of liver injury or liver failure comes from their secretion of various cytokines rather than their differentiation into functional cells and replacement of damaged parenchyma. There are some potential risks and adverse effects in the application of MSCs in the treatment of liver diseases. For example, hepatic stellate cells and myofibroblasts can also be derived from MSCs, raising the possibility that MSC therapies may promote hepatic fibrosis[17]. It has also been reported that malignant transformation may occur after MSC transplantation[18].

Studies of iPSCs in Liver Diseases

iPSCs are a type of pluripotent cell that can be created from fully differentiated cells, as first demonstrated by Takahashi and Yamanaka via the introduction of four specific genes[19]. Since iPSCs can be derived directly from adult tissues, they not only forego the destruction of embryos, but can also be made in a patient-specific manner. These autologous cells could be used to perform transplants without the risk of immune rejection. The emergence of iPSCs has become one of the hotspots in stem cell research[20].

Song et al. first demonstrated that iPSCs can be differentiated into HLCs by the administration of multiple growth factors in a time-dependent manner. In this study, human iPSCs were induced to differentiate into hepatocytes. In the process, hepatocyte marker expression and liver-related functions of differentiated cells were monitored and compared with primary hepatocytes and HLCs derived from human ESCs. It was found that human iPSC-derived HLCs (hiPSC-HLCs) expressed hepatocyte markers alpha fetoprotein (AFP) and albumin, and exhibited hepatocyte functions such as production of glycogen comparable to human ESC-derived HLCs. This result confirmed that human iPSCs can be effectively induced to differentiate to HLCs in vitro comparably to human ESCs[21].

Asgari et al. conducted an in vivo study of hiPSC-HLCs in which human iPSCs were induced via growth factor-mediated differentiation to form functional HLCs. These were then transplanted into CCl_4-injured mouse livers which were evaluated for improvement in liver function. It was found that CCl_4-injured mice transplanted with hiPSC-HLCs showed a significant increase in serum albumin levels after 1 week. Moreover, the levels of LDH and serum bilirubin at 1 and 5 weeks status post transplantation were significantly lower than controls. Meanwhile, human serum albumin and albumin-positive transplanted cells were detected in the sera and livers of the recipient mice, indicating that the transplanted hiPSC-HLCs developed a functional integration into the mouse liver. This study demonstrated the functional potential of hiPSC-HLCs in vivo[22].

In order to obtain iPSC-derived functional HLCs more quickly and efficiently, Chen et al. discovered a rapid three-step differentiation process. In the endodermal induction step, HGF was added to synergize with activin A and Wnt3a. The expression of Foxa2, an endodermal marker, was increased by 39.3% compared with cultures without HGF. This significantly improved the efficiency of human iPSC differentiation into HLCs that have gene expression profiles similar to mature hepatocytes. In a model of lethal fulminant hepatic failure caused by CCl_4 in NOD-SCID mice, iPSC-derived HLCs obtained via this method were transplanted into mouse livers, which resulted in a survival rate of 71% versus 0% of controls, thus proving the functional potential of hepatocyte-like cells derived in this manner[23].

Zhu et al. transfected Oct4, Sox2, and Klf4 into human fibroblasts to generate induced multipotent progenitor cells (iMPCs) and then induced them to differentiate into hepatocytes (iMPC-Heps). IMPC-Heps were transplanted into the immunodeficient, FAH-deficient mice via intrasplenic injection. The treatment effectively alleviated the liver damage caused by type-I hereditary tyrosinemia with improved survival, prolonged time free from NTBC therapy, and reduced required dosage of NTBC[24].

Since human iPSC-HLCs can be produced on a large scale, cell transplantation based on iPSC-HLCs is expected to be used for the treatment of patients with liver failure. However, in the traditional intraportal or intrasplenic transplantation method, there is a possibility for off-target engraftment. To solve this problem, Nagamoto et al. developed a hepatocyte sheet transplantation technique. They cultured human iPSC-HLCs in temperature-responsive culture dishes and then obtained a cell sheet formed by temperature change-dependent cell harvesting. This cell sheet was orthotopically transplanted onto the liver surface of mouse model status post two-thirds partial hepatectomy. Two weeks later, the level of human albumin in the sera of mice subjected to cell sheet transplantation was significantly higher than that of mice status post conventional intrasplenic injection. In the sheet group, human DNA fragments were detected via PCR only in the liver. In addition, in a mouse model of CCl_4-induced acute hepatic failure, the survival rate of mice status post human iPSC-HLCs sheet transplantation (63.2%) was significantly higher than that of mice having undergone intrasplenic injection (33.3%)[25].

Furthermore, Takebe et al. have succeeded in generating a three-dimensional, vascularized, and functional "human liver" by transplantation of "liver buds" (LBs) created in vitro from human iPSCs. This is the first reported macroscopic functional human tissue derived from pluripotent stem cells[26]. They first differentiated human iPSCs into hepatocytes in vitro and then co-cultured them with human MSCs and human umbilical vein endothelial cells. Hepatocytes interacted with endothelial cells and MSCs and self-organized into three-dimensional LBs which were mechanically stable and could be manipulated physically. Next, the human iPSC-derived LBs (4–7 mm) were transplanted into the mesentery of immune-deficient mice. The

blood vessels of the iPSC-derived LBs connected to the host blood vessels spontaneously within 48 h. This angiogenesis stimulated further maturation of the LBs, which eventually formed a functional tissue resembling the adult liver. It was found that this "liver tissue" had functions such as human-specific protein production and drug metabolism. Moreover, transplantation of 12 human iPSC-derived LBs significantly improved the survival of mice after gancyclovir-induced acute hepatic failure compared with both transplantation of human fetal liver cell-derived LBs and transplantation of human adult-hepatocytes[26].

Currently, iPSCs have shown a great potential benefit in preclinical studies of liver disease. They demonstrate similar pluripotency to ESC while also permitting the possibility of autogenous transplantation. Again, the potential for tumorigenesis has yet to be adequately alleviated. Due to the current use of retroviruses in the preparation of some iPSCs, it is unavoidable that some incompletely reprogrammed cells will be produced. These cells may differ in gene expression patterns from truly pluripotent stem cells. These differences may impair the directional differentiation potential of cells and result in abnormal gene expression[27].

Chimerism

Most of the therapies described above involve cell transplant into post-natal recipients to replace liver function or enhance liver regeneration. However, stem cells may also be transplanted during embryonic or fetal stages of development, thus allowing them to differentiate and develop in vivo and ultimately to eliminate diseases or construct organs derived solely from engrafted stem cells. This technique of early embryonic or fetal injection, which leads to chimerism, has been shown to achieve tolerization between allogeneic or even xenogeneic cell combinations[28].

Xiang et al reported the first study of pluripotent stem cell (PSC)-derived viable interspecies chimerism between two distant rodent species. They injected ESCs of *Apodemus sylvaticus* into blastocysts of *Mus musculus*. There are 14.59 million years of evolutionary divergence and 18% genomic divergence between *Apodemus sylvaticus* and *Mus musculus*. After birth, *Apodemus sylvaticus*-derived cells were widely distributed in almost all major organs of chimeric offspring including the gonads. In some tissues, the contribution rate of *Apodemus sylvaticus* cells was as great as 40%[29].

In vitro generation of whole organs cannot be achieved currently, but the possibility of in vivo organogenesis with organ-specific chimerism is becoming more feasible. Kobayashi et al. injected mouse wild-type PSCs into Pdx1 (-/-) (pancreatogenesis-disabled) mouse blastocysts and found that the graft PSCs filled the "developmental niche" of the host pancreas which resulted in a normal pancreas almost entirely derived from donor cells. They further injected rat wild-type PSCs into blastocysts of Pdx1 (-/-) mice resulting in the development of a rat pancreas with normal function within the mouse host. For the first time, this study reported the use of interspecies blastocyst complementation to produce donor-derived organs in heterogeneous environments[30].

The use of blastocyst complementation to produce donor-derived organs involves an important mechanism called cell competition. Cell competition was first proposed by Morata and Ripoll in 1975. They found that Drosophila cells carrying RpS17 mutations were eliminated by more metabolically dominant wild-type cells[31]. Subsequent studies in mammals have also confirmed that cells with lower replication rates can survive only in a homogeneous environment. When surrounded by more adaptive cells, their population is outcompeted and eventually eliminated. The phenomenon of cell competition is widespread and conserved in the animal kingdom[32]. Therefore, interspecies blastocyst complementation can be effectively applied in the practice of in vivo production of xenogeneic organs, and may result in transplantable organs to treat human diseases[28].

Matsunari et al. performed blastocyst complementation in pigs and confirmed that when functionally normal allogeneic PSCs were introduced into a pancreaticogenesis-disabled porcine blastocyst, the exogenous PSCs could develop into the missing organ[33]. This confirmed the feasibility of using blastocyst complementation to develop allogeneic organs in large mammals. Our group, led by Nyberg, also performed blastocyst complementation experiments in pigs. We have succeeded in curing FAH-deficiency via pig–pig blastocyst complementation (Fig. 1, manuscript in preparation). As a result, the piglet can survive normally in the absence of both NTBC and immunosuppression.

The transplant of allogeneic PSCs can successfully form chimeric large mammals; however, human chimerae will be required to produce clinically transplantable grafts. Wu et al. systematically evaluated the chimeric competency of several types of human PSCs (hPSCs) using a more diverse mammal population: the ungulates. They used a CRISPR-Cas9-mediated interspecies blastocyst complementation system to transplant human PSCs into both cattle and pig blastocysts. However, the graft contributed little to post-implantation pig embryos[34]. Recently, Huang et al. found that hPSCs had a very limited contribution to the formation of interspecies chimerae due to graft apoptosis, which was an initial barrier in interspecies chimerism using hPSCs. However, the increased expression of BMI1 (a polycomb factor) in hPSCs could result in successful integration of hPSCs into mouse pre-implantation embryos engraftment in the blastocysts of different species, including mouse, rabbit, and swine[35].

Organ generation via interspecies blastocyst complementation may play a role in solving the shortage of donor organs. In addition, human–animal chimerae can be used to study the pathogenesis of human diseases or to conduct in vivo drug testing. However, it is currently unclear whether chimerae with sufficient human contribution can be formed

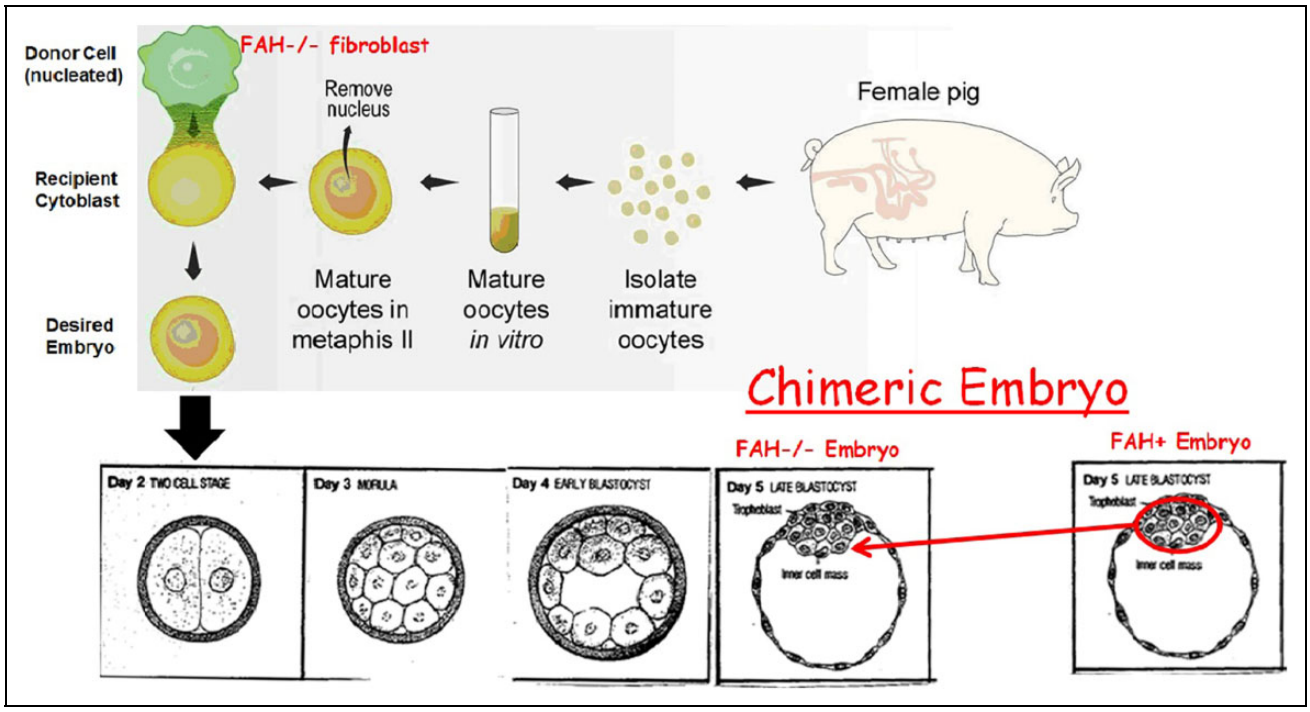

Figure 1. New possibilities for stem cell therapy in chimeric embryos cloning and blastocyst complementation (Nyberg group from Mayo Clinic).

and whether xenogeneic contributions to chimeric organs will preclude transplantation[28].

Discussion

Stem cells hold great promise for cell therapy because of their proliferation and differentiation potential. This promise is especially great in the field of liver disease, as once liver disease progress to the end stage, conventional treatment has little effect, and we lack effective drugs and an adequate supply of transplantable organs. Although great progress has been made in preclinical studies and clinical applications of bio-artificial liver devices based on primary hepatocytes, there remains a serious shortage of primary hepatocytes because they cannot proliferate and maintain function indefinitely in vitro[1,36]. However, HLCs or other hepatic precursor cells derived from stem cells can not only be produced in large quantities, but also have the ability to replace damaged hepatocytes and improve liver function status post transplantation. In the future, co-culture of various stem cells and endothelial cells to produce "liver buds" may be used for transplantation and liver regeneration. Furthermore, generation of transplantable human organs derived from human–animal chimerae may become possible. Nowadays, most stem cell-related studies in the field of liver disease are still based on basic research and preclinical research. There are many scientific, medical, ethical, political, financial, and other challenges yet to be solved. For the benefit of patients and future generations, scientists need to translate the achievements of regenerative medicine into clinical application in the future.

Author Contributions

Wei Zhou wrote the paper. Erek D. Nelson, Anan A. Abu Rmilah, and Bruce P. Amiot modified the manuscript. Scott L. Nyberg supervised the project. All authors reviewed and contributed to the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr. Nyberg owns stock and intellectual property rights with Liver Cell Technologies. The authors declare no other competing interests.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This project is supported by the Mayo Foundation for Medical Education and Research, NIH (R01 DK10667) and Minnesota Regenerative Medicine.

ORCID iD

Wei Zhou https://orcid.org/0000-0002-5626-2304

References

1. Chen HS, Joo DJ, Shaheen M, Li Y, Wang Y, Yang J, Nicolas CT, Predmore K, Amiot B, Michalak G, Mounajjed T, et al. Randomized trial of spheroid reservoir bioartificial liver in

porcine model of posthepatectomy liver failure. Hepatology. 2019;69(1):329–342.
2. Eom YW, Kim G, Baik SK. Mesenchymal stem cell therapy for cirrhosis: present and future perspectives. World J Gastroenterol. 2015;21(36):10253–10261.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282(5391):1145–1147.
4. Cai J, Zhao Y, Liu Y, Ye F, Song Z, Qin H, Meng S, Chen Y, Zhou R, Song X, Guo Y, et al. Directed differentiation of human embryonic stem cells into functional hepatic cells. Hepatology. 2007;45(5):1229–1239.
5. Li F, Liu P, Liu C, Xiang D, Deng L, Li W, Wangensteen K, Song J, Ma Y, Hui L, Wei L, et al. Hepatoblast-like progenitor cells derived from embryonic stem cells can repopulate livers of mice. Gastroenterology. 2010;139(6):2158–2169.
6. Basma H, Soto-Gutiérrez A, Yannam GR, Liu L, Ito R, Yamamoto T, Ellis E, Carson SD, Sato S, Chen Y, Muirhead D, et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology. 2009;136(3): 990–999.
7. Touboul T, Hannan NR, Corbineau S, Martinez A, Martinet C, Branchereau S, Mainot S, Strick-Marchand H, Pedersen R, Di Santo J, Weber A, et al. Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology. 2010;51(5):1754–1765.
8. Möbus S, Yang D, Yuan Q, Lüdtke TH, Balakrishnan A, Sgodda M, Rani B, Kispert A, Araúzo-Bravo MJ, Vogel A, Manns MP, et al. MicroRNA-199a-5p inhibition enhances the liver repopulation ability of human embryonic stem cell-derived hepatic cells. J Hepatol. 2015;62(1):101–110.
9. Rao M. Tumorigenesis and embryonic stem cell-derived therapy. Stem Cells Dev. 2007;16(6):903–904.
10. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315–317.
11. Lin H, Xu R, Zhang Z, Chen L, Shi M, Wang FS. Implications of the immunoregulatory functions of mesenchymal stem cells in the treatment of human liver diseases. Cell Mol Immunol. 2011;8(1):19–22.
12. Wang YH, Wu DB, Chen B, Chen EQ, Tang H. Progress in mesenchymal stem cell-based therapy for acute liver failure. Stem Cell Res Ther. 2018;9(1):227.
13. Wang J, Cen P, Chen J, Fan L, Li J, Cao H, Li L. Role of mesenchymal stem cells, their derived factors, and extracellular vesicles in liver failure. Stem Cell Res Ther. 2017;8(1):137.
14. Aurich I, Mueller LP, Aurich H, Luetzkendorf J, Tisljar K, Dollinger MM, Schormann W, Walldorf J, Hengstler JG, Fleig WE, Christ B. Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. Gut. 2007;56(3):405–415.
15. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, Okochi H, Ochiya T. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology. 2007;46(1):219–228.
16. Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC, Yang VW, Lee OK. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology. 2008;134(7):2111–2121.
17. Yang L, Chang N, Liu X, Han Z, Zhu T, Li C, Yang L, Li L. Bone marrow-derived mesenchymal stem cells differentiate to hepatic myofibroblasts by transforming growth factor-β1 via sphingosine kinase/sphingosine 1-phosphate (S1P)/S1P receptor axis. Am J Pathol. 2012;181(1):85–97.
18. Wu XZ, Chen D. Origin of hepatocellular carcinoma: role of stem cells. J Gastroenterol Hepatol. 2006;21(7):1093–1098.
19. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663–676.
20. Hockemeyer D, Jaenisch R. Induced pluripotent stem cells meet genome editing. Cell Stem Cell. 2016;18(5):573–586.
21. Song Z, Cai J, Liu Y, Zhao D, Yong J, Duo S, Song X, Guo Y, Zhao Y, Qin H, Yin X, et al. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. Cell Res. 2009;19(11):1233–1242.
22. Asgari S, Moslem M, Bagheri-Lankarani K, Pournasr B, Miryounesi M, Baharvand H. Differentiation and transplantation of human induced pluripotent stem cell-derived hepatocyte-like cells. Stem Cell Rev. 2013;9(4):493–504.
23. Chen YF, Tseng CY, Wang HW, Kuo HC, Yang VW, Lee OK. Rapid generation of mature hepatocyte-like cells from human induced pluripotent stem cells by an efficient three-step protocol. Hepatology. 2012;55(4):1193–1203.
24. Zhu S, Rezvani M, Harbell J, Mattis AN, Wolfe AR, Benet LZ, Willenbring H, Ding S. Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature. 2014; 508(7494):93–97.
25. Nagamoto Y, Takayama K, Ohashi K, Okamoto R, Sakurai F, Tachibana M, Kawabata K, Mizuguchi H. Transplantation of a human iPSC-derived hepatocyte sheet increases survival in mice with acute liver failure. J Hepatol. 2016;64(5): 1068–1075.
26. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, Zhang RR, Ueno Y, Zheng YW, Koike N, Aoyama S, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013;499(7459):481–484.
27. Müller LU, Daley GQ, Williams DA. Upping the ante: recent advances in direct reprogramming. Mol Ther. 2009;17(6):947–953.
28. Wu J, Greely HT, Jaenisch R, Nakauchi H, Rossant J, Belmonte JC. Stem cells and interspecies chimaeras. Nature. 2016;540(7631):51–59.
29. Xiang AP, Mao FF, Li WQ, Park D, Ma BF, Wang T, Vallender TW, Vallender EJ, Zhang L, Lee J, Waters JA, et al. Extensive contribution of embryonic stem cells to the development of an evolutionarily divergent host. Hum Mol Genet. 2008;17(1):27–37.
30. Kobayashi T, Yamaguchi T, Hamanaka S, Kato-Itoh M, Yamazaki Y, Ibata M, Sato H, Lee YS, Usui J, Knisely AS,

Hirabayashi M, et al. Generation of rat pancreas in mouse by interspecific blastocyst injection of pluripotent stem cells. Cell. 2010;142(5):787–799.
31. Morata G, Ripoll P. Minutes: mutants of drosophila autonomously affecting cell division rate. Dev Biol. 1975;42(2):211–221.
32. Amoyel M, Bach EA. Cell competition: how to eliminate your neighbours. Development. 2014;141(5):988–1000.
33. Matsunari H, Nagashima H, Watanabe M, Umeyama K, Nakano K, Nagaya M, Kobayashi T, Yamaguchi T, Sumazaki R, Herzenberg LA, Nakauchi H. Blastocyst complementation generates exogenic pancreas in vivo in apancreatic cloned pigs. Proc Natl Acad Sci U S A. 2013;110(12): 4557–4562.
34. Wu J, Platero-Luengo A, Sakurai M, Sugawara A, Gil MA, Yamauchi T, Suzuki K, Bogliotti YS, Cuello C, Morales Valencia M, Okumura D, et al. Interspecies chimerism with mammalian pluripotent stem cells. Cell. 2017;168(3): 473–486.
35. Huang K, Zhu Y, Ma Y, Zhao B, Fan N, Li Y, Song H, Chu S, Ouyang Z, Zhang Q, Xing Q, et al. BMI1 enables interspecies chimerism with human pluripotent stem cells. Nat Commun. 2018;9(1):4649.
36. Glorioso JM, Mao SA, Rodysill B, Mounajjed T, Kremers WK, Elgilani F, Hickey RD, Haugaa H, Rose CF, Amiot B, Nyberg SL. Pivotal preclinical trial of the spheroid reservoir bioartificial liver. J Hepatol. 2015;63(2):388–398.