

Clinical Application of Stem Cell Therapy for Liver Cirrhosis: Progress, Pitfalls, and Prospects

Prenali D. Sattwika¹, Fahmi Indrarti², Putut Bayupurnama^{2}*

¹ Department of Internal Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

² Division of Gastroenterohepatology, Department of Internal Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

***Corresponding Author:**

Putut Bayupurnama, MD., PhD. Division of Gastroenterohepatology, Department of Internal Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada - Dr. Sardjito General Hospital. Jl. Kesehatan no. 1, Yogyakarta 55243, Indonesia. email: pututby@yahoo.com.

ABSTRACT

Liver cirrhosis is the advanced stage of liver disease accounting for high morbidity and mortality rates worldwide. Liver transplantation for liver cirrhosis has several limitations, rendering stem cell transplantation as a potential therapy. Clinical trials of stem cell applications for liver cirrhosis are being established using various types of stem cells. This review will provide a current report of the achievements, limitations, and future directions of stem cell transplantation. Current progress of clinical trials is valuable in defining the best type of stem cells, mode of delivery, the number and frequency of cells to be injected, and determining potential candidates for cell therapy. Some of the encountered pitfalls are the limited homing and differentiation potential of stem cells, the use of non-xenofree culture system, and the risk for tumorigenesis in certain types of stem cells. The prospective developments of liver stem cell transplantation are the generation of genetically modified stem cells and the formation of liver organoids for treating liver cirrhosis.

Keywords: stem cells, liver cirrhosis, regenerative medicine.

INTRODUCTION

Distortion of liver architecture due to viruses, toxins, or chemical agents in the case of liver cirrhosis results in declining function of the liver. Deaths due to severe complications of liver cirrhosis are estimated to reach 1.03 million people annually worldwide.¹ While hepatitis B and C infections are the main cause of liver cirrhosis, alcoholic liver disease also contributes to the development of liver cirrhosis.

Anti-fibrotic drugs are being investigated for the management of patients with liver cirrhosis. These drugs may bring clinical improvements for the patients with no overt reversal of the fibrotic state.² Orthotopic liver transplantation

remains the feasible solution for replacing the cirrhotic liver. However, several limitations are encountered: the shortage of the organ, the high procedural cost, and the risk for organ rejection. The development of cell therapy has emerged as a promising alternative for the management of liver cirrhosis. Hepatocytes have been studied for cell therapy.³ In spite of that, due to the limited donor organ, inability to be expanded in cell culture, poor engraftment, and proneness for damage under cryopreservation, the finding of another source of cells is of the utmost importance. The proliferation and differentiation ability of stem cells bring benefits for ameliorating liver cirrhosis. Unfortunately, there is still a long way

to go before standard clinical application of stem cell transplantation can be established. Exactly how these cells can restore or replace cirrhotic areas also remains inconclusive. This review will provide a current report of the progress, pitfalls, and prospects of liver stem cell transplantation to give clinical insights and possible future directions in the treatment of liver cirrhosis.

STEM CELLS FOR LIVER CIRRHOSIS

The liver consists of various types of cells including hepatocytes, Kupffer cells, and hepatic stellate cells. Some residing cells in the liver may serve as endogenous stem cell sources that can be activated during injury. Any injury to the liver may cause inflammation and promote the activation of hepatic stellate cells to produce fibrin that accumulates in the space of Disse leading to liver fibrosis.⁴

In the context of exogenous stem cells, there are three large classifications of stem cells that comprise embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). ESCs and iPSCs represent prospective sources of stem cells due to their robust ability in differentiation. Ethical consideration then hampers the use of ESCs that are taken from embryonic tissue. We expect iPSCs to be the ideal source for treating liver cirrhosis, however teratogenicity potential has become the hurdle of developing this cell line, therefore, the study of cell biology in the case of iPSCs are still under investigation. Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are ASCs that are commonly used in the clinical trials of treatment for liver cirrhosis.

ASCs can be harvested from healthy donors (allogeneic cells) or from the patients themselves (autologous cells). Allogeneic stem cells may provide good quality of stem cells at the cost of the difficulty to match the human leukocyte antigen (HLA) antigens to prevent any rejection. Autologous stem cells seem to be easier to gain. Stem cells are proposed to have several mechanisms in the treatment of liver fibrosis: mediation of inflammatory microenvironments by paracrine effect, inhibition of hepatic stellate cell activation, apoptosis induction of hepatic stellate cells, the differentiation capacity into

hepatocytes, and facilitation in regeneration of residual hepatocytes.²

PROGRESS

Clinical studies have emerged in elucidating the potential of stem cells in treating liver cirrhosis.

Type of Stem Cells

ESCs have the ability to differentiate into hepatocyte-like cells and serve as a potential source of cells for treating liver cirrhosis, unfortunately these cells were not further recommended due to ethical consideration. ASCs, comprising of HSCs, MSCs, and endothelial progenitor cells (EPCs), have arisen as potential cells for stem cell therapy. HSCs can be obtained from bone marrow^{5,6} and peripheral blood,⁷ whereas MSCs can be isolated from adipose tissue, umbilical cord, and bone marrow.^{1,8,9}

Bone marrow is the main source of HSCs that can be identified from the expression of cluster of differentiation (CD)34⁺ as their surface marker. Peripheral mobilization by granulocyte colony stimulating factor (G-CSF) makes it possible to obtain the cells from peripheral apheresis and was shown to be safe in patients with end stage liver disease.¹⁰ Injection of HSCs in patients with liver disease was expected to facilitate liver regeneration by differentiating into functioning hepatocytes and genetic reprogramming of resident hepatocytes by cell fusion. Nevertheless, the advantage of HSCs injection is more likely due to the paracrine effect and the role of macrophages in phagocytosing dead cells and producing collagenase.¹¹

MSCs serve as the best potential source of stem cells for liver cirrhosis. They can be expanded *ex vivo* while maintaining their differentiation potential. MSCs have also the ability to migrate into injured areas and the immunomodulatory properties to escape immune recognition. MSCs were shown to differentiate into hepatocytes in animal models of liver cirrhosis, however, the paracrine mechanism through the secretion of cytokines and growth factors seem to dominate their function in liver regeneration.¹¹

Administration of bone marrow-derived MSCs in patients with hepatitis B-associated

liver cirrhosis facilitated an increase in regulatory T (Treg) cells and a decrease in helper T (Th)17 cells. This regulation of Treg/Th17 cell balance could mediate improvement of liver function following stem cell transplantation.¹² **Figure 1** depicts possible interaction of hepatic stellate cells and MSCs in term of MSCs transplantation upon liver cirrhosis.^{13,14,15}

Liver-derived stem cells consist of oval cells and hepatoblasts that are involved in liver regeneration. Hepatoblasts are bipotent cells that can differentiate into duct cells or hepatocytes. Their small population in liver architecture, with oval cells constituting 0.3-0.7% and hepatoblasts only 0.1% of the liver, makes this source of stem cells not practical.¹⁶

Mode of Delivery

Stem cells are transplanted in several ways that include peripheral injection,⁵ intrahepatic artery injection,⁷ intrasplenic vein injection,⁸ intraportal vein injection,^{7,17} and percutaneous intrahepatic injection.¹⁸ Intrahepatic and intrasplenic injection of bone marrow-derived MSCs have comparable safety and a short-term efficacy profile.⁸ A study of MSCs biodistribution following intravenous injection of MSCs was performed with the use of ¹¹¹In-oxide labelling. Immediately after transfusion, there was accumulation of radioactivity in both lungs of all patients. On the 10th day of observation, radioactivity increased in the liver and spleen along with reduction of radioactivity in the lung. Spleen accumulation of MSCs was found to be higher in quantity compared to the liver in all patients.¹⁹

Cells Number and Frequency

The number of the cells to be injected and the frequency of injection remains the question to be answered in cell therapy. An average of 2×10^7 bone marrow-derived MSCs could be injected safely to patients with liver cirrhosis.⁸ Autologous bone marrow-derived mononuclear cells of $1.6-13.1 \times 10^8$ were injected through the intrahepatic artery of patients with advanced liver disease.⁶ Another study injected different doses of peripheral blood-derived CD34⁺ cells with range of 1×10^6 to 2×10^8 cells via portal vein and hepatic artery routes.⁷ Suk et al. performed transhepatic artery injection of bone marrow-derived MSCs

for alcoholic cirrhosis patients revealing that two injections did not improve patients' condition compared to single injection.²⁰

Published Clinical Trials

A meta-analysis of stem cells therapy for liver cirrhosis has been published recently.²¹ This meta-analysis showed that MSCs therapy could improve laboratory parameters of patients with liver cirrhosis. There were improvements in the level of albumin, alanine aminotransferase, total bilirubin, prothrombin time, and end-stage liver disease score; however, there were no significant changes in terms of cholinesterase level, prothrombin activity, and international normalized ratio. This meta-analysis suggested the safety profile and efficacy of stem cells injection for the patients with liver cirrhosis in the first 6 months. Multiple injections of stem cells did not result in beneficial effects compared to single injection. Intraarterial injection was shown to bring better efficacy in comparison to other modes of delivery. In terms of stem cell source, bone marrow-derived stem cells provided better improvement of liver cirrhosis.

Caution should be taken into consideration when interpreting the results of clinical studies as some studies did not provide a control group of comparable cirrhotic patients⁶ with only short-term periods of follow-up. Publications derived from small-scale studies with peripheral injection may result in minimal cell delivery toward the liver. The choice of undifferentiated or differentiated bone marrow-derived MSCs injection showed no significant difference in the efficacy.⁹ One clinical study showed safety and efficacy of bone marrow CD34⁺ cells during 12 months follow-up.²² Long-term follow-up of cirrhotic patients receiving bone marrow MSCs did not present favorable outcomes.²³

Although phase I-II clinical trials of stem cell treatments prove the safety profile,²² stem cells transplantation is not without risk. Treatment related therapy should be also taken into consideration. Intrahepatic injection of stem cells could result in lethal side effects, for instance the development of radiocontrast nephropathy leading to hepatorenal syndrome.²⁴ A larger scale of randomized controlled trial comparing

the effects of HSCs infusions to GCSF treatment showed no greater beneficial effects received by cirrhotic patients and the patients are even more likely to suffer from treatment-related adverse events compared to standard care.²⁵

Defining Potential Candidates for Therapy

Before applying stem cell therapy for patients with liver cirrhosis, it is very crucial to determine the patients that potentially will get the most benefit from the transplantation. Conditioning regimen for preparing stem cell transplantation may include the use of immunosuppressive therapy that could put the patients at risk of infection. Current clinical studies of stem cells show that stem cells are able to improve clinical conditions and laboratory parameters of cirrhotic patients but are yet to reverse the cirrhotic state. Therefore, this method may be beneficial for those waiting for liver transplantation or as an add-on therapy of standard medications. A small-scale study showed that the injection of bone marrow-derived CD133⁺ may enhance hepatic regeneration before performing hepatectomy in the case of hepatocellular carcinoma or metastatic liver mass.¹⁷ Cirrhotic patients of Child Pugh C aged less than 60 can be considered as the candidates for stem cell therapy. Those patients with advanced complications of cirrhotic liver (moderate to severe hepatic encephalopathy and recurrent variceal bleeding), bleeding tendency, active infection condition, hepatocellular carcinoma, and hepatic/portal/splenic thrombus are considered as non-favorable patients for stem cell transplantation. Strict cut-off point of creatinine level should be decided to prevent patients with underlying kidney problems from receiving contrast for the delivery stem cells that may lead to contrast-induced nephropathy.²⁴

PITFALLS

This part covers the limitations of stem cell therapy in alleviating liver cirrhosis.

Homing

Once stem cells are injected into the patients with liver cirrhosis, homing efficacy will be the first aspect to consider. Following cell transplantation, only 1-3% of the liver was repopulated by stem cells.²⁶ In order to give the

best effect, stem cells should remain in the liver. Intravenous injection is one of the most common methods in the administration of stem cells. Unfortunately, stem cells may be phagocytosed by reticuloendothelial cells in the circulation. Furthermore, these cells will pass through the capillary bed of the lung before reaching hepatic circulation, putting up an obstacle to achieve effective homing. Peripheral injection of bone marrow MSCs did not give beneficial effect for cirrhotic patients compared to control.⁵ Therefore, although technically more difficult, intraarterial injection is preferred to distant intravenous injection.²¹

To promote engraftment, intrahepatic injection of stem cells seems to be the best way to deliver cells since there will be less cell entrapment in the circulation. Cell culture conditioning, for instance hypoxic preconditioning, also contributes in improving the homing ability of stem cells. Preventing high cell confluence during passaging will also maintain homing efficacy as high confluence of culture can increase the production of tissue inhibitor metalloproteinase-3. The use of cells from early passages may also provide better homing efficacy.¹ Homing may also be enhanced by modifying MSCs by liver specific receptors.¹¹

Differentiation Potential

Although the injections of stem cells to patients with liver cirrhosis were shown to improve their clinical condition, differentiation potential of the stem cells into hepatocytes still is in question. Autologous bone marrow infusion can stimulate the activation and differentiation of hepatic progenitor cells. However, this effect was not sustained more than 6 months as liver biopsy showed histological pictures returning to the baseline before infusion.²⁷ Transdifferentiation of stem cells into hepatocytes may be driven by some transcription factors and cellular signalling. Hepatocyte nuclear factor (HNF)3 β and HNF4 α are among transcription factors that play an important role in hepatic differentiation. HNF3 β is a family of forkhead box transcription factors that promote hepatic differentiation of stem cells as evidenced by the production of alpha fetoprotein, albumin, and tyrosine aminotransferase. HNF4 α induces the

differentiation into the hepatocyte phenotypes. Wnt signalling inhibition may also drive hepatic differentiation of stem cells. Another concern is that transplanted MSCs may be differentiated into myofibroblast-like cells that may further worsen the liver's fibrotic state.²⁶

Non-xeno-free culture system

The preparation of stem cells, from the isolation of cells to the transplantation, needs several reagents processed from animal products. This process brings the potential of infection transmission and increased risk of cells rejection. Techniques for preparing xeno-free culture system have been developed.²⁸ Unfortunately, the xeno-free system cannot be applied widely because the preparation of the cells in the beginning step requires several growth hormones which are animal-derived products.

Tumorigenesis

The risk for teratoma formation is one of the major hurdles for the application of iPSCs. This tumor may arise from the undifferentiated cells injected along with differentiated ones. Accordingly, there is a method to purge and separate the undifferentiated from differentiated cells. The problem is that even single cells remaining in the injection process may cause the teratoma formation. Furthermore, genome instability of stem cells may cause mutation of cells during culturing, increasing the possibility to become teratogenic cells. One of the solutions for this issue is the method of the viral vector transduction system to insert a suicide gene and oncolytic virus to kill the tumorigenic cells.²⁹

The teratogenic potential of MSCs transplantation should also be considered as MSCs may promote tumor growth by transdifferentiating into malignant cells, suppressing the anti-tumor immune system, and secreting growth factors for cancer cells as well as promoting the secretion of angiogenic factors, for instance fibroblast growth factor, vascular endothelial growth factor, and platelet-derived growth factor.²⁶

PROSPECTS

The study of cell biology related to stem cells has been evolving. In the future, there will

be new discoveries playing important roles for cell therapy.

Genetically modified stem cells

Stem cells can be modified in such a way that some genes will be transferred and will influence how the cells behave in case of injury. Genetic modification may increase survival and function of stem cells once they are transplanted. Genetically modified-stem cells are expected to be able to protect themselves in term of inflammation following transfusion, immune rejection, hypoxia condition, and apoptosis. Gene modification can be obtained by transgenic methods using specific vectors, Cre/Lox P system to knock out target gene, antisense inhibition, small interfering RNA (siRNA) gene silencing, microRNA technology, or the use of cell specific promoters.³⁰

Liver organoids

Liver organoids are expected to possess the ability to replace the diseased tissue. The study of liver organoids is now under the preclinical stage. Hepatocytes are the main cells responsible for liver regeneration following injury of the liver. In addition, there are also ductal populations that may differentiate into bi-potent progenitor. During liver regeneration, several signalling pathways are needed to direct the differentiation that may include Notch (Sattwika et al., unpublished work), Wnt, hepatocyte growth factor, and fibroblast growth factor. In order to create supportive microenvironments to develop liver organoids, an interaction between embryonic endoderm and mesoderm is needed.³¹ Therefore, ASCs alone are not enough to produce such complex interaction. Another solution is to prepare co-cultures of iPSCs-derived hepatocytes with MSCs. Takebe et al. has shown outstanding formation of liver organoids from iPSCs.³² In the future, liver organoids are expected to replace cirrhotic areas in patients with liver cirrhosis.

CONCLUSION

Current basic researches and clinical trials of stem cell therapy for liver cirrhosis show that stem cell transplantation may be a potential alternative to liver transplantation. From the perspective of clinicians, big-scale randomized

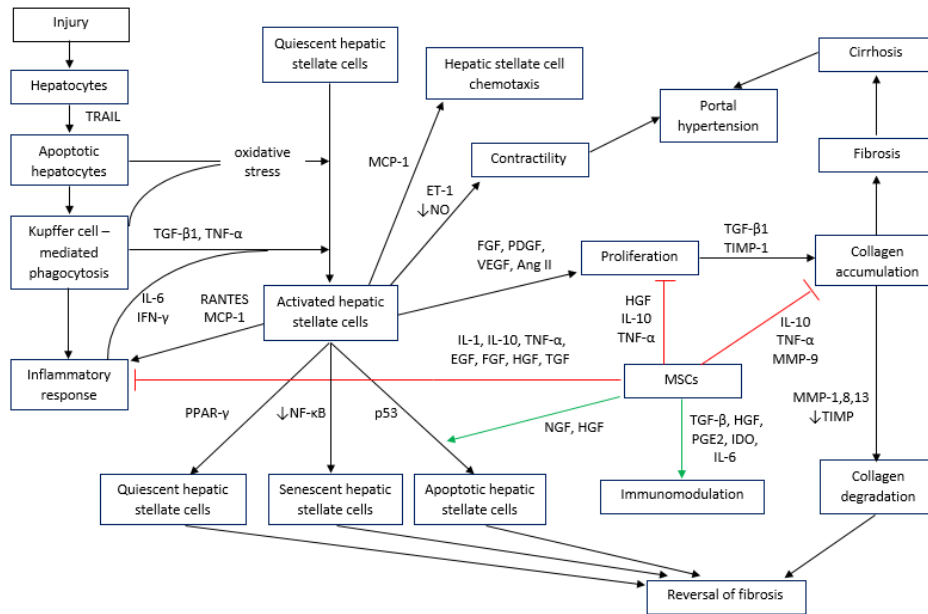


Figure 1. Schematic diagram of the proposed interaction between hepatic stellate cells and mesenchymal stem cells (MSCs) during liver injury. Vulnerable hepatocytes undergo apoptosis when exposed to injury. Apoptotic bodies of hepatocytes are engulfed by resident Kupffer cells. Activated Kupffer cells then promote inflammatory response and activation of hepatic stellate cells. Transformation of quiescent to activated hepatic stellate cells (myofibroblasts) also results from oxidative stress as a consequence of formation of apoptotic bodies. Activated hepatic stellate cells further enhance inflammatory response due to their chemotactic, contractile, and proliferative properties. Contractility of hepatic stellate cells results in an increase in portal pressure, whereas proliferative property is responsible for increased production and accumulation of collagen leading to liver fibrosis. Fibrotic state may eventually result in liver cirrhosis. Cessation of injury, clearance of activated hepatic stellate cells (i.e. by transformation into quiescent, senescent, or apoptotic hepatic stellate cells), and degradation of collagen are all expected to reverse fibrosis. MSCs may contribute to improve the fibrotic state through their paracrine mechanism to suppress inflammatory condition, inhibit the activation of hepatic stellate cells, and induce the apoptosis of hepatic stellate cells.^{13,14,15} TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; TNF- α : tumor necrosis factor alpha; TGF- β : transforming growth factor beta; IFN- γ : interferon gamma; IL-6: interleukin-6; RANTES: regulated upon activation, normal T cell expressed and secreted; MCP-1: monocyte chemotaxis protein-1; PDGF: platelet derived growth factor; EGF: epidermal growth factor; bFGF: basic fibroblast growth factor; VEGF: vascular endothelial growth factor; ET-1: endothelin 1; NO: nitric oxide; Ang II: angiotensin II; MMP: matrix metalloproteinase; TIMP: tissue inhibitors of metalloproteinase; HGF: hepatocyte growth factor; NGF: nerve growth factor; IL-1: interleukin-1; IL-10: interleukin-10; PGE2: prostaglandin E2; IDO: indoleamine 2,3-dioxygenase; PPAR- γ : peroxisome proliferator-activated receptor gamma; NF- κ B: nuclear factor kappa B; p53: tumor protein 53.

controlled trials will be definitely needed to elucidate the best type of stem cells, the best route, and the best cell number and frequency of injection. From the aspect of scientists, further studies to explore the mechanisms underlying the role of stem cells in the case of liver cirrhosis is of importance, as well as to answer the limitation of stem cell therapy as discussed above. The discovery of genetically modified stem cells and liver organoids may be beneficial for liver cirrhosis patients in the next several decades.

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CONFLICT OF INTERESTS

The author(s) declare that they have no conflict of interests.

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REFERENCES

- Zhang Y, Li Y, Zhang L, et al. Mesenchymal stem cells: potential application for the treatment of hepatic cirrhosis. *Stem Cell Res Ther.* 2018;9(1):59.
- Berardis S, Sattwika PD, Najimi M, Sokal EM. Use of mesenchymal stem cells to treat liver fibrosis: current situation and future prospects. *World J Gastroenterol.* 2015;21(3):742–758.
- Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: from liver transplantation to cell factory. *J Hepatol.* 2015;62(S1):S157–S169.

4. Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology*. 2010;134(6):1655–69.
5. Mohamadnejad M, Alimoghaddam K, Bagheri M, et al. Randomized placebo-controlled trial of mesenchymal stem cell transplantation in decompensated cirrhosis. *Liver Int*. 2013;33(10):1490–6.
6. Lyra AC, Soares MB, da Silva LF, et al. Feasibility and safety of autologous bone marrow mononuclear cell transplantation in patients with advanced chronic liver disease. *World J Gastroenterol*. 2007;13(7):1067–73.
7. Gordon MY, Levičar N, Pai M, et al. Characterization and clinical application of human CD34⁺ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. *Stem Cells*. 2006;24(7):1822–30.
8. Amer M-EM, El-Sayed SZ, El-Kheir WA, et al. Clinical and laboratory evaluation of patients with end-stage liver cell failure injected with bone marrow-derived hepatocyte-like cells. *Eur J Gastroenterol Hepatol*. 2011;23(10):936–41.
9. El-ansary M, Abdel-aziz I. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in egyptian patients with HCV induced liver cirrhosis. *Stem Cell Rev Rep*. 2012;8:972–81.
10. Gaia S, Smedile A, Omedè P, et al. Feasibility and safety of G-CSF administration to induce bone marrow-derived cells mobilization in patients with end stage liver disease. *J Hepatol*. 2006;45(1):13–9.
11. Kwak K-A, Lee S-P, Yang J-Y, Park Y-S. Current perspectives regarding stem cell-based therapy for liver cirrhosis. *Can J Gastroenterol Hepatol*. 2018;2018:1–14.
12. Xu L, Gong Y, Wang B, et al. Randomized trial of autologous bone marrow mesenchymal stem cells transplantation for hepatitis B virus cirrhosis: regulation of Treg/Th17 cells. *J Gastroenterol Hepatol*. 2014;29(8):1620–8.
13. Cohen-Naftaly M, Friedman SL. Current status of novel antifibrotic therapies in patients with chronic liver disease. *Therap Adv Gastroenterol*. 2011;4(6):391–417.
14. Lin H, Xu R, Zhang Z, et al. Implications of the immunoregulatory functions of mesenchymal stem cells in the treatment of human liver diseases. *Cell Mol Immunol*. 2011;8(1):19–22.
15. Eom YW, Shim KY, Baik SK. Mesenchymal stem cell therapy for liver fibrosis. *Korean J Intern Med*. 2015;2(2):510–6.
16. Nicolas C, Wang Y, Luebke-Wheeler J, Nyberg S. Stem cell therapies for treatment of liver disease. *Biomedicines*. 2016;4(1):2.
17. am Esch JS, Knoefel WT, Klein M, et al. Portal application of autologous CD133⁺ bone marrow cells to the liver: a novel concept to support hepatic regeneration. *Stem Cells*. 2005;23(4):463–70.
18. Bahk JY, Piao Z, Jung JH, Han H. Treatment of the end stage liver cirrhosis by human umbilical cord blood stem cells: preliminary results. In: *Stem Cells in Clinic and Research*, Dr Ali Gholamrezaezhad (Ed). InTech. 2011. p. 469–500.
19. Gholamrezaezhad A, Mirpour S, Bagheri M, Mohamadnejad M. In vivo tracking of ¹¹¹In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. *Nucl Med Biol*. 2011;38(7):961–7.
20. Suk KT, Yoon J-H, Kim MY, et al. Transplantation with autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: phase 2 trial. *Hepatology*. 2016;64(6):2185–97.
21. Zhao L, Chen S, Shi X, Cao H. A pooled analysis of mesenchymal stem cell-based therapy for liver disease. *Stem Cell Res Ther*. 2018;9:1–13.
22. Levicar N, Pai M, Habib NA, et al. Long-term clinical results of autologous infusion of mobilized adult bone marrow derived CD34⁺ cells in patients with chronic. *Cell Prolif*. 2008;41(1):115–25.
23. Peng L, Xie D, Lin B, et al. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. *Hepatology*. 2011;820–8.
24. Mohamadnejad M, Namiri M, Bagheri M, et al. Phase 1 human trial of autologous bone marrow-hematopoietic stem cell transplantation in patients with decompensated cirrhosis. *World J Gastroenterol*. 2007;13(24):3359–63.
25. Newsome PN, Fox R, King AL, et al. Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial. *Lancet Gastroenterol Hepatol*. 2017;1253(17):4–6.
26. Liu WH, Song FQ, Ren LN, et al. The multiple functional roles of mesenchymal stem cells in participating in treating liver diseases. *J Cell Mol Med*. 2015;19(3):511–20.
27. Kim JK, Park YN, Kim JS, et al. Autologous bone marrow infusion activates the progenitor cell compartment in patients with advanced liver cirrhosis. *Cell Transplant*. 2010;19(10):1237–46.
28. Shahjalal HM, Shiraki N, Sakano D, et al. Generation of insulin-producing beta-like cells from human iPS cells in a defined and completely xeno-free culture system. *J Mol Cell Biol*. 2014;6(5):394–408.
29. Mitsui K, Ide K, Takahashi T, Kosai KI. Viral vector-based innovative approaches to directly abolishing tumorigenic pluripotent stem cells for safer regenerative medicine. *Mol Ther Methods Clin Dev*. 2017;5(June):51–8.
30. Phillips MI, Tang YL. Genetic modification of stem cells for transplantation. *Adv Drug Deliv Rev*. 2008;60(2):160–72.
31. Huch M, Koo B-K. Modeling mouse and human development using organoid cultures. *Development*.

2015;142(18):3113–3125.

32. Takebe T, Sekine K, Kimura M, et al. Massive and reproducible production of liver buds entirely from human pluripotent stem cells. *Cell Rep.* 2017;21(10):2661–70.