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Mini Review

Methods of Liver Stem Cell Therapy in Rodents as Models of Human Liver Regeneration in Hepatic Failure

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Abstract

Cell therapy is a promising intervention for treating liver diseases and liver failure. Different animal models of human liver cell therapy have been developed in recent years. Rats and mice are the most commonly used liver failure models. In fact, rodent models of hepatic failure have shown significant improvement in liver function after cell infusion. With the advent of stem-cell technologies, it is now possible to re-programme adult somatic cells such as skin or hair-follicle cells from individual patients to stem-like cells and differentiate them into liver cells. Such regenerative stem cells are highly promising in the personalization of cell therapy. The present review article will summarize current approaches to liver stem cell therapy with rodent models. In addition, we discuss common cell tracking techniques and how tracking data help to direct liver cell therapy research in animal models of hepatic failure.

Introduction

The liver is one of the critical organs in the body and performs many important functions including homeostasis, synthesis and storage of glucose and proteins, detoxification and immune defence.[1] Hepatocytes comprise the majority of liver cells. Functional disorders of these cells are related to many devastating diseases such as hepatitis, cirrhosis and hepatocellular carcinoma. Liver related diseases have a heavy burden on socioeconomics and affect 1.7% of population. Cirrhosis is an irreversible hepatocyte failure caused by long-term infection with hepatitis viruses, alcohol abuse,[2] autoimmune inflammation and exposure to metabolic metals like iron and copper.[3] Although the liver is a naturally regenerative organ, at the end stage failure of liver disease such as fibrosis and cirrhosis, therapeutic intervention is necessary.[4,5] Liver transplantation is considered the most efficient therapeutic strategy in liver failure.[1,6] However, because of the lack of appropriate donors,[1,2] around 15% of list waiting patients die.[6] Furthermore, risks associated with operation, immune suppression,[1] immunological rejection and high-costs,[5] and the need for high technology and an experienced team are obvious limitations of liver transplantation. According to these obstacles, cell therapy has been proposed as a novel alternative method for liver transplantation.

Cell therapy is defined as cell transplantation to damaged organs for repair. The transplanted cells will replace or enhance the function of damaged tissues or organs. The major candidate cells for cell therapy are primary autologous mesenchymal cells, genetically identical or syngeneic cells, immortalized hepatocytes[7] and stem cells.[2,8] Currently, cellular transplantation is developing for liver damage therapy[9] and in comparison to liver transplantation has many advantages including overcoming the shortage of liver donors[1,6,10] and immunological rejection,[1,10] and a minimally invasive approach.[7]

Research in the area of cell replacement therapy in the liver, particularly with stem cells, is at the beginning stages. The development and evaluation of novel cell therapy for liver disease requires the use of an appropriate animal model. In this paper we focus on the principles and methods of liver stem cell therapy in rodents as models of human liver regeneration in hepatic failure.

Induction of liver damage

Experimental animal models of liver damage can be made using exogenous factor induction or surgical removal of the liver.

Drug induced damage

Acetaminophen (N-acetyl-p-aminophenol [APAP]) is the most common pharmaceutical poison that causes severe necrosis in hepatocytes.[11-13] This necrosis in hepatocytes is followed by increasing mitochondrial permeability transition (MPT).[12,13] The cytotoxic effects of APAP in the hepatocytes can be assessed by measuring the release of alanine aminotransferase (ALT), mitochondrial membrane

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potential using JC-1 or propidium iodide labeling.[12] Koffman et al. have shown a dose dependent effect of APAP, administered intraperitoneally, on the destruction of liver in C57BL/6J.[14] A single 300 mg/kg intraperitoneal injection of APAP is appropriate to cause severe acute parenchymatous liver injury in the rodents.[15,16]

Chemical induced damage

Carbon tetrachloride (CCl_4) intoxication is a common method for induced liver injury in rodents that was originally described by Proctor and Chatamra.[17] Injection of doses between 0.5-1 mL/kg CCl4 dissolved in olive[18-20] or corn oil[5,21] twice a week for 4-8 weeks[18,22] have been used for liver injury in small animal models.[12,20] CCl4 can be injected to rodents intraperitoneally[12,23,24] or subcutaneously.[5,18,20,21]

Paracetamol,[25] ally alcohol (AA)[26] and dimethylnitroseamine (DMN) are other chemical agents for inducing liver damage in rodents.[27] Mixture of D-galactoseamine (D-gal) and lipopolysaccharide (LPS) 0.5 mg/g and 1 ng/g, respectively, in 1 ml of saline via intraperitoneal have also been used for inducing fulminant hepatic failure (FHF) in mice.[28]

Surgery

Hepatectomy is another strategy to prepare rodent models with liver damage. In this method a one-third partial hepatectomy is performed under general anaesthesia, and is followed by a 2 mg/kg body weight 2-acetylaminofluoren administration to inhibit liver regeneration (Figure 1).[29]

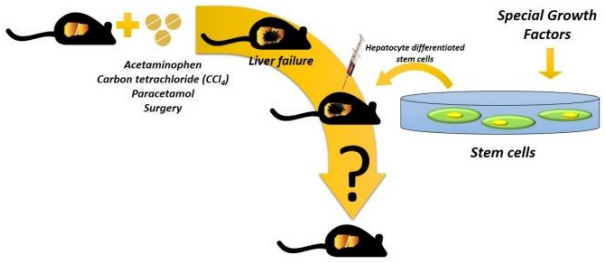

Figure 1. Schematic representation of liver stem cell therapy in rodents model. Stem cells can be differentiated to hepatocytes in culture using specific condition and growth factors. Exogenous inducing factors or surgical removal of the liver are used to produce experimental animal model of acute liver failure. Finally, it is possible to transplant differentiated cells to liver disease models through intrasplenic or intraportal infusion or tail vein injection.

Isolation and differentiation of stem cells

Stem cells are undifferentiated cells that are able to self-renew and differentiate into mature cell types of the specific tissue. Stem cells can be isolated from a variety of natural sources, such as blastocytes, somatic tissues, or whole blood. The first successful isolation of human embryonic stem cells was reported in 1998.[30] Gradient separation, as with Percoll and isolation by enzymatic digests, as with trypsin and collagenase are the most basic techniques for stem cells. Isolation and differentiation procedures are still improved and expanding in their fields of application.

Stem cell *in vitro* differentiation is a novel therapeutic strategy in regenerative medicine. This strategy is based on the condition known to promote terminal differentiation of stem cells. This section provides an overview of the isolation and differentiation of different types of stem cells such as embryonic stem cells, adult stem cells, and induced pluripotent stem cells.

Embryonic stem cells

Embryonic stem cells (ESC) are pluripotent cells derived from the inner cell mass (ICM) of blastocysts, and can differentiate into all three germ layers.[31] ESC are proliferated in an undifferentiated state by culturing them on feeder layers of mouse embryonic fibroblast cells (MEF) or in a medium containing leukaemia inhibitory factor (LIF). In order to differentiate ESC to cell linages, MEF or LIF is removed from the culture medium. ESC spontaneously differentiate and aggregate to form an embryoid body (EB) in hanging drop culture.[32] EB contains all three germ layers which are capable of differentiating to several kinds of cells.[29,32] For example, supplementation of ESC medium with sodium butyrate, recombinant mouse hepatocyte growth factor (rMuHGF) and dexamethasone resulted in hepatic differentiation in 19 days.[33] Differentiated cells efficiently expressed liver biomarkers such as albumin (ALB), α- fetoprotein (AFP), α-1-antitrypsin (AAT) and transthyretin (TTR). Other researchers reported a more efficient differentiation protocol by adding the mid-stage factor oncostatin M (OSM).[32] The synthesis of urea is also an important hepatocytes marker that is detectable from the ninth day of differentiation, reaching its maximum level on day 18.[29]

Adult stem cells

Mesenchymal stem cells (MSC) are the most available and important adult stem cells. MSC isolated from adult tissues such as bone marrow, adipose tissue, umbilical cord blood and placenta can be differentiated into functional hepatocytes.[21] In recent studies the immunomodulatory effects of MSC have been documented.[34] MSC that play a critical role in the bone marrow niche have the ability to self-renew or differentiate to other lineages.[35] MSC are positive for CD90, CD105 and CD73 and negative for CD45, CD34, CD14, CD11b, CD79a and human leukocyte antigen (HLA)-DR.[36] Fluorescence-activated cell sorting showed gene expression of CD90 and CD29 but not CD45 in MCS from rats.[37] The natural environments of MSC are hypoxic and published data showed hypoxia preconditioning to have supporting effects on the cell survival and genetic instability of these cells, confirming a new insight towards overcoming poor engraftment after transplantation at the bed side.[38]

MSC can differentiate into hepatocytes by culturing on a specific medium containing growth factors such as FGF, HGF, EGF, insulin and dexamethasone.[37] The first

replacement of the medium is performed on the third day of differentiation protocol.[18,22,39] Different concentrations of growth factors including human hepatocyte growth factor (rhHGF), human fibroblast growth factor-4 (rhFGF-4) and EGF have been used to differentiate MSC into hepatocytes.[21,40] For assessing the differentiation of MSC to liver cells, hepatocytes marker genes such as ALB and α-fetoproteins analysed during *in vitro* differentiation.[5,37]ESC and MSC are two more common types of stem cells that are used to differentiate to hepatocytes.

Induced pluripotent stem cells

Induced pluripotent stem cells (iPSC) are actually patient specific pluripotent cells. iPSC were originally created by introduction of four genes, Oct4, Sox2, Kfl4, and cMyc, into a differentiated mouse cell.[41] These reprogrammed cells were pluripotent. Technologies for the generation of iPSC are continuously evolving. For example, it has recently been shown that the presence of a single transcription factor, Oct4, was enough to induce pluripotency in adult cells.[42] iPSC are now being used for disease modelling, drug development and organ synthesis. iPSC have been efficiently used to generate liver tissue for transplantation. A co-culture of iPSC with endothelial and MSC resulted in functional liver buds.[43] For example, in one study, baicalin led to erythroid differentiation of CD133+ hematopoietic stem cells (HSC). Thus, baicalin application can be effective in the treatment of a wide range of disease.[44] Then, HSC niches and other factors around HSC can promote malignancies,[45-47] so it may be stem cells has notable clinical applications, especially in transplantation.[44]

The methods and ways of transplantation of functional mature cells

Generated functional mature cells from stem cells could be successfully transplanted into laboratory rat models. Transplanted cells could adhere, survive and regenerate the liver organ. The methods of transplantation of functional mature cells are discussed as follows.

Intrasplenic

A cell count of1×10^6-6×10^6 cells/ml in 0.5-1ml PBS could be injected through the lower pole of the spleen in laboratory rats or mice. To prevent bleeding and cell leakage after cell injection the lower pole of the spleen is ligated.[18,22,29,40]

Tail

A cell count of 5×10^5 suspended in 100µl PBS could be injected into the tail vein of rodents.[1,19,28]

Portal vein

Zhao and colleagues showed that intravenous injection of MSC is effective in the treatment of liver fibrosis in comparison with intrahepatic or intraperitoneal injection. As in intravenous injection liver lobules were normal, but in intrahepatic and intraperitoneal injection severe collagen deposition was observed[5]. A cell count of 1×10^6 suspended in 30µl of PBS or DMEM could be injected through the portal vein.[20,21,26]

Homing and efficacy of cell therapy

Histopathological methods such as Haematoxylin and Eosin (H&E),[22,28] Masson Trichrome[21,22] and Sirius Red[22] staining were used for detection of tissue structure changes after cell therapy in liver failure.

The homing and engraftment of the transplanted cells to the target organ is key to the success of the cell therapy. Tracing of individual transplanted cells in the body of the recipient is particularly important in studies of cell therapy.

Tracing of transplanted cells

Several labelling techniques are currently used to trace transplanted cells in cell therapy. Carboxy fluorescein diacetate succinimidyl ester (CFSE)[39] and 4,6-diamidino-2-phenylindole, dihydrochloride (DAPI),[24] chloromethyl-benzamidodialkylcarbocyanine (CM- Dil)[40] fluorescent dyes are commonly used to examine cell localization.

The red fluorochrome PKH26, which mainly binds to the cell membrane, is an efficient cell tracer to locate transplanted cells.[20] The liver cell suspension could be easily labelled after around 4 minutes of incubation with PKH26[1]. This fluorescent dye is not toxic, and labelled cells retain both biological and proliferative activity. The characteristics of PKH26 are compatible with rhodamine or phycoerythrin detection systems.[1]

Super paramagnetic particles of iron oxide (SPIO), such as Fe_3O_4- Poly- L- Lysin (PLL), could be used for tracking in cell therapy. In this method, the labelling efficiency of cells could be assessed *in vitro* by Prussian blue staining and atomic absorption spectrometry. The magnetically labelled cells are tracked *in vivo* by MR imaging.[18,19]

Alu sequence detection by polymerase chain reaction (PCR) and green fluorescent protein (GFP) fusions are other useful techniques for tracking and locating transplanted cells.[9,19,21,28]

Functional assay of transplanted cells

Characterization of cell viability and cell identity after administering the cells is very important in the long-term follow-up study of liver cell therapy. Mortality rate is a reliable indicator of transplant functionality and overall recipient health. Other cellular indicators include Y-chromosome and hepatic markers.

Detection of the Y-chromosome in the liver tissue of female recipients from male rodents is related to the efficacy of cell therapy. Both fluorescent *in situ* hybridization (FISH) and real time quantitative polymerase chain reaction (qPCR) may be used to identify and quantify Y chromosome material or a male-specific SRY.[29] Investigations of immunohistochemical expression of hepatic markers, including ALB, AFP, CK18 and CK19, are popular quantitative functional assays of transplanted cells.[26,28]

Conclusion

Research is ongoing to identify an optimal strategy for long-term cell therapy treatment in liver failure. Rodents are suitable animal models of liver regeneration in hepatic failure. In fact, rodent models of hepatic failure have shown significant improvement in liver function after cell infusion. The major steps of liver stem cell therapy in rodents are induction of liver damage, stem cell *in vitro* differentiation, and transplantation of functional mature cells. *In vivo* identification and tracking of transplanted cells in animal models are important to determine their homing and fate. These parameters are actually related to and may even predict, the success or failure of liver stem cell therapy.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Piryaei A, Rezazadeh Valojerdi M, Baharvand H. Therapeutic Potential of Mouse Bone Marrow Mesenchymal Stem Cells in Carbon Tetrachloride (CCl4)-Induced Liver Fibrosis. *J Iran Anat Sci* 2010;7(28-29):99-112.
2. Levicar N, Dimarakis I, Flores C, Tracey J, Gordon MY, Habib NA. Stem cells as a treatment for chronic liver disease and diabetes. *Handb Exp Pharmacol* 2007;(180):243-62. doi: 10.1007/978-3-540-68976-8_11
3. Wu XB, Tao R. Hepatocyte differentiation of mesenchymal stem cells. *Hepatobiliary Pancreat Dis Int* 2012;11(4):360-71.
4. Pournasr B, Farzaneh Z, Shahsanvani M, Baharvand H. Liver Development and In vitro Differentiation of Embryonic Stem Cells to Hepatocytes. *Yakhteh Medical Journal* 2010;11(4):348-73.
5. Zhao Q, Ren H, Zhu D, Han Z. Stem/progenitor cells in liver injury repair and regeneration. *Biol Cell* 2009;101(10):557-71. doi: 10.1042/BC20080105
6. Atta SA. Hepatocyte Transplantation. *Euroasian J Hepato-Gastroenterol* 2013;3(1):59-63.
7. Jameson E. Cellular Transplantation for Liver Diseases. *Gastroen Res* 2008;1(1):8-13. doi: 10.4021/gr2008.11.1243
8. Zhou WL, Medine CN, Zhu L, Hay DC. Stem cell differentiation and human liver disease. *World J Gastroenterol* 2012;18(17):2018-25. doi: 10.3748/wjg.v18.i17.2018
9. Seppen J, Filali EE, Elferink RO. Small animal models of hepatocyte transplantation. *Methods Mol Biol* 2009;481:75-82. doi: 10.1007/978-1-59745-201-4_7
10. Nussler A, Konig S, Ott M, Sokal E, Christ B, Thasler W, et al. Present status and perspectives of cell-based therapies for liver diseases. *J Hepatol* 2006;45(1):144-59. doi: 10.1016/j.jhep.2006.04.002
11. Botta D, Shi S, White CC, Dabrowski MJ, Keener CL, Srinouanprachanh SL, et al. Acetaminophen-induced liver injury is attenuated in male glutamate-cysteine ligase transgenic mice. *J Biol Chem* 2006;281(39):28865-75. doi: 10.1074/jbc.m605143200
12. James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatotoxicity. *Drug Metab Dispos* 2003;31(12):1499-506. doi: 10.1124/dmd.31.12.1499
13. Reid AB, Kurten RC, McCullough SS, Brock RW, Hinson JA. Mechanisms of acetaminophen-induced hepatotoxicity: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. *J Pharmacol Exp Ther* 2005;312(2):509-16. doi: 10.1124/jpet.104.075945
14. Kofman AV, Morgan G, Kirschenbaum A, Osbeck J, Hussain M, Swenson S, et al. Dose- and time-dependent oval cell reaction in acetaminophen-induced murine liver injury. *Hepatology* 2005;41(6):1252-61. doi: 10.1002/hep.20696
15. Shen K, Chang W, Gao X, Wang H, Niu W, Song L, et al. Depletion of activated hepatic stellate cell correlates with severe liver damage and abnormal liver regeneration in acetaminophen-induced liver injury. *Acta Biochim Biophys Sin (Shanghai)* 2011;43(4):307-15. doi: 10.1093/abbs/gmr005
16. Yin H, Cheng L, Holt M, Hail N Jr, Maclaren R, Ju C. Lactoferrin protects against acetaminophen-induced liver injury in mice. *Hepatology* 2010;51(3):1007-16. doi: 10.1002/hep.23476
17. Proctor E, Chatamra K. High yield micronodular cirrhosis in the rat. *Gastroenterology* 1982;83(6):1183-90.
18. Ju S, Teng GJ, Lu H, Zhang Y, Zhang A, Chen F, et al. In vivo MR tracking of mesenchymal stem cells in rat liver after intrasplenic transplantation. *Radiology* 2007;245(1):206-15. doi: 10.1148/radiol.2443061290
19. Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H, et al. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. *Hepatology* 2004;40(6):1304-11. doi: 10.1002/hep.20452
20. Zhan Y, Wang Y, Wei L, Chen H, Cong X, Fei R, et al. Differentiation of hematopoietic stem cells into hepatocytes in liver fibrosis in rats. *Transplant Proc* 2006;38(9):3082-5. doi: 10.1016/j.transproceed.2006.08.132
21. Chang YJ, Liu JW, Lin PC, Sun LY, Peng CW, Luo GH, et al. Mesenchymal stem cells facilitate recovery from chemically induced liver damage and decrease

liver fibrosis. *Life Sci* 2009;85(13-14):517-25. doi: 10.1016/j.lfs.2009.08.003
22. Rabani V, Shahsavani M, Gharavi M, Piryaei A, Azhdari Z, Baharvand H. Mesenchymal stem cell infusion therapy in a carbon tetrachloride-induced liver fibrosis model affects matrix metalloproteinase expression. *Cell Biol Int* 2010;34(6):601-5. doi: 10.1042/cbi20090386
23. Khurana S, Mukhopadhyay A. Characterization of the potential subpopulation of bone marrow cells involved in the repair of injured liver tissue. *Stem Cells* 2007;25(6):1439-47. doi: 10.1634/stemcells.2006-0656
24. Zhao W, Li JJ, Cao DY, Li X, Zhang LY, He Y, et al. Intravenous injection of mesenchymal stem cells is effective in treating liver fibrosis. *World J Gastroenterol* 2012;18(10):1048-58. doi: 10.3748/wjg.v18.i10.1048
25. Maheswari C, Maryammal R, Venkatanarayanan R. Hepatoprotective Activity of "Orthosiphon stamineus" on Liver Damage Caused by Paracetamol in Rats. *Jordan J Biol Sci* 2008;1(3):105-8.
26. Sato Y, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, et al. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood* 2005;106(2):756-63. doi: 10.1182/blood-2005-02-0572
27. George J, Rao KR, Stern R, Chandrakasan G. Dimethylnitrosamine-induced liver injury in rats: the early deposition of collagen. *Toxicology* 2001;156(2-3):129-38. doi: 10.1016/s0300-483x(00)00352-8
28. Yu J, Cao H, Yang J, Pan Q, Ma J, Li J, et al. In vivo hepatic differentiation of mesenchymal stem cells from human umbilical cord blood after transplantation into mice with liver injury. *Biochem Biophys Res Commun* 2012;422(4):539-45. doi: 10.1016/j.bbrc.2012.04.156
29. Chinzei R, Tanaka Y, Shimizu-Saito K, Hara Y, Kakinuma S, Watanabe M, et al. Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes. *Hepatology* 2002;36(1):22-9. doi: 10.1053/jhep.2002.34136
30. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;282(5391):1145-7. doi: 10.1126/science.282.5391.1145
31. Poh YC, Chen J, Hong Y, Yi H, Zhang S, Chen J, et al. Generation of organized germ layers from a single mouse embryonic stem cell. *Nat Commun* 2014;5:4000. doi: 10.1038/ncomms5000
32. Hamazaki T, Iiboshi Y, Oka M, Papst PJ, Meacham AM, Zon LI, et al. Hepatic maturation in differentiating embryonic stem cells in vitro. *FEBS Lett* 2001;497(1):15-9. doi: 10.1016/s0014-5793(01)02423-1
33. Yibo Y, Zhong B, Weiwei Q, Yang S, Yongcong W, Yixuan F, et al. In vitro differentiation of mouse embryonic stem cells into functional hepatocytes by sodium butyrate, hepatocyte growth factor and dexamethasone under chemically defined conditions. *Afr J Biotechnol* 2011;10(46):9493-500.
34. Lotfinegad P, Shamsasenjan K, Movassaghpour A, Majidi J, Baradaran B. Immunomodulatory Nature and Site Specific Affinity of Mesenchymal Stem Cells: a Hope in Cell Therapy. *Adv Pharm Bull* 2014;4(1):5-13. doi: 10.5681/apb.2014.002
35. Mohammadian M, Shamsasenjan K, Lotfi Nezhad P, Talebi M, Jahedi M, Nickkhah H, et al. Mesenchymal Stem Cells: New Aspect in Cell-Based Regenerative Therapy. *Adv Pharm Bull* 2013;3(2):433-7. doi: 10.5681/apb.2013.070
36. Sousa BR, Parreira RC, Fonseca EA, Amaya MJ, Tonelli FM, Lacerda SM, et al. Human adult stem cells from diverse origins: an overview from multiparametric immunophenotyping to clinical applications. *Cytometry A* 2014;85(1):43-77. doi: 10.1002/cyto.a.22402
37. Meier RP, Müller YD, Morel P, Gonelle-Gispert C, Buhler LH. Transplantation of mesenchymal stem cells for the treatment of liver diseases, is there enough evidence? *Stem Cell Res* 2013;11(3):1348-64. doi: 10.1016/j.scr.2013.08.011
38. Ejtehadifar M, Shamsasenjan K, Movassaghpour A, Akbarzadehlaleh P, Dehdilani N, Abbasi P, et al. The Effect of Hypoxia on Mesenchymal Stem Cell Biology. *Adv Pharm Bull* 2015;5:In Press.
39. Xia X, Chen W, Ma T, Xu G, Liu H, Liang C, et al. Mesenchymal stem cells administered after liver transplantation prevent acute graft-versus-host disease in rats. *Liver Transpl* 2012;18(6):696-706. doi: 10.1002/lt.23414
40. Sun S, Chen G, Xu M, Qiao Y, Zheng S. Differentiation and migration of bone marrow mesenchymal stem cells transplanted through the spleen in rats with portal hypertension. *PLoS One* 2013;8(12):e83523. doi: 10.1371/journal.pone.0083523
41. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126(4):663-76. doi: 10.1016/j.cell.2006.07.024
42. Kim JB, Greber B, Arauzo-Bravo MJ, Meyer J, Park KI, Zaehres H, et al. Direct reprogramming of human neural stem cells by OCT4. *Nature* 2009;461(7264):649-53. doi: 10.1038/nature08436
43. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013;499(7459):481-4. doi: 10.1038/nature12271
44. Abbasi P, Shamsasenjan K, Movassaghpour A, Akbarzadeh P, Dehdilani N, Ejtehadifar M. The Effect of Baicalin, A PPAR Activator, on Erythroid

Differentiation of CD133+ Hematopoietic Stem Cells in Umbilical Cord. *Cell J* 2014;17(1):1-17.
45. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. *Blood* 2009;114(16):3367-75. doi: 10.1182/blood-2009-06-225326
46. Liu S, Otsuyama K, Ma Z, Abroun S, Shamsasenjan K, Amin J, et al. Induction of Multilineage Markers in Human Myeloma Cells and Their Down-Regulation by Interleukin 6. *Int J Hematol* 2007;85(1):49-58. doi: 10.1532/ijh97.06132
47. Iqbal MS, Otsuyama K, Shamsasenjan K, Asaoku H, Mahmoud MS, Gondo T, et al. Constitutively lower expressions of CD54 on primary myeloma cells and their different localizations in bone marrow. *Eur J Haematol* 2009;83(4):302-12. doi: 10.1111/j.1600-0609.2009.01284.x