Hanyang Medical Reviews

Hanyang Med Rev 2014;34:145-152
http://dx.doi.org/10.7599/hmr.2014.34.4.145
pISSN 1738-429X eISSN 2234-4446

Liver Stem Cells

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Liver transplantation has been regarded as the definitive curative approach for pathologic liver conditions from the acute stage to the chronic end stage for decades. Recently, translational research has been focused on liver stem cell transplantation, using various cell therapies, due to the potential benefit of natural host liver regeneration. Many studies are ongoing utilizing and evaluating the use of either fetal-liver-derived stem cells or oval cells, however many obstacles still remain. Extensive research identifying and characterizimg stem/progenitor cells for potential application to *in vitro* cell therapy, whereas many questions remain concerning the isolation and identification of adult liver stem cells with adequate capacity for proliferation and the regeneration of injured liver. Recent approaches to liver regeneration include the production of hepatocyte-like cells from other stem cell sources such as mesenchymal stem cells and embryonic stems cells. Another major target for liver regeneration studies include the generation of liver stem cells from induced pluripotent stem cells (IPSC) We review the current data concerning characterization of stem cells and progenitor cells for their capacity to support their potential for re-population and regeneration of normal adult liver from liver damaged due to injury and/or disease.

Key Words: Liver; Stem Cells; Cell Transplantation; Liver Regeneration

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Received 31 August 2014
Revised 26 September 2014
Accepted 10 October 2014

INTRODUCTION

All organs are composed of various kinds of cells that cooperate together to comprise the tissues that provide the structure and function of the organ. Due to normal aging, disease, and environmental insult, the functional capacity and even the structure of the organs are degraded over time. Tissue regeneration is essential for tissue repair and maintenance of organ function in the face of the ongoing insults.

In its normal physiological function, liver is an essential organ providing homeostatic stability for the entire organism, performing functions such as metabolic regulation, synthesis of essential hormones, digestive fluids, blood components and more; detoxification and purification of the blood and other tissue fluids; as well as clearance of senescent and diseased blood cells. It faces constant insult and injury in the course of its normal function, and therefore, unlike other organs, liver demonstrates unique regenerative capacities. For instance, in partial hepatectomy, hepatocyte proliferation can fully regenerate the liver without participation of any stem cells. However, in pathologic conditions like liver cirrhosis, in which liver damage results in the fibrotic formation of scar tissue, the liver fails to regenerate [1]. In such a situation, organ transplantation has been the only treatment available to recover the loss of liver and liver function [1].

There are several significant problems in liver transplantation. First, there are few donors so that the supply cannot match the demand. Second, liver transplantation is a massively invasive surgery with many complications causing significant morbidity and mortality. Third, allograft can cause donor-receiver cross match problems [2].

To overcome these limitations, it has been suggested that transplantation of cellular components such as hepatocytes or hepatic stem cells has the potential to regenerate and cure end stage liver disease [2]. Research has demonstrated that in early development, hepatoblasts which can differentiate into two cell lineages (hepatocyte and cholangiocyte), emerge and function as stem cells [1].

Other studies have suggested that in adult liver, oval cells emerge after massive hepatic injury and proliferate into hepatocytes in the terminal developmental stage (in adult liver). However, it is unclear that this oval cell actually participates in liver repopulation [1].

Human stem cells have the potential to differentiate into many different lineages of specialized cells and function as a reservoir for the maintenance, repair and regeneration of organ tissue. Although adult hepatic stem cells are not clearly identified as yet and their existence is still controversial, recent studies suggest both that there is potential for stem cell differentiation to liver cells from various sources, and also direct reprogramming of either hepatic progenitors or adult hepatocytes to provide therapeutic regeneration of impaired liver. This review will discuss about the types of known stem cells giving rise to liver regeneration and its clinical application so far.

BASIC PROPERTIES OF STEM CELLS

Two stem cell types exist, embryonic stem cells (ESC) and adult stem cells. In the early developmental stage of mammals, the ultimate progenitor cell is the zygote and in early development, mitotic division of the zygote produces the trophoblast, with an inner cell mass consisting of pluripotent cells called embryonic stem cells. Embryonic stem cells undergo further proliferation and differentiation to generate multipotent stem cell lineages that are the source for all the progenitor cells which then replicate and differentiate into adult tissue cells that constitute the organs [3].

It has been demonstrated that many cells of the adult organisim retain the ability of stem cells. These adult stem cells are different from other cells because they can self-renew the population of stem cells through proliferation while maintaining the ability to differentiate to multiple lineages of differentiated cells. A stem cell population existing in an adult organism gives rise to various cells of the organ tissues, continuously supplying specific terminally differentiated cells needed to maintain homeostasis of the body. Usually stem cells have four major characteristics: (1) self-renewal capacity, (2) multipotency, (3) long term tissue regeneration, and (4) serial transplantability [1]. To maintain their function continuously, adult stem cells are able to undergo asymmetric division, wherein one of the daughter cells remains a stem cell, unchanged, to serve as a reservoir for future proliferation and regeneration. The other daughter cell undergoes a transformation and differentiation to become a multipotent progenitor of a specific cell lineage [4]. These progenitor cells, daughters of the original stem cell, often maintain capacity for rapid proliferation, but have a restricted capacity for differentiation along a particular lineagee, and so are described as multipotent instead of pluripotent. The significant differences are that they no longer demonstrate the capacity for continual self-renewal and serial transplantability, and generally participate in short term tissue regeneration [1]. In general, fully differentiated mammalian somatic cells are thought to be non-proliferative. One of the exceptions is the hepatocyte which proliferates to regenerate liver after hepatectomy or severe hepatic injury.

REGENERATION CAPACITY OF LIVER

Liver is a crucial organ of the body, which performs numerous essential functions. It demonstrates a remarkable and unique regenerative ability, compared to other organs, in that it can restore its initial mass even after 2/3 hepatectomy. Liver regeneration of mammals, including humans, occurs by increasing size of the remaining lobe due to proliferation of adult hepatocytes, a process of compensatory hyperplasia of the resected lobe [5]. Compensatory hyperplasia leading to liver regeneration, is unique to other organs, in that differentiated hepatocytes constitute the initial response to injury, and progenitor cells of the liver remain as a reservoir [5]. Normal hepatocytes are in a quiet status exhibiting very slow turnover rate (1-2 times/year) [1]. However, after acute massive damage, the liver regenerates very fast (in 7-10 days) in rodents [5]. In humans, a similar process occurs with a slower rate (-3 month) [1]. Although a physiologic model for liver regeneration has not been determined precisely, it is believed that there are two essential components: (1) physiologically, hepatocytes maintain a slow turnover for homeostatic replacement and (2) under normal conditions, liver parenchymal replacement is conducted almost exclusively by adult hepatocytes with a minor contribution of stem cells [6]. With experimentally induced damage, hepatocytes first proliferate within 1-2 days, followed by other hepatic cell groups such as Stellate cells and Kupffer cells [5]. The involvement of stem cells in natural liver homeostasis is believed to be very limited, but their role needs more precise identification [1].

CELL TYPES FOR LIVER REGENERATION

Liver parenchyma consists of polyploid epithelial cells called hepatocytes [5]. They originate from endodermal hepatoblasts that

can differentiate into two adult cell types; hepatocyte or cholangiocyte [6]. Hepatocytes have numerous functions, for example, the expression of cytochrome P450 for metabolic regulation, the production of albumin, the storage of glycogen, the creation of urea, and the conjugation of bilirubin [7]. More importantly, for our review, they can proliferate nearly indefinitely to restore liver damage under specific circumstances [8]. Consequently, the transplantation of hepatocytes has shown promising results in the repair of massive recurrent liver injury by having a greater potential for transplanted cells to proliferate than host hepatocytes [1]. Surprisingly, hepatocytes have also demonstrated the ability to differentiate into cholangiocytes, which are the biliary tract epithelial cells [9]. From these results, hepatocytes might be considered to fulfill the four criteria for stem cells. However, since in the normal conditions transplanted hepatocytes can not reproduce a normal liver, they do not correspond completely to the stem cell properties [10,11].

However, it is difficult to apply hepatocytes to cell transplantation therapy for two major reasons. The first is that they have not been able to completely restore the normal hepatic functions that have been lost in end stage liver disease. The second reason is that hepatocytes are unstable in short term culture and so are difficult to multiply in *in vitro* culture for transplantation in sufficient quantities to rapidly reconstitute liver function [12]. Many studies seeking to multiply adult hepatocytes are ongoing, including supplying a suitable extracellular matrix for cell attachment that allows hepatocytes maintain functionality while proliferating. Still, the regeneration of adult hepatocytes for transplantation *in vitro* has not been possible and is not promising at this time [2].

1. Oval cells as hepatic progenitor cells

There appear to be quiescent stem cells in the adult liver which replicate massively in response to prolonged liver injury [2], which are called oval cells. The term "oval cells" was coined because of their oval shape described by Farber [13]. They are also called hepatic adult stem cells or hepatic progenitor cells-precursor cells [6]. Originating from portal areas around the canals of Hering, they are induced to proliferate when hepatocyte regeneration is impaired as in chronic liver diseases. Oval cell activation is thought to proceed via four steps: (1) activation, (2) further amplification of oval cells, (3) migration of progenitor cells, and (4) differentiation to either hepatocytes or cholangiocytes [5]. Oval cells may have stem cell traits because they repopulate the liver after transplantation, but the low rate of repopulation limits their clinical application [1]. Also they are hard to isolate and purify and may generate tumors [14]. Still, research with oval cells suggests the possible existence of liver stem cells. The identifcation of self-renewing, stable populations of liver stem cells capable of liver regeneration and creating culture conditions for their generation in transplantable numbers are the task of future studies [1].

2. Fetal hepatocytes (hepatoblasts) as liver stem/progenitor cells

Hepatoblasts are the progenitor cells in the fetal liver [15]. In early gestational stage, proliferating endodermal stem cells start differentiating into hepatic epithelial lineages. These cells are now called hepatoblasts and are able to proliferate massively. Hepatoblasts can divide into two lineages, hepatocytes and cholangiocytes [1]. The difference between hepatoblasts and fetal hepatocytes is that hepatoblasts can reproduce massively *in vitro* and can become both hepatocyte and cholangiocytes *in vivo*. Hepatoblasts have shown promise in regenerating injured liver in animal transplantation studies [16]. However, there are ethical issues concerning availability.

Fetal hepatocytes can also replicate multiple times *in vitro* in contrast to adult hepatocytes [17]. In the study of Sandhu et al. [10] using fetal liver epithelial cells, transplanted liver epithelial cells engrafted to the host liver forming bile canaliculi after partial hepatectomy. And demonstrated three characteristics of stem cells: (1) massive proliferation, (2) bipotency, and (3) *in vivo* long term repopulation [10]. However, these cells have not demonstrated self-renewal or serial transplantability [18]. Functionally, fetal hepatocytes have low ability to eliminate ammonia (49%) and produce urea (1.1%) compared with adult hepatocytes [19]. Together with ethical problems, the possibility of tumor formation [20], and functional immaturity [21] limit the clinical application of these cells.

3. Bone marrow MSC-derived hepatic lineage cells

Comparing the 2 stem cell types referred above, adult stem cells have relative merits over embryonic stem cells in that they have low risk of forming teratoma and can avoid ethical problems. In many studies, mesenchymal stem cells from diverse sources are proved capable to trans-differentiate to hepatocyte-like cells [22]. It is reported that bone marrow MSC cells travelling through the circulatory system will differentiate into hepatocytes after arriving at the liver [1]. In particular, studies using hematopoietic stem cells pre-homed to the bone marrow showed the nearly 100% en-

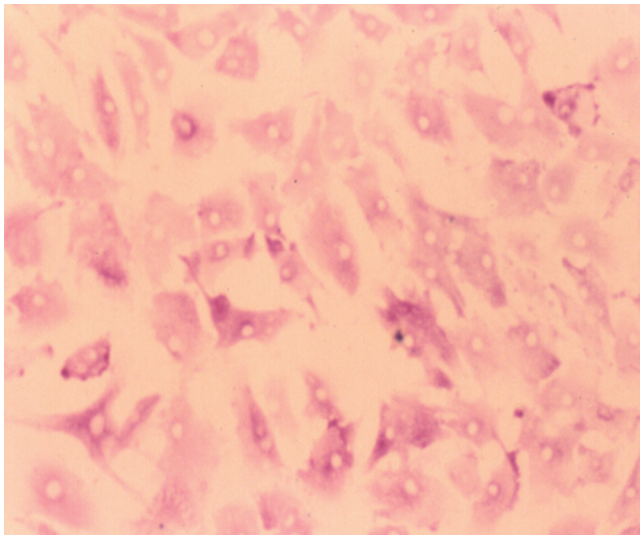

Fig. 1. Hepatocyte-like cells originate from human MSC. Polygonal hepatocyte like cells stained with PAS indicating glycogen storage were found after three weeks of treatment of cytokine cocktail for human mesenchymal stem cells. Picture image, 200 magnification. MSC, Mesenchymal Stem Cells.

graftment efficiency [23]. Many other studies have been done with human cord blood cells [24], adult progenitor cells [25], and mesenchymal stem cells [26] have consistently had problems with a low liver repopulation rate. Mesenchymal stem cells are known to have other roles in liver repair, improving hepatic fibrosis and helping to restore liver function [27]. However, mesenchymal stem cells propensity to trans-differentiate to myofibroblasts is a stubborn obstacle [28]. Liver regeneration studies in more normal, clinical situations are needed [1]. Fig. 1 shows the capacity to generate hepatocyte-like cells from human MSC.

4. Pluripotent stem cell-derived hepatic lineage cells

ESCs of the inner cell mass of the trophoblast are pluripotent, self-renewing primitive cells [29]. Their reproductive integrity has the potential to supply unlimited somatic cells for use in regenerative medicine [30]. Many studies in animal models demonstrate the successful transformation of hepatocyte-like cells from ESCs [31]. Transplanted into the liver, the differentiated hepatic lineage cells showed further differentiation into mature hepatocytes and cholangiocytes [32]. But like other stem cells, the repopulation rate of the transplanted cells is low. Further research is necessary [1]. Ethical controversies, immunological compatibility, and risks of teratoma formation currently preclude the use of ESC in cell therapy [33]. Also, a selection technique for isolationg hepatic differentiated cells from many different cells is required [30].

Induced pluripotent stem cells (iPSC) have been generated from adult epidermal fibroblasts by using four viral vectors (KLF4, Oct4, Sox2 and Myc) [34,35]. iPSCs share the characteristics of ESCs in that they are both pluripotent and self-renewable [34,35]. It is hoped that using iPSC's generated from the patient's own adult fibroblasts will provide a solution to the immunologic and ethical problems that accompany the use of embryonic stem cells. Many studies have successfully generated hepatocyte-like cells from iPSCs [36]. Researchers have also generated iPSC from hepatic lineage cells at different stages of liver development and compared their capacity tp re-differentiate into hepatocyte-like cells. As found by those studies, iPSCs seem to have stage-specific memory from the donor cells that affects the capacity to differentiate into hepatocyte-like cells [37]. This legacy from the donor cells may be the cause of a low capacity for hepatic differentiation from stem cells that needs to be overcome. iPSC can supply sufficient amounts of hepatocyte-like cells needed in autologous transplantation [38], but in rodent study, the engraftment and the repopulation of the transplanted hepatocytes was not satisfactory [6]. Improving the engrafting efficiency and liver repopulation rate of the transplanted cells are the obstacles preventing clinical use at this time [38]. Also, the risk of using viral vectors, somatic cells' epigenetic transcriptional memory and possibility of teratoma formation are further limiting factors for using iPSC in clinical applications [34]. In spite of these limitations, iPSCs are readily available for many studies *in vitro* and further studies may find ways to overcome these problems [2]. Other recent studies have demonstrated the possibility to produce hepatocyte-like cells by direct lineage reprogramming of adult fibroblasts from both human and mouse without a previous pluripotent state through special viral vectors [39]. Fig. 2 shows hepatocyte-like cells originating from human pluripotent stem cells.

CLINICAL APPLICATION OF LIVER CELLS DERIVED FROM VARIOUS SOURCE

In congenital liver metabolic diseases and acute liver failure, hepatocyte transplantation has been investigated as treatment [40]. Allogenic hepatocytes can be directly induced to position in the liver and repopulate it, though, again, low restoration rate and high cellular loss limit the therapy [41]. Successful studies using hepatocytes in transplantation increased the anticipation to apply them in clinical liver diseases [42]. Hepatocyte transplantation showed

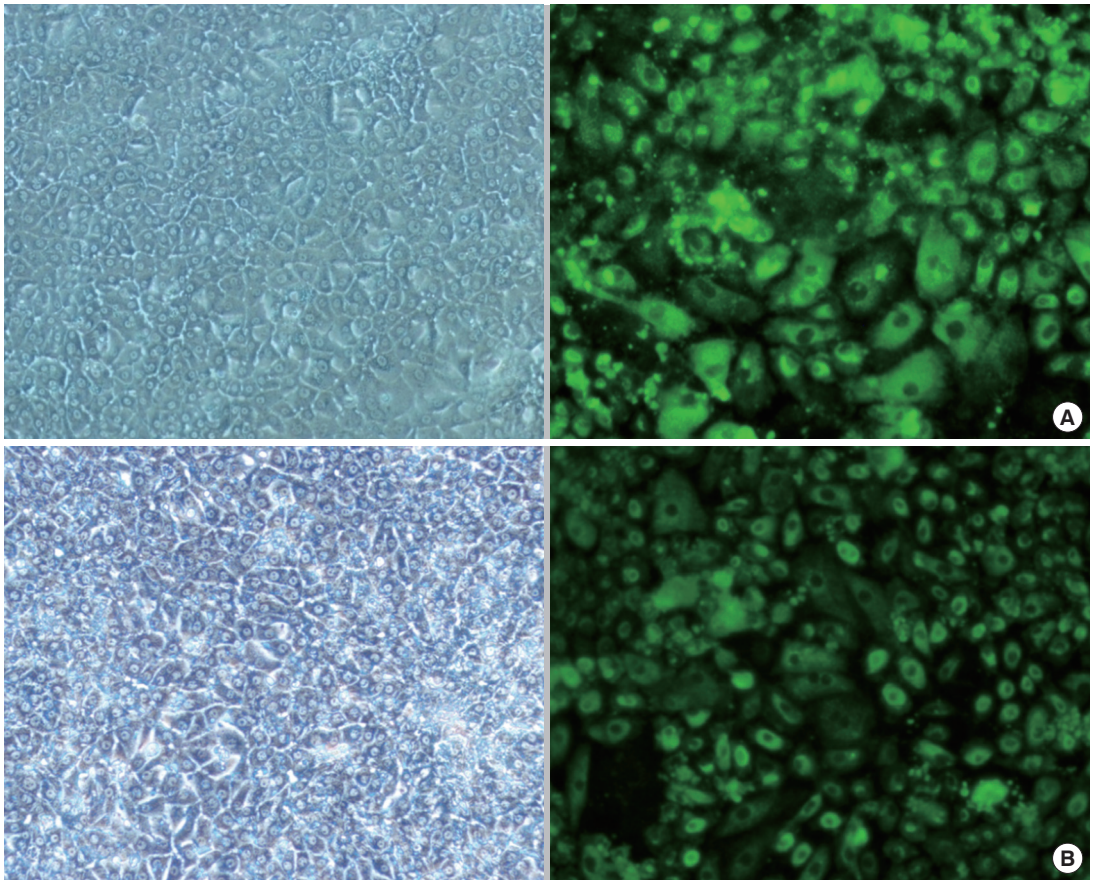

Fig. 2. Hepatocyte-like cells originate from human pluripotent stem cell. (A) shows morphology of human embryonic stem cell derived hepatocyte like cells (left panel) and expression of albumin as one of the major markers of hepatocytes by immunostaining (right panel). Picture image, 200 magnification. (B) presents hepatocyte like cells (left panel) and albumin expression by immunostaining (right panel) derived from human IPSC after embryoid body formation, endoderm enrichment, and hepatocyte specification protocol for 5 weeks culture period. Picture image, 200 magnification.

successful outcome in some metabolic liver diseases [43], but there was only a limited expansion of the transplanted cells. Table 1 summarizes many cell types and their characteristics [2].

FUTURE CHALLENGE (3D PRINTING, DECELLULARIZED MATRIX)

Many researchers are interested in liver stem cells, because they may open the door to new curative management of acute or chronic liver diseases. Liver stem cells are believed to reside in the liver stroma, and can proliferate and differentiate giving arise to liver when hepatocytes proliferation is suppressed. Because of their ability to regenerate functional liver tissue, attempts to transplant the selected liver stem cells are regarded as a hopeful method for future treatment. The clinical outcome of many trials are now being evaluated. Until now, one limitation has been insufficient liver repopulation rate from transplanted cells *in vivo*. Also, *in vitro* attempts to multiply hepatocytes grown in culture, have resulted in the degeneration of hepatic function and loss of hepatocytic characteristics. These are persisting problems to be solved whether the transplanted cells are primary isolated or differentiated from other stem cells [2].

As an effort to improve the latter problem of *in vitro* generation, creating better culture conditions that are more similar to the human liver is currently being investigated. Regulating factors such as the interactions of cell-cell communication or signals from the extracellular-matrix, are being incorporated as components of the culturing media [44,45]. Constructing a model in which hepatocytes can preserve their functional structural hepatocyte properties would be an amazing development in liver regeneration studies [2]. With the development of 3-dimensional printing technology, *in vitro* culture of hepatocytes in a 3-D tissue culture matrix

Table 1. Cell types studied in liver regeneration and their characteristics

Cell	Origin	Properties	Problems
Regenerating hepatocyte [1,2,5]	Adult liver	High host compatibility	Poor proliferation *in vitro* culture Low availability
Oval cells [1,2]	Portal areas of adult liver	Bipotency	Hard to isolate Low availability Possible tumor formation
Hepatoblast [2]	Early gestational stage fetal liver	Massive proliferation *in vitro* culture Bipotency *in vivo*	Ethical concerns Low availability
Fetal hepatocyte [2]	Fetal liver	Easy to isolate Multiple proliferation *in vitro* culture	Functional immaturity Low availability Ethical concerns Possible tumor formation
Mesenchymal stem cells [1,2,5]	Adult tissue (e.g. bone marrow etc.)	High availability No tumor formation No ethical concerns	Transdifferentiation to myofibroblasts Low hepatic differentiation
Embryonic stem cells [2]	Embryo	High availability Multiple proliferation *in vitro* culture High hepatic differentiation	Ethical concerns Possible tumor formation
Induced pluripotent stem cells [2,5]	Somatic cells (e.g. fibroblast etc.)	No ethical concerns High availability Multiple proliferation *in vitro* culture High hepatic differentiation	Possible tumor formation

mimicking the normal liver may allow a more realistic culture environment [46]. Also studies using decellularized tissue matrix from liver that keeps intact the microvascular and matrix formations, may furnish the necessary signals to maintain hepatocyte function during *in vitro* multiplication. Continued advances in these kinds of studies provides hope proceeding toward successful liver transplantation [47].

CONCLUSION AND PERSPECTIVE

The main concerns in liver regeneration are finding appropriate cell lines for transplantation. Of the four major properties of stem cells, liver repopulation rate and functional maturity of derived hepatocyte are the focus of continuing efforts [2]. There have been numerous studies to identify stem cells in adult liver including research of adult hepatocytes, oval cells, and mesenchymal stem cells. Efforts to differentiate hepatocytes from pluripotent stem cells have also been made. Embryonic stem cells and induced pluripotent stem cells are the target of these trials. Many studies show promising outcomes and possibility, but still we have a long way to go. Establishing a protocol to differentiate functionally grown hepatocytes in sufficient numbers for regenerating liver function is a key future task, and to do so, better understanding of cellular signal transduction, cell to cell interactions, and cell to matrix interactions are needed [48]. Further research about liver stem cells has direct application to other purposes, such as investigations of normal hepatocyte physiology, models of disease pathology, drug screening and efficacy testing, and so on, providing ample reason to continue our research.

ACKNOWLEDGMENTS

This study was supported by the project titled "Multidisciplinary assessment in radiation injury and application of regenerative medicine (50586-2014)" by the Ministry of Science, ICT and Future Planning (MSIP), Korean Government.

REFERENCES

1. Oertel M, Shafritz DA. Stem cells, cell transplantation and liver repopulation. Biochim Biophys Acta 2008;1782:61-74.
2. Palakkan AA, Hay DC, Anil Kumar PR, Kumary TV, Ross JA. Liver tissue engineering and cell sources: issues and challenges. Liver Int 2013;33:666-76.
3. 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;282:1145-7.
4. Marshman E, Booth C, Potten CS. The intestinal epithelial stem cell. Bioessays 2002;24:91-8.
5. Russo FP, Parola M. Stem cells in liver failure. Best Pract Res Clin Gastroenterol 2012;26:35-45.

6. Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. Gastroenterology 2009;137:466-81.
7. Lemaigre FP. Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies. Gastroenterology 2009; 137:62-79.
8. Dan YY, Yeoh GC. Liver stem cells: a scientific and clinical perspective. J Gastroenterol Hepatol 2008;23:687-98.
9. Yamamoto H, Quinn G, Asari A, Yamanokuchi H, Teratani T, Terada M, et al. Differentiation of embryonic stem cells into hepatocytes: biological functions and therapeutic application. Hepatology 2003;37:983-93.
10. Sandhu JS, Petkov PM, Dabeva MD, Shafritz DA. Stem cell properties and repopulation of the rat liver by fetal liver epithelial progenitor cells. Am J Pathol 2001;159:1323-34.
11. Rajvanshi P, Kerr A, Bhargava KK, Burk RD, Gupta S. Studies of liver repopulation using the dipeptidyl peptidase IV-deficient rat and other rodent recipients: cell size and structure relationships regulate capacity for increased transplanted hepatocyte mass in the liver lobule. Hepatology 1996;23:482-96.
12. Walldorf J, Aurich H, Cai H, Runge D, Christ B, Strom S, et al. Expanding hepatocytes in vitro before cell transplantation: donor age-dependent proliferative capacity of cultured human hepatocytes. Scand J Gastroenterol 2004;39:584-93.
13. Farber E. Similarities in the Sequence of Early Histological Changes Induced in the Liver of the Rat by Ethionine, 2-Acetylamino-fluorene, and 3′-Methyl-4dimethylaminoazobenzene. Cancer Res 1956;16:142.
14. Dumble ML, Croager EJ, Yeoh GC, Quail EA. Generation and characterization of p53 null transformed hepatic progenitor cells: oval cells give rise to hepatocellular carcinoma. Carcinogenesis 2002;23:435-45.
15. Mahieu-Caputo D, Allain JE, Branger J, Coulomb A, Delgado JP, Andreoletti M, et al. Repopulation of athymic mouse liver by cryopreserved early human fetal hepatoblasts. Hum Gene Ther 2004;15:1219-28.
16. Malhi H, Irani AN, Gagandeep S, Gupta S. Isolation of human progenitor liver epithelial cells with extensive replication capacity and differentiation into mature hepatocytes. J Cell Sci 2002;115:2679-88.
17. Wege H, Le HT, Chui MS, Liu L, Wu J, Giri R, et al. Telomerase reconstitution immortalizes human fetal hepatocytes without disrupting their differentiation potential. Gastroenterology 2003;124:432-44.
18. Oertel M, Menthena A, Dabeva MD, Shafritz DA. Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. Gastroenterology 2006;130:507-20.
19. Poyck PP, van Wijk AC, van der Hoeven TV, de Waart DR, Chamuleau RA, van Gulik TM, et al. Evaluation of a new immortalized human fetal liver cell line (cBAL111) for application in bioartificial liver. J Hepatol 2008; 48:266-75.
20. Diekmann S, Bader A, Schmitmeier S. Present and Future Developments in Hepatic Tissue Engineering for Liver Support Systems: state of the art and future developments of hepatic cell culture techniques for the use in liver support systems. Cytotechnology 2006;50:163-79.
21. Poyck PP, Hoekstra R, van Wijk AC, Attanasio C, Calise F, Chamuleau RA, et al. Functional and morphological comparison of three primary liver cell types cultured in the AMC bioartificial liver. Liver Transpl 2007; 13:589-98.
22. Saulnier N, Lattanzi W, Puglisi MA, Pani G, Barba M, Piscaglia AC, et al. Mesenchymal stromal cells multipotency and plasticity: induction toward the hepatic lineage. Eur Rev Med Pharmacol Sci 2009;13:71-8.
23. Jang YY, Collector MI, Baylin SB, Diehl AM, Sharkis SJ. Hematopoietic stem cells convert into liver cells within days without fusion. Nature cell biology 2004;6:532-9.
24. Danet GH, Luongo JL, Butler G, Lu MM, Tenner AJ, Simon MC, et al. C1qRp defines a new human stem cell population with hematopoietic and hepatic potential. Proc Natl Acad Sci U S A 2002;99:10441-5.
25. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-9.
26. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 2004;103:1669-75.
27. 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;40:1304-11.
28. Di Bonzo LV, Ferrero I, Cravanzola C, Mareschi K, Rustichell D, Novo E, et al. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. Gut 2008;57:223-31.
29. 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;282:1145-7.
30. Dalgetty DM, Medine CN, Iredale JP, Hay DC. Progress and future challenges in stem cell-derived liver technologies. Am J Physiol Gastrointest Liver Physiol 2009;297:G241-8.
31. Woo DH, Kim SK, Lim HJ, Heo J, Park HS, Kang GY, et al. Direct and indirect contribution of human embryonic stem cell-derived hepatocyte-like cells to liver repair in mice. Gastroenterology 2012;142:602-11.
32. Heo J, Factor VM, Uren T, Takahama Y, Lee JS, Major M, et al. Hepatic precursors derived from murine embryonic stem cells contribute to regeneration of injured liver. Hepatology 2006;44:1478-86.
33. Payne CM, Samuel K, Pryde A, King J, Brownstein D, Schrader J, et al. Persistence of functional hepatocyte-like cells in immune-compromised mice. Liver Int 2011;31:254-62.
34. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-72.
35. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126: 663-76.
36. Liu H, Kim Y, Sharkis S, Marchionni L, Jang YY. In vivo liver regeneration potential of human induced pluripotent stem cells from diverse origins. Sci Transl Med 2011;3:82ra39.
37. Lee SB, Seo D, Choi D, Park KY, Holczbauer A, Marquardt JU, et al. Contribution of hepatic lineage stage-specific donor memory to the differential potential of induced mouse pluripotent stem cells. Stem Cells 2012; 30:997-1007.
38. Forbes SJ, Alison MR. Regenerative medicine. Knocking on the door to successful hepatocyte transplantation. Nat Rev Gastroenterol Hepatol 2014;11:277-8.
39. Du Y, Wang J, Jia J, Song N, Xiang C, Xu J, et al. Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. Cell stem cell 2014;14:394-403.
40. Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. Transplantation 2003;76:735-8.
41. Han B, Lu Y, Meng B, Qu B. Cellular loss after allogenic hepatocyte transplantation. Transplantation 2009;87:1-5.
42. Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. Transplantation 2006;82:441-9.
43. Muraca M, Gerunda G, Neri D, Vilei M-T, Granato A, Feltracco P, et al. Hepatocyte transplantation as a treatment for glycogen storage disease

type 1a. The Lancet 2002;359:317-8.
44. Matsumura T, Kauffman FC, Meren H, Thurman RG. O2 uptake in periportal and pericentral regions of liver lobule in perfused liver. Am J Physiol 1986;250:G800-5.
45. Malik R, Selden C, Hodgson H. The role of non-parenchymal cells in liver growth. Semin Cell Dev Biol 2002;13:425-31.
46. Shan J, Stevens KR, Trehan K, Underhill GH, Chen AA, Bhatia SN. Hepatic tissue engineering. In Monga SPS, ed. Molecular Pathology of Liver Diseases: Springer; 2011:321-42.
47. Uygun BE, Soto-Gutierrez A, Yagi H, Izamis M-L, Guzzardi MA, Shulman C, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nature medicine 2010;16:814-20.
48. Hay DC, Pernagallo S, Diaz-Mochon JJ, Medine CN, Greenhough S, Hannoun Z, et al. Unbiased screening of polymer libraries to define novel substrates for functional hepatocytes with inducible drug metabolism. Stem Cell Res 2011;6:92-102.