Use of Stem Cells for Liver Diseases—Current Scenario

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ABSTRACT

End-stage liver disease and liver failure are major health problems worldwide leading to high mortality and morbidity and high healthcare costs. Currently, orthotropic liver transplantation is the only effective treatment available to the patients of end-stage liver disease. However, a serious shortage of liver donors, high cost, and risk of organ rejection are the major obstacles to liver transplantation. Because of the ability of stem cells for differentiation into any tissue type, they have huge potential in therapy of various end-stage or degenerative diseases and traumatic injuries. Stem cell therapy has the potential to provide a valuable adjunct and alternative to liver transplantation and has immense potential in the management of end stage liver disease and liver failure. Stem cell therapy can be mediated by either a direct contribution to the functional hepatocyte population with embryonic, induced pluripotent, or adult stem cells or by promotion of endogenous regenerative processes with bone marrow-derived stem cells. Initial translational studies have been encouraging and have suggested improved liver function in advanced chronic liver disease and enhanced liver regeneration after portal vein embolization and partial hepatic resection. Stem cells infusion in cirrhotic patients has improved liver parameters and could form a viable bridge to transplantation. The present review summarizes basic of stem cell biology relevant to clinicians and an update on recent advances on the management of liver diseases using stem cells.

Keywords: Hepatocyte transplantation, liver transplantation, stem cell
Received: 01.06.2011; *Accepted:* 04.06.2011
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Abbreviations: AFP: alpha (α)-fetoprotein; BM: bone marrow; EPCAM: epithelial cell adhesion molecule; ES: embryonic stem; FSCs: fetal stem cells; hAECs: human amniotic epithelial cells; HPC: hepatic progenitor cells; HSC: hematopoietic stem cells; ICAM: intercellular adhesion molecule; iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stem cells; NCAM: neural cell adhesion molecule; UCB: umbilical cord blood
doi: 10.1016/S0973-6883(11)60114-X

INTRODUCTION

End-stage liver disease and liver failure are major health problems worldwide leading to high mortality and morbidity, and high healthcare costs. Alcohol, hepatitis viruses, diabetes, and obesity are important causes of end-stage liver disease and liver failure. Currently, orthotropic liver transplantation is the only effective treatment available to the patients of end-stage liver disease.[1] However, a serious shortage of liver donors, high cost, and risk of organ rejection are the major obstacles to liver transplantation. Therefore, alternative methods, with the potential to substitute for liver transplantation or bridge the patients awaiting transplantation, are urgently required. Stem cell therapy has the potential to provide a valuable adjunct and alternative to liver transplantation and has immense potential in the management of end-stage liver disease and liver failure.[2,3] The present review summarizes basic of stem cell biology relevant to clinicians and an update on recent advances on the management of liver diseases using stem cells.

WHAT ARE STEM CELLS?

Stem cells are defined as undifferentiated cells capable of proliferation, self-maintenance, and differentiation into functional progeny with flexibility or plasticity in these options. They play a crucial role in the development and regeneration. Stem cells are found at all developmental stages, from embryonic stem (ES) cells that differentiate into all cell types found in the human body to adult stem cells that are responsible for tissue regeneration.[4] Adult stem cells have been found in the bone marrow (BM), peripheral blood, umbilical cord, liver, skin, gastrointestinal tract, pancreas, cornea, and retina of the eye, and the dental pulp of teeth.[5]

Stem cells are characterized by two special properties: self-renewal and potency.[6] Self-renewal implies the ability to go through numerous cycles of cell division while maintaining the undifferentiated state. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or

a brain cell. Potency specifies the potential of the stem cells to differentiate into different cell types. They have the remarkable potential to develop into many different cell types in the body during early life and growth. In addition, in many tissues they serve as internal repair system, dividing essentially without limit, to replenish other cells. Stem cells may be divided into 5 different groups according to their potential for differentiation: totipotent, pluripotent, multipotent, oligopotent, or unipotent (Table 1).

There are important differences between adult stem cells and fetal stem cells—adult stem cells are small in number, are already partially committed to a lineage, and their main function is to maintain tissue homeostasis as they are. In contrast, fetal stem cells are higher in number, with expansion potential and differentiation abilities to form a complete organism.[4]

Stem cells and undifferentiated progenitor cells play an important role in both tissue homeostasis and tissue regeneration. The ability of the human body to self-repair and replace the cells and tissues of some organs is often evident (as in the case of the skin), and is mediated by adult stem cells. Adult stem cells are typically quiescent or pass slowly through the cell cycle, but they can be activated in response to cell loss and wounding. To a great extent the stem cells migration towards injured tissue is mediated by damaged tissue factors. The tissue factors lead to gene activation and other functional reactions of stem cells such as movement to a specific district, differentiation into a particular cell type or resting in specific niches and eventually alteration of the gene expression patterns.[5] Therefore, stem cells can be induced to become cells with specialized functions such as liver cells, cardiac myocytes, neurons, and insulin-producing pancreatic β-cells.

USE OF STEM CELLS IN REGENERATIVE MEDICINE

Current research is on to understand and to indentify the cellular cross-talk and molecular processes that involves the stem cells. The aim of the scientific research is to be able to reproduce the cellular cross-talk and molecular processes of stem cells in the laboratory and apply the results obtained in the treatment of degenerative pathologies, i.e. neurological disorder such as Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple sclerosis, musculoskeletal disorder, diabetes, eye disorder, autoimmune diseases, liver cirrhosis, lung disease and cancer.[7]

Because of the ability of stem cells for differentiation into any tissue type, they have huge potential in therapy of various end-stage or degenerative diseases and traumatic injuries. Therefore, stem cells field of research, with the name of 'regenerative medicine' has emerged rapidly in recent years with leading to great interest among clinicians and scientists.[8] Therefore, stem cell therapy has the potential to help tissue regeneration while providing minimally invasive procedures and few complications.

USE OF STEM CELLS IN LIVER DISEASES

In liver disease scenario, stem cell therapy sounds particularly attractive for its potential to support tissue regeneration. Stem cell therapy can be mediated by either a direct contribution to the functional hepatocyte population with embryonic, induced pluripotent, or adult stem cells or by promotion of endogenous regenerative processes with BM-derived stem cells. Preclinical studies have demonstrated a range of endogenous repair processes that can be exploited through stem cell therapy. Initial translational

Table 1 Potency of stem cells.

Name	Potency	Example
Totipotent	Can differentiate into embryonic and extra-embryonic cell types. Such cells can construct a complete, viable, organism. The zygote and the earliest embryonic stem cells are totipotent, from which the trophoblast and all three germ layers (endoderm, mesoderm, and ectoderm) necessary for future development of an organism are derived.	Zygote and the earliest embryonic stem cells.
Pluripotent	Can differentiate into all cells (all three germ layers). But, they lack the potential to form into extra-embryonic tissue. Embryonic stem cells are considered pluripotent instead of totipotent because they do not have the ability to become part of the extra-embryonic membranes or the placenta.	Embryonic stem cells.
Multipotent	Can differentiate cells of different lineages within a single germ layer. They constitute the adult stem cell population in later life.	Adult hematopoietic stem cells that can become red and white blood cells and platelets.
Oligopotent	Can differentiate into cells of only a few lineages.	Oval stem cells of liver that can form into hepatocytes and cholangiocytes.
Unipotent	Can produce only one cell type but have the property of self-renewal required to be labeled a stem cell.	Muscle stem cells and skin stem cells.

studies have been encouraging and have suggested improved liver function in advanced chronic liver disease and enhanced liver regeneration after portal vein embolization and partial hepatic resection. Stem cells infusion in cirrhotic patients has improved liver parameters, such as decrease in transaminase and bilirubin, and increase in albumin.[9,10] After stem cell infusion, proliferation indexes, such as α-fetoprotein (AFP) and proliferating cell nuclear antigen have significantly increased, suggesting that stem cells can enhance and accelerate hepatic regeneration.[11]

Commonly, for therapeutic and research purposes, stem cells come from following main sources[7]:

- Adult stem cells
- Induced pluripotent stem cells
- Embryonic stem cells
- Fetal stem cells
- Umbilical cord stem cells

USE OF ADULT STEM CELLS

Adult or somatic stem cells are found among differentiated cells in tissues or organs, throughout the body, that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissues or organs. These stem cells have been found in brain, BM, blood, blood vessels, skeletal muscles, skin, and the liver. They remain in a quiescent or non-dividing state for years until activated by disease or tissue injury. The primary roles of adult stem cells are to maintain and repair the tissue in which they are found. They can divide or self-renew indefinitely, enabling them to generate a range of cell types from the originating organ, or they can even regenerate the entire original organ.

Unlike ES cells, which are defined by their origin (cells from the pre-implantation stage embryo), the origin of adult stem cells in some mature tissues is still under investigation. It is generally thought that adult stem cells are limited in their ability to differentiate based on their tissue of origin, but there is some evidence to suggest that they can differentiate to become other cell types.

Hematopoietic stem cells, mesenchymal stem cells, and hepatic stem cells are the most common adult stem cells being used in research and clinical practice for liver diseases.

Hematopoietic Stem Cells

Hematopoietic stem cells (HSC) have been the center of intensive research and they are probably the most studied and best-understood stem cell within the body.[12] The identification and isolation of HSCs is possible with immune capture of CD34, a surface protein that distinguishes stem cells from other hematopoietic cells.[13] Not being at the top of the stem cell hierarchy, HSCs were initially thought to possess a restricted differentiation potential and therefore to be able to generate only cells of the hematopoietic system. However, BM stem cells have been shown to contribute to parenchymal liver cell populations, and although this may not be functionally significant, it has sparked interest in the field of autologous stem cell infusion as a possible treatment for cirrhosis.[14]

Both rodent and human HSCs have been induced to differentiate into hepatocytes in vitro. Most of the protocols to induce CD34+ HSCs differentiation into hepatocytes employed growing media conditioned with growth factors and mitogens (e.g. hepatocyte growth factors, fibroblast growth factor, oncostatin M and culture layers specific for hepatocyte growth, like matrigel). To reproduce the pathophysiological conditions of liver injury, some studies also employed cholestatic serum or co-culture with chemically damaged liver tissue.[15] Although these studies showed some HSC 'transdifferentiation' into hepatocytes, the reported percentage of hepatocytes derived from HSCs did not exceed 5%.

Differentiation of hepatocyte like cells from umbilical cord blood derived HSC were also observed to show high expression of genes related to hepatocytes. The quantities of albumin and AFP at day 0 were low and upon differentiation the cells were able to produce albumin and AFP at high levels. This strategy can also be used as cell replacement therapy for liver diseases.[16]

Recent animal studies conducted showed that HSC transplantation can lead to regression of liver fibrosis. Although, there have been many animal studies on BM-derived HSC administration, clinical studies on humans are few and involve small number of patients (Table 2). They can be divided into studies performed in patients with and without an underlying chronic liver disease. In patients with liver malignancies arisen on a 'healthy' liver, the intraportal injection of CD133+ BM stem cells (a subpopulation of stem cells with both hematopoietic and endothelial progenitor characteristics) improved liver regeneration after extensive resection and segmental portal vein embolization.[11,22] Injection of CD34+ HSCs directly into the liver vascular system of patients with cirrhosis,[19] and infusion of autologous BM through a peripheral vein[18] was also studied. These studies have documented a slight improvement in liver function and clinical conditions, however, these studies have a limitation of small number of patients and lack of a control group. Reports of stem cell transplantation and phase 1 trials of BM transplantation in humans for liver diseases are exciting but require more robust verification.[32]

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) originally derived from BM, however have been isolated from other tissues also, such as adipose tissue, periosteum, synovial membrane, synovial fluid muscle, dermis, deciduous teeth, pericytes, trabecular bone, infrapatellar fat pad, and articular cartilage.[33,34] MSCs are fusiform, fibroblast-like, and in their

Table 2 Human studies on use of adult stem cells for treatment of liver diseases.

Author	Year	Number of patients	Treatment	Results
Am Esch et al[11]	2005	3 vs 3 controls	Infusion of autologous bone marrow-derived CD133+ cells in patients who were undergoing partial hepatectomy for liver cancer, to expand a remnant segment of liver.	Patients receiving the infusion of bone marrow cells (which likely contained both hematopoietic stem cells and epithelial cells) exhibited 2.5-fold higher mean proliferation rates when compared with a group of three consecutive patients who did not receive bone marrow cells.
Yannaki et al[17]	2006	2	Patients with end-stage liver disease treated with autologous mobilized peripheral blood hematopoietic stem cells.	Lasting amelioration in the clinical course of the disease during the 30 months of follow-up.
Terai et al[18]	2006	9	Liver cirrhosis patients underwent autologous bone marrow cell infusion via the peripheral vein.	Significant improvements in serum albumin levels and total protein at 24 weeks. Significantly improved Child-Pugh scores at 4 and 24 weeks.
Gordon et al[19]	2006 2008	5	Infusion of autologous CD34+ cells via either the portal vein or the hepatic artery in patients suffering from cirrhosis.	Three of the 5 patients showed improvement in serum bilirubin and 4 of 5 in serum albumin. Long-term results published subsequently showing that 4 of 5 patients maintained improved clinical parameters for roughly 12 months post-infusion.
Mohamadnejad et al[20]	2007	4	Patients with decompensated cirrhosis were infused through the hepatic artery 3–10 million CD34+ cells which were isolated from their bone marrow.	Development of hepatorenal syndrome and death of 1 patient, with the remaining 3 patients showing no evidence of significant clinical improvement.
Mohamadnejad et al[21]	2007	4	Patients with decompensated cirrhosis were infused bone marrow derived mesenchymal stem cells through a peripheral vein.	Procedure well-tolerated. Definite therapeutic effect in 2 of 4 patients.
Fürst et al[22]	2007	6 vs 7 controls	Infusion of autologous bone marrow derived CD133+ cells with portal vein embolization in patients who were undergoing extended right hepatectomy for liver cancer, to expand a remnant segment of liver.	The CD133+ administration substantially increased hepatic regeneration compared with portal vein embolization alone.
Lyra et al[10]	2007	10	Patients of Child-Pugh B and C infused into hepatic artery autologous bone marrow mononuclear cells.	Improved liver function seen up to 4 months from infusion.
Khan et al[23]	2008	4	Patients with chronic liver disease were injected autologous bone marrow stem cells (CD34+) into hepatic artery.	Patients showed improvements in serum albumin, bilirubin and ALT after 1 month from the cell infusion.
Pai et al[9]	2008	9	Patients with alcoholic liver cirrhosis received autologous CD34+ cells into the hepatic artery.	Significant decreases in serum bilirubin 4, 8, and 12 week post-infusion. Transaminases showed improvement through the study period and at 1 week post-infusion. The Child-Pugh score improved in 7 out of 9 patients, while 5 patients had improvement in ascites on imaging.
Kharaziha et al[24]	2009	8	Patients with end stage liver disease injected with autologous mesenchymal stem cells into peripheral or portal vein.	Improved liver function, model for end-stage liver disease score, and creatinine, up to 24 weeks.
Ismail et al[25]	2010	10 vs 10 controls	Patients having liver cirrhosis with hepatocellular carcinoma randomly received autologous stem cells or placebo followed by liver resection.	Group receiving pre-operative stem cell therapy had shown a significant improvement in all parameters of liver function and had no postoperative complications.
Kim et al[26]	2010	10	Patients with advanced liver cirrhosis due to hepatitis B underwent autologous bone marrow infusion.	Serum albumin, hemoglobin, quality of life, liver volume, and Child-Pugh scores improved. Clinical improvement was sustained for >6 months, histological changes in the liver returned to baseline by 6 months.

Table 2 (Continued)

Author	Year	Number of patients	Treatment	Results
Lyra et al[27]	2010	15 vs 15 controls	Liver cirrhosis patients on transplant waiting list were infused into the hepatic artery autologous mononuclear-enriched bone marrow stem cells.	Improved liver function in the first 90 days.
Salama et al[28]	2010	48	Patients with end stage liver disease were infused autologous hematopoietic stem cells into hepatic artery or portal vein.	Borderline significant improvements in the serum albumin levels at the end of the 6 month study.
Salama et al[29]	2010	90 vs 50 controls	Autologous CD34+ and CD133+ stem cell infusion in the portal vein.	Near normalization of liver enzymes and improvement in synthetic function were observed in 54.5% of treated patients; 13.6% of the patients showed stable states in the infused group.
Saito et al[30]	2011	5 vs 5 controls	Patients with alcoholic liver cirrhosis received autologous bone marrow cells intravenously.	Liver function and Child-Pugh score improved. The degree of fibrosis decreased in 4 of 5 patients.
Nikeghbalian et al[31]	2011	6	Patients with end-stage liver disease subjected to intraportal administration of autologous bone marrow-derived CD133+ or mononuclear cells.	There were no adverse effects during the short- and long-term follow-up. No significant alterations of liver function parameters, liver enzymes, serum albumin, creatinine, serum bilirubin and/or liver volume after transplantation of both types of autologous cells in these patients.

initial growth in vitro they form colonies. Stem cell characteristics of MSCs are based on their ability to differentiate into multiple cell types including osteoblasts, chondrocytes, endothelial cells, hepatic cells and even neuron-like cells.[35,36] MSCs are of great therapeutic potential due to their capacity of self-renewal and multilineage differentiation. Recent studies have shown that MSC have far greater differentiative abilities than previously thought. They, in fact, appear to be capable of giving rise to cells of all three germinal layers,[37] including albumin-producing hepatocyte-like cells in vitro and in vivo.[38–41]

There is no specific marker or combination of markers that specifically identifies MSCs. These cells have been defined by using a combination of phenotypic markers and functional properties. Controversy still exists over the in-vivo phenotype of MSC; however, ex-vivo expanded MSCs do not express the hematopoietic markers CD14, CD31, CD34, CD45, or CD133. Along with these phenotypic characteristics, MSCs secrete various anti-apoptotic, immunomodulatory, angiogenic, antiscarring, and chemoattractant bioactive molecules, providing a basis for their use as tools to create local regenerative environments in vivo.[42] Experimental and clinical data also demonstrated an immunoregulatory function of BM-derived MSC, which may contribute to the reduction of the incidence of graft versus host disease following hematopoietic stem cell transplantation.

Mesenchymal stem cells can be a rescue for liver diseases as they differentiate to hepatocytes, stimulate the regeneration of endogenous parenchymal cells, and enhance fibrous matrix degradation.[43] The transplantation of autologous BM-derived mesenchymal stem cells holds great potential for treating hepatic cirrhosis. Umbilical cord blood-derived mesenchymal stem cells were also effective in improving insulin resistance in CCl4-induced liver cirrhosis and thereby contributing to glucose homeostasis.[44] Transplantation of MSCs alone and along with baicalin was able to promote partial recovery of liver function, suppression of liver inflammation, and had the best therapeutic effect for hepatic fibrosis.[45]

In spite of great potential of MSCs in therapy of liver diseases, only a few clinical studies on the administration of MSCs to cirrhotic patients have been published up to now (Table 2). Although preliminary results seem to be encouraging, the number of treated patients is too small and the study design is not completely appropriate to demonstrate safety and efficacy of MSC therapy in liver cirrhosis. Well designed, randomized, controlled studies are needed to confirm preliminary results and eventually clear doubts.

Hepatic Stem Cells

Hepatocytes are the main cells of the liver. Normally, these cells are non-proliferative, however, in response to cell loss they enter the cell cycle and undergo rapid self-renewal to regenerate liver tissue. Some of this expansion in cell numbers is the result of clonal expansion, as shown by studies of hepatocyte transplantation.[46] The hepatocyte can therefore be regarded as a functional stem cell for the

liver. More severe damage or blockage of normal hepatocyte regeneration after injury activates a second regenerative program within the liver. Cells from the intrahepatic biliary tree proliferate and give rise to bipotential oval cells that differentiate into both new hepatocytes and biliary cells.[47]

Liver stem cells or oval cells were first described by Farber[48] in 1956 as 'small oval cells with scanty light basophilic cytoplasm and pale blue-staining nuclei' with a high nuclear/cytoplasmic ratio and an ovoid nucleus.[49] Oval cells represent a heterogeneous population of bipotential, transiently amplifying cells originating in the canal of Hering, and activate in the liver as a result of injury or insult. The number of quiescent (or dormant) oval cells is very low under physiological conditions,[50] but is greatly increased in response to specific types of liver injury. Hepatic stem cells constitute approximately 0.5–2.5% of liver parenchyma of all donor ages. These cells behave like bipotential progenitor cells and are able to differentiate into hepatocytes and cholangiocytes.[49]

Identification and isolation of oval cells provides difficulties, mostly because the differences in expression of specific markers by oval cells in mice, rats, and humans. Due to significant differences in the anatomical structure of murine and human canals of Hering, the human equivalent of oval cells are named liver precursor cells or hepatic progenitor cells (HPC).[49,51] HPCs are difficult to track and isolate because of the lack of definitive markers, and require further in-vitro testing by an epithelial colony-forming assay to assess the precursor properties of isolated cells. HPCs have been reported to express CD133, CK-19, CD44, claudin 3 and have been recently successfully isolated using two novel HPC-specific surface markers, epithelial cell adhesion molecule (EPCAM) and neural cell adhesion molecule (NCAM).[49,52] They are negative for AFP, intercellular adhesion molecule (ICAM) 1, and for markers of adult liver cells (cytochrome P450s), hemopoietic cells (CD45), and mesenchymal cells (vascular endothelial growth factor receptor and desmin). In situ studies reveal that EPCAM+liver stem cells are located in the ductal plates of fetal liver. Once isolated, these cells are capable of self-renewal and clonogenic expansion, as well as differentiation into both hepatocytic and biliary lineages in defined culture conditions.[8]

Hepatic progenitor cells transplantation is a promising alternative to liver transplantation for patients with end-stage liver disease.[53]

Animal studies showed that, purified EPCAM+liver stem cells when transplanted are able to engraft the livers of immunodeficient adult mice yielding mature human liver tissue.[52] Transplantation of freshly isolated EPCAM+ cells expanded in culture into NOD/SCID mice results in mature liver tissue expressing human-specific proteins and proved to be good candidate for liver cell therapies.[52]

USE OF INDUCED PLURIPOTENT STEM CELLS

Recently, breakthrough has been obtained to reprogram adult differentiated cells into stem cells, known as induced pluripotent stem cells (iPSCs).[54–56] In 2006, Yamanaka and co-workers demonstrated that both mouse embryonic fibroblasts and tail tip fibroblasts could be reprogrammed into a pluripotent state similar to that observed in ES cells.[57] This was achieved by the retroviral transduction of Oct4, Sox2, Klf4, and c-Myc genes. iPS cells are defined as adult somatic cells that have been genetically reprogrammed to an ES cell-like state by being forced to express genes and factors important for maintaining the defining properties of ES cells. Subsequently in 2007, human somatic cells were successfully reprogrammed into iPS cells.[58,59] There are a few ways of creating iPSCs, i.e. genomic modification, protein introduction, and treatment with chemical agents.[60]

Human-iPS cells have the hallmarks of ES cell attributes including: morphology, unlimited self-renewal, expression of key pluripotency genes, and a normal karyotype. Human iPS cell generation from developmentally diverse origins like endoderm, mesoderm, and ectoderm along with multistage hepatic differentiation protocols regarded as the most promising way to create stem cells.[61]

It has been demonstrated that human-iPS cells can be differentiated into specialized cell lineages of all three embryonic germ layers, such as motor neurons,[62] hepatocytes,[63] pancreatic insulin-producing cells,[64] cardiomyocytes,[65] etc. This wide differentiation potential provides fascinating possibilities for their use in regenerative medicine, in addition to their role in study of human development, genetic diseases, and drug discovery.[66] Human-iPS cells can be made to differentiate into cells of hepatic lineage which presents possibilities for treating liver diseases by autologous cell therapies that would avoid immune rejection and enable correction of gene defects. iPS technology can also be used for drug development, tissue engineering, and the development of bio-artificial livers.[67]

Therefore, iPSCs may hold great promise for a potentially abundant source of hepatocytes; however, directing their differentiation into specific, fully functional adult cell lineages remains a significant challenge.[68,69] New discoveries in the mechanisms of liver development have provided novel insights into hepatocyte differentiation of stem cells for therapeutic applications.[50] Efforts in programming human iPSCs, to generate hepatocytes de novo are founded on understanding how hepatocytes normally develop and differentiate in the embryo and how hepatocytes arise during regeneration in adults, in response to tissue damage and disease.[70,71] Many animal studies have reveled differentiation of murine iPS cells into hematopoietic-like and liver-like embryoid bodies. Liver-like embryoid bodies provided evident cure for coagulation factor

deficiencies and liver diseases.[72] Differentiation of fetal hepatocyte from iPS cells and ES cells display specific hepatic functions like ammonia metabolism, excretion of indocyanin green and are capable to engraft and express hepatic proteins 2 months after transplantation into newborn uPAxrag2gc−/− mouse liver.[73]

However, modulating the human genome and over expression has been associated with tumorigenesis,[74,75] there is a risk that the differentiated cells might also be tumorigenic when transplanted into patients. The insertion of transgenes into functional genes of the human genome can be detrimental.[76]

Transplantation of mouse iPS cell-derived endothelial cells and endothelial progenitor cells into the livers of irradiated hemophilia A mice have increased survival rates and plasma factor VIII.[77] To date, there is no report on the transplantation of iPS cell-derived hepatocytes into animal models, but successful differentiation of human-iPS cells into hepatocytes[63,78,79] has paved the way for the future application of patient-specific iPS cells to be utilized as cell therapies for liver diseases.[67]

USE OF EMBRYONIC STEM CELLS

Embryonic stem cells, as their name suggests, are derived from embryos. They are derived from a 4 or 5 day old human embryo that is in the blastocyst phase of development. However, these embryos are not derived from eggs fertilized in a woman's body. Most of these embryos develop from eggs that have been fertilized in vitro at an in vitro fertilization clinic. The embryos are usually extras where several eggs are fertilized in a test tube, but only one is implanted into a woman. These extra embryos are then donated for research purposes with informed consent of the donors.

Blastocyst consists of an inner cell mass (embryoblast) and an outer cell mass (trophoblast). The outer cell mass becomes part of the placenta, and the inner cell mass is the group of cells that will differentiate to become all the structures of an adult organism. This latter mass is the source of pluripotent ES cells. These cells are isolated by placing the inner cell mass into a culture dish containing a nutrient-rich broth. Lacking the necessary stimulation to differentiate, they begin to divide and replicate while maintaining their ability to become any cell type in the human body. Eventually, these undifferentiated cells can be stimulated to create specialized cells.

Embryonic stem cells possess the most potent differentiation potential, with their capacity for self-renewal theoretically providing an unlimited supply of hepatocytes to support regeneration of the injured liver. Directed differentiation of ES cells to liver cells is a promising strategy for obtaining hepatocytes that can be used for cell transplantation. ES cells were very well compared with adult liver progenitor cells and found suitable for hepatocyte-like cell generation.[80] In vitro hepatocyte differentiation of ES cells requires a profound understanding of normal development during embryonic hepatogenesis.[81] In vitro differentiation has been well documented in generating functional but immature hepatocytes.[82,83] When they are transplanted into rodent models of toxin-induced hepatic injury and partial hepatectomy, there is evidence of engraftment and differentiation into hepatocyte-like cells with some contribution to regeneration, but generally at low levels with minimal hepatocyte function.[82,84,85] In comparison with the transplantation of adult hepatocytes, however, fetal liver progenitors and ES cell-derived hepatic precursors currently appear less efficient at generating liver tissue in vivo.[86]

The discovery of human ES cells[87] had raised the hopes for curing diseases that have poor prognoses. However, exploiting the therapeutic potential of ES cells in a clinical setting presents a number of challenges. These challenges relate to stem cell safety, efficacy, and bioethics, and they have not been sufficiently overcome till date. The potential for teratoma formation is likely to remain a concern until long-term trials can provide evidence of phenotypic stability and safety.[88] A clinical trial of human ES cell-derived oligodendrocyte progenitors in spinal cord injury patients was placed on hold pending more data regarding its safety. The ethical dilemmas may continue to prohibit research in some countries, but there has been a gradual relaxation around the world as the therapeutic possibilities are realized.

USE OF FETAL STEM CELLS

Fetal stem cells (FSCs) are multipotent cells with the same functional properties of ASCs, but they are located in the fetal tissue.[7] They can be isolated from fetal blood and BM as well as from other fetal tissues, including liver and kidney. FSCs have been subdivided into hemopoietic FSCs, located in blood, liver, BM, mesenchymal FSCs located in blood, liver, BM, lung, kidney and pancreas, endothelial FSCs found in BM and placenta, epithelial FSCs located in liver and pancreas and neural FSCs located in brain and spinal cord.[89] Fetal blood is a rich source of hemopoietic stem cells, which proliferate more rapidly than those in cord blood or adult BM. Obviously, the only source of FSCs, relatively feasible and safe for fetus is fetal blood which can be obtained under ultrasound guidance. First trimester fetal blood also contains a population of non-hemopoietic mesenchymal stem cells, which support hemopoiesis and can differentiate along multiple lineages. In terms of eventual downstream application, both fetal HSC and MSC have advantages over their adult counterparts, including better intrinsic homing and engraftment, greater multipotentiality and lower immunogenicity. Fetal stem cells are less ethically contentious than ES cells and their differentiation potential appears greater than adult stem cells.[89]

Human amniotic epithelial cells (hAECs) harvested from term-delivered fetal membranes could be induced to differentiate into cardiomyocytic, myocytic, osteocytic, adipocytic (mesodermal), pancreatic, hepatic (endodermal), neural, and astrocytic (neuroectodermal) cells in vitro, as defined by phenotypic, mRNA expression, immunocytochemical, and/or ultrastructural characteristics.[90] Fetal BM-derived MSCs are able to differentiate into functional hepatocyte-like cells and may serve as a source of cells for liver disease therapy.[91] After hepatocyte induction, fetal MSCs expressed the hepatocyte-specific markers, α-fetoprotein, and cytokeratin 18, as demonstrated by immunofluorescence staining. They also demonstrated in vitro functions characteristic of liver cells, including albumin production, urea secretion, and glycogen storage.[91] The use of human liver progenitor or stem cells from fetus abrogates the issue of forced differentiation as in the case of induced pleuripotent cells, as fetal progenitors have undergone sufficient morphological and physiological differentiation so that they are committed to a hepatic fate, and yet they retain their 'stemness' by maintaining their bipotentiality, proliferative capacity, and transplantability.[92]

USE OF UMBILICAL CORD BLOOD STEM CELLS

In human umbilical cord blood (UCB) there are two different types of stem cells, i.e. hematopoietic (UC-HS) and mesenchymal (UC-MS).[7,93] Although, UCB stem cells are biologically analogous to their adult counterpart, it has been pointed out that UCB cells are characterized by a higher immunological tolerance than their adult counterpart.[94] UC-MS can produce cytokines which facilitate grafting in the donor, in vitro SC survival and it is more efficient than BM MSC graft.[95]

Immature hepatic precursors have been identified within human cord blood, which can derive engraftable bipotent progenitors. A stem cell subset CD133+/CD34+/OV6 (low) expressing a surface-marker profile consistent with that of fetal liver cells has been isolated. Upon induction of hepatic commitment by a medium containing cytokines and factors involved in vivo oval-cell activation, a heterogeneous cell population displaying characteristics of functional oval cell-like bipotent hepatic progenitors can be obtained.[96]

CONCLUSION

Stem cells are one of the most fascinating areas in regenerative medicine today. The field of stem cell science for hepatology is rapidly evolving as new research and clinical trials are being carried out. Exploring the therapeutic potential of stem cells in treatment of end-stage liver disease and liver failure will benefit millions of people who suffer from these diseases. Within the next 10 years stem cell therapeutics for liver diseases will be firmly established in hepatology.

REFERENCES

1. Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet* 2008;371:838–51.
2. Lorenzini S, Gitto S, Grandini E, Andreone P, Bernardi M. Stem cells for end stage liver disease: how far have we got? *World J Gastroenterol* 2008;14:4593–9.
3. Chen Z, Qi LZ, Zeng R, Li HY, Dai LJ. Stem cells and hepatic cirrhosis. *Panminerva Med* 2010;52:149–65.
4. Vats A, Bielby RC, Tolley NS, Nerem R, Polak JM. Stem cells. *Lancet* 2005;366:592–602.
5. Moore KA, Lemischka IR. Stem cells and their niches. *Science* 2006;311:1880–5.
6. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 2000;100:157–68.
7. Lodi D, Iannitti T, Palmieri B. Stem cells in clinical practice: applications and warnings. *J Exp Clin Cancer Res* 2011;30:9.
8. Mimeault M, Hauke R, Batra SK. Stem cells: a revolution in therapeutics-recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies. *Clin Pharmacol Ther* 2007;82:252–64.
9. Pai M, Zacharoulis D, Milicevic MN, et al. Autologous infusion of expanded mobilized adult bone marrow-derived CD34+ cells into patients with alcoholic liver cirrhosis. *Am J Gastroenterol* 2008; 103:1952–8.
10. Lyra AC, Soares MBP, da Silva LFM, et al. Feasibility and safety of autologous bone marrow mononuclear cell transplantation in patients with advanced chronic liver disease. *World J Gastroenterol* 2007;13:1067–73.
11. am Esch JS 2nd, 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;23:463–70.
12. Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am J Pathol* 2006;169: 338–46.
13. Körbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002;346:738–46.
14. Gilchrist ES, Plevris JN. Bone marrow-derived stem cells in liver repair: 10 years down the line. *Liver Transpl* 2010;16:118–29.
15. Yamazaki S, Miki K, Hasegawa K, Sata M, Takayama T, Makuuchi M. Sera from liver failure patients and a demethylating agent stimulate transdifferentiation of murine bone marrow cells into hepatocytes in coculture with nonparenchymal liver cells. *J Hepatol* 2003;39:17–23.
16. Sellamuthu S, Manikandan R, Thiagarajan R, et al. In vitro transdifferentiation of human umbilical cord derived hematopoietic stem cells into hepatocyte like cells using combination of growth factors for cell based therapy. *Cytotechnology* 2011;63:259–68.
17. Yannaki E, Anagnostopoulos A, Kapetanos D, et al. Lasting amelioration in the clinical course of decompensated alcoholic cirrhosis with boost infusions of mobilized peripheral blood stem cells. *Exp Hematol* 2006;34:1583–7.
18. Terai S, Ishikawa T, Omori K, et al. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. *Stem Cells* 2006;24:2292–8.
19. Gordon MY, Levicar 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;24:1822–30.
20. 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;13:3359–63.

21. Mohamadnejad M, Alimoghaddam K, Mohyeddin-Bonab M, et al. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. *Arch Iran Med* 2007;10:459–66.
22. Fürst G, Schulte am Esch J, Poll LW, et al. Portal vein embolization and autologous CD133+ bone marrow stem cells for liver regeneration: initial experience. *Radiology* 2007;243:171–9.
23. Khan AA, Parveen N, Mahaboob VS, et al. Safety and efficacy of autologous bone marrow stem cell transplantation through hepatic artery for the treatment of chronic liver failure: a preliminary study. *Transplant Proc* 2008;40:1140–4.
24. Kharaziha P, Hellström PM, Noorinayer B, et al. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I–II clinical trial. *Eur J Gastroenterol Hepatol* 2009;21:1199–205.
25. Ismail A, Fouad O, Abdelnasser A, Chowdhury A, Selim A. Stem cell therapy improves the outcome of liver resection in cirrhotics. *J Gastrointest Cancer* 2010;41:17–23.
26. 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;19:1237–46.
27. Lyra AC, Soares MBP, da Silva LFM, et al. Infusion of autologous bone marrow mononuclear cells through hepatic artery results in a short-term improvement of liver function in patients with chronic liver disease: a pilot randomized controlled study. *Eur J Gastroenterol Hepatol* 2010;22:33–42.
28. Salama H, Zekri A-R, Zern M, Bahnassy A, Loutfy S, et al. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage chronic liver diseases. *Cell Transplant* 2010;19:1475–86.
29. Salama H, Zekri A-RN, Bahnassy AA, et al. Autologous CD34+ and CD133+ stem cells transplantation in patients with end stage liver disease. *World J Gastroenterol* 2010;16:5297–305.
30. Saito T, Okumoto K, Haga H, et al. Potential therapeutic application of intravenous autologous bone marrow infusion in patients with alcoholic liver cirrhosis. *Stem Cells Dev [Internet]* 2011 May 11 [cited 2011 May 21]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/21417817.
31. Nikeghbalian S, Pournasr B, Aghdami N, et al. Autologous transplantation of bone marrow-derived mononuclear and CD133(+) cells in patients with decompensated cirrhosis. *Arch Iran Med* 2011;14:12–7.
32. Kallis YN, Alison MR, Forbes SJ. Bone marrow stem cells and liver disease. *Gut* 2007;56:716–24.
33. Satija NK, Singh VK, Verma YK, et al. Mesenchymal stem cell-based therapy: a new paradigm in regenerative medicine. *J Cell Mol Med* 2009;13:4385–402.
34. Gucciardo L, Lories R, Ochsenbein-Kölble N, Done' E, Zwijsen A, Deprest J. Fetal mesenchymal stem cells: isolation, properties and potential use in perinatology and regenerative medicine. *BJOG* 2009;116:166–72.
35. Kassem M, Kristiansen M, Abdallah BM. Mesenchymal stem cells: cell biology and potential use in therapy. *Basic Clin Pharmacol Toxicol* 2004;95:209–14.
36. Lange C, Bassler P, Lioznov M-V, et al. Liver-specific gene expression in mesenchymal stem cells is induced by liver cells. *World J Gastroenterol* 2005;11:4497–504.
37. Porada CD, Zanjani ED, Almeida-Porad G. Adult mesenchymal stem cells: a pluripotent population with multiple applications. *Curr Stem Cell Res Ther* 2006;1:365–9.
38. Chamberlain J, Yamagami T, Colletti E, et al. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. *Hepatology* 2007;46:1935–45.
39. Lee K-D, Kuo TK-C, Whang-Peng J, Chung Y-F, Lin C-T, Chou S-H, et al. In vitro hepatic differentiation of human mesenchymal stem cells. *Hepatology* 2004;40:1275–84.
40. Sato Y, Araki H, Kato J, et al. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood* 2005;106:756–63.
41. Schwartz RE, Reyes M, Koodie L, et al. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002;109:1291–302.
42. Meirelles L da S, Nardi NB. Methodology, biology and clinical applications of mesenchymal stem cells. *Front Biosci* 2009;14:4281–98.
43. Christ B, Dollinger MM. The generation of hepatocytes from mesenchymal stem cells and engraftment into the liver. *Curr Opin Organ Transplant [Internet]* 2010 Dec 9 [cited 2011 May 20]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/21150616.
44. Jung KH, Uhm Y-K, Lim YJ, Yim S-V. Human umbilical cord blood-derived mesenchymal stem cells improve glucose homeostasis in rats with liver cirrhosis. *Int J Oncol* 2011;39:137–43.
45. Qiao H, Tong Y, Han H, et al. A novel therapeutic regimen for hepatic fibrosis using the combination of mesenchymal stem cells and baicalin. *Pharmazie* 2011;66:37–43.
46. Overturf K, al-Dhalimy M, Ou CN, Finegold M, Grompe M. Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. *Am J Pathol* 1997;151:1273–80.
47. Alison M. Liver stem cells: a two compartment system. *Curr Opin Cell Biol* 1998;10:710–5.
48. 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-4-dimethylaminoazobenzene. *Cancer Res* 1956;16:142–8.
49. Kisseleva T, Gigante E, Brenner DA. Recent advances in liver stem cell therapy. *Curr Opin Gastroenterol* 2010;26:395–402.
50. Dabeva MD, Shafritz DA. Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. *Am J Pathol* 1993;143:1606–20.
51. Theise ND, Saxena R, Portmann BC, et al. The canals of Hering and hepatic stem cells in humans. *Hepatology* 1999;30:1425–33.
52. Schmelzer E, Zhang L, Bruce A, et al. Human hepatic stem cells from fetal and postnatal donors. *J Exp Med* 2007;204:1973–87.
53. Alison MR, Choong C, Lim S. Application of liver stem cells for cell therapy. *Semin Cell Dev Biol* 2007;18:819–26.
54. Giorgetti A, Montserrat N, Rodriguez-Piza I, Azqueta C, Veiga A, Izpisúa Belmonte JC. Generation of induced pluripotent stem cells from human cord blood cells with only two factors: Oct4 and Sox2. *Nat Protoc* 2010;5:811–20.
55. Si-Tayeb K, Noto FK, Sepac A, et al. Generation of human induced pluripotent stem cells by simple transient transfection of plasmid DNA encoding reprogramming factors. *BMC Dev Biol* 2010;10:81.
56. Yamanaka S. A fresh look at iPS cells. *Cell* 2009;137:13–7.
57. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663–76.
58. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;131:861–72.
59. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007;318:1917–20.
60. Soto-Gutierrez A, Tafaleng E, Kelly V, Roy-Chowdhury J, Fox IJ. Modeling and therapy of human liver diseases using induced pluripotent stem cells: how far have we come? *Hepatology* 2011;53:708–11.
61. Chun YS, Chaudhari P, Jang Y-Y. Applications of patient-specific induced pluripotent stem cells; focused on disease modeling, drug screening and therapeutic potentials for liver disease. *Int J Biol Sci* 2010;6:796–805.
62. Karumbayaram S, Novitch BG, Patterson M, et al. Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells* 2009;27:806–11.

63. Si-Tayeb K, Noto FK, Nagaoka M, et al. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology* 2010;51:297–305.
64. Zhang D, Jiang W, Liu M, et al. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res* 2009;19:429–38.
65. Zhang J, Wilson GF, Soerens AG, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009; 104:e30–41.
66. Nishikawa S-ichi, Goldstein RA, Nierras CR. The promise of human induced pluripotent stem cells for research and therapy. *Nat Rev Mol Cell Biol* 2008;9:725–9.
67. Asgari S, Pournasr B, Salekdeh GH, Ghodsizadeh A, Ott M, Baharvand H. Induced pluripotent stem cells: a new era for hepatology. *J Hepatol* 2010;53:738–51.
68. Yamashita JK. ES and iPS cell research for cardiovascular regeneration. *Exp Cell Res* 2010;316:2555–9.
69. Rashid ST, Corbineau S, Hannan N, et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J Clin Invest* 2010;120:3127–36.
70. McDevitt TC, Palecek SP. Innovation in the culture and derivation of pluripotent human stem cells. *Curr Opin Biotechnol* 2008;19: 527–33.
71. 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.
72. Kasuda S, Tatsumi K, Sakurai Y, et al. Expression of coagulation factors from murine induced pluripotent stem cell-derived liver cells. *Blood Coagul Fibrinolysis* 2011;22:271–9.
73. Touboul T, Vallier L, Weber A. Robust differentiation of fetal hepatocytes from human embryonic stem cells and iPS. *Med Sci (Paris)* 2010;26:1061–6.
74. Foster KW, Liu Z, Nail CD, et al. Induction of KLF4 in basal keratinocytes blocks the proliferation-differentiation switch and initiates squamous epithelial dysplasia. *Oncogene* 2005;24:1491–500.
75. Hochedlinger K, Yamada Y, Beard C, Jaenisch R. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. *Cell* 2005;121:465–77.
76. Nair V. Retrovirus-induced oncogenesis and safety of retroviral vectors. *Curr Opin Mol Ther* 2008;10:431–8.
77. Xu D, Alipio Z, Fink LM, et al. Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. *Proc Natl Acad Sci USA* 2009;106:808–13.
78. Song Z, Cai J, Liu Y, et al. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. *Cell Res* 2009; 19:1233–42.
79. Sullivan GJ, Hay DC, Park I-H, et al. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. *Hepatology* 2010;51:329–35.
80. Funakoshi N, Duret C, Pascussi J-M, et al. Comparison of hepatic-like cell production from human embryonic stem cells and adult liver progenitor cells: CAR transduction activates a battery of detoxification genes. *Stem Cell Rev [Internet]*. 2011 Jan 6 [cited 2011 May 20]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/21210253.
81. Chen X, Zeng F. Directed hepatic differentiation from embryonic stem cells. *Protein Cell* 2011;2:180–8.
82. Agarwal S, Holton KL, Lanza R. Efficient differentiation of functional hepatocytes from human embryonic stem cells. *Stem Cells* 2008; 26:1117–27.
83. Yamamoto H, Quinn G, Asari A, et al. Differentiation of embryonic stem cells into hepatocytes: biological functions and therapeutic application. *Hepatology* 2003;37:983–93.
84. Moriya K, Yoshikawa M, Ouji Y, et al. Embryonic stem cells reduce liver fibrosis in CCl4-treated mice. *Int J Exp Pathol* 2008;89:401–9.
85. Heo J, Factor VM, Uren T, et al. Hepatic precursors derived from murine embryonic stem cells contribute to regeneration of injured liver. *Hepatology* 2006;44:1478–86.
86. Haridass D, Yuan Q, Becker PD, et al. Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice. *Am J Pathol* 2009; 175:1483–92.
87. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;282: 1145–7.
88. Teramoto K, Hara Y, Kumashiro Y, et al. Teratoma formation and hepatocyte differentiation in mouse liver transplanted with mouse embryonic stem cell-derived embryoid bodies. *Transplant Proc* 2005;37:285–6.
89. O'Donoghue K, Fisk NM. Fetal stem cells. *Best Pract Res Clin Obstet Gynaecol* 2004;18:853–75.
90. Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, Manuelpillai U. Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod* 2007; 77:577–88.
91. Wei X, Wang CY, Liu QP, et al. In vitro hepatic differentiation of mesenchymal stem cells from human fetal bone marrow. *J Int Med Res* 2008;36:721–7.
92. Riehle KJ, Dan YY, Campbell JS, Fausto N. New concepts in liver regeneration. *J Gastroenterol Hepatol* 2011;26(Suppl 1):203–12.
93. Ruhil S, Kumar V, Rathee P. Umbilical cord stem cell: an overview. *Curr Pharm Biotechnol* 2009;10:327–34.
94. Mihu CM, Mihu D, Costinus Ciucă D, Suşman S, Ciortea R. Isolation and characterization of stem cells from the placenta and the umbilical cord. *Rom J Morphol Embryol* 2008;49:441–6.
95. In't Anker PS, Scherjon SA, Kleijburg-van der Keur C, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells* 2004;22:1338–45.
96. Crema A, Ledda M, De Carlo F, et al. Cord blood CD133 cells define an OV6-positive population that can be differentiated in vitro into engraftable bipotent hepatic progenitors. *Stem Cells Dev [Internet]* 2011 Mar 23 [cited 2011 May 20]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/21291316.