

Chronic Liver Disease: Stem Cell Therapy

Gamal MA Hassan, Hamdy Sliem, Abousree T Ellethy, Mahmoud E Salama

Gamal MA Hassan, Anatomy MD, Pediatric MSc, Suez Canal University, Ismailia, Egypt

Hamdy Sliem, Internal Medicine, Qassim University, Saudi Arabia

Abousree T Ellethy, Biochemistry, Qassim University, Saudi Arabia

Mahmoud E Salama, Physiology, Qassim University, Saudi Arabia

Correspondence to: Hamdy Sliem, Internal Medicine MD, Qassim University, Saudi Arabia. Hamdy.sliem@yahoo.com

Telephone: +01-818-364-3230 Fax: +01-818-364-4573

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ABSTRACT

Chronic liver diseases (CLD) affect hundreds of millions of patients worldwide. Stem Cells (SCs) therapy to treat chronic liver diseases is resorted and is considered as the dream of the future. Although SCs are a promising means for treatment of liver diseases, studies are still at the beginning of this era. SCs are undifferentiated cells capable of renewing themselves throughout their life and of generating one or more types of differentiated cells. Different types of SCs with hepatic differentiation potential are theoretically eligible for liver cell replacement. These include Embryonic and fetal liver SCs, induced pluripotent SCs, hepatoblasts, annex SCs (pluripotent SCs obtained from umbilical cord and umbilical cord blood, placenta and amniotic fluid), and adult SCs, such hepatic progenitor cells, hematopoietic SCs, and mesenchymal stem cell. The optimal SCs delivery route should be easy to perform, less invasive and traumatic, minimum side effects, and with high cells survival rate. Liver SCs can be transplanted through several routes: Intrahepatic and percutaneous intrahepatic artery catheterization in acute liver failure, and umbilical vein catheterization, percutaneous intrahepatic route, and portal vein or intrahepatic artery catheterization in metabolic liver diseases. Whatever the source or delivery route of SCs, how they can be manipulated for therapeutic interventions in a variety of hepatic diseases is of course of great interest in future studies. Although all clinical trials to date have shown some improvement in liver function and CD34+ cells have been used safely for bone marrow transplantation for over 20 years, only randomized controlled clinical trials will be able to fully assess the potential clinical benefit of adult SCs therapy for patients with CLD.

INTRODUCTION

For decades, the role of stem or progenitor cells in liver regeneration has been controversial. Observations of mitotic division by hepatocytes and cholangiocytes in human and other vertebrate livers confirmed that these cell compartments had regenerative potential. It was assumed by many, though not all, investigators that this regenerative capacity of mature cells was sufficient to explain the extensive regenerative capacity of this organ. Currently, liver transplantation is the most effective therapy for patients with end stage liver disease (ESLD). However, its potential benefits are hampered by many drawbacks, such as the relative shortage of donors, operative risk, post-transplant rejection, recurrence of the pre-existing liver disease, and high costs^[1]. For these reasons, various alternatives to liver transplantation have been evaluated. Stem Cells (SCs) therapy to treat chronic liver diseases is resorted and is considered as the dream of the future. The current article was intended to provide an overview of the up to date status of SCs and the prospects of liver regeneration therapy.

LIVER DISEASE

Liver diseases affect hundreds of millions of patients worldwide. The most common causes are chronic hepatitis C and B, alcoholism, non-alcoholic fatty liver disease, autoimmune, and drug-induced hepatic disorders. Many of these conditions can be prevented or treated, but if not, they can lead to progressive liver injury, liver fibrosis and ultimately cirrhosis (disorganized proliferation of hepatocytes and biliary cells, excessive scarring, and loss of the three-dimensional architecture of the hepatic lobule, which leads to portal hypertension, liver failure, and, in some instances, cancer). The risk of developing hepatocellular carcinoma is significantly

increased in patients with hepatitis virus infection, alcoholic liver disease, or genetic hemochromatosis^[2]. ESLD is the final stage of acute or chronic liver damage and is irreversibly associated with liver failure. ESLD can develop rapidly, over days or weeks (acute and sub-acute liver failure, respectively), or gradually, over months or years (chronic liver failure). Identification of the cell-surface molecules of liver progenitors has made it possible to identify and isolate liver progenitors, making the liver a unique model for SCs biology^[3]. Among the liver diseases that may be treated by SCs are: hepatitis, non-alcoholic fatty liver disease, glycogen storage disease type II, Wilson's disease, primary sclerosing cholangitis, cirrhosis, and cancer of the liver.

STEM CELLS

A stem cell is an undifferentiated cell capable of renewing itself throughout its life and of generating one or more types of differentiated cells. While embryonic SCs are the only ones to be totipotent, adult tissues with high cellular turnover (e.g. skin, gut mucosa and bone marrow) retain a population of SCs with restricted differentiation potential that constantly supply the tissue with new cells^[4,5]. In addition, it has the ability to repopulate a tissue or organ after transplantation and the potential to be serially transplanted^[6]. SCs normally do not proliferate rapidly and the offspring of them are referred to as progenitor cells. Progenitor cells may be further classified as early and late. The early progenitors can possess characteristics similar to SCs and may be multi-potent. In contrast, the late progenitors appear to have progressed along the differentiation pathway and may only produce one or two cell types only. Progenitor cells are not self-renewing or capable of serial transplantation^[7].

Based on the ability to differentiate, SCs can be classified into three broad categories, (1) Totipotent SCs which are found only in early embryos. Each cell can form a complete organism (e.g., identical twins); (2) Pluripotent SCs which exist in the undifferentiated inner cell mass of the blastocyst and can form any of the over 200 different cell types found in the body; (3) Multi-potent SCs which are derived from fetal tissue, cord blood, and adult SCs^[8]. In general, SCs have three characteristics: Plasticity: Potential to change to other cell types; Homing: Travel to site of tissue damage; and Engraftment: Unite with other tissues.

The term, "plasticity" has been coined to emphasize the process in which pluripotent SCs are able to differentiate into multiple cell lineages, and is supported by the finding of intrahepatic chimerism in transplant recipients as well as the apparent repopulation of injured livers with bone marrow derived cells. The term, "trans-differentiation", is used to focus on differentiation of cells from one organ specific lineage to those of another organ^[9].

Different types of SCs with hepatic differentiation potential are theoretically eligible for liver cell replacement. These include Embryonic and fetal liver SCs (ESCs), induced pluripotent SCs (iPSs), hepatoblasts, annex SCs (pluripotent SCs obtained from umbilical cord and umbilical cord blood, placenta and amniotic fluid), and adult SCs, such as hepatic progenitor cells (HPCs), hematopoietic SCs (HSCs), and mesenchymal stem cell (MSCs). Despite encouraging results *in vitro*, the use of hepatocyte like cells derived from these stem/progenitor cell populations is still confined to preclinical studies, given the scarce tissue-specific functionality and, up to now, the lack of evidence of strong liver repopulation levels in animal models^[10].

Embryonic and Fetal Liver Stem Cells (ESCs)

ESCs are found in an early embryo called a blastocyst. A blastocyst is a hollow sphere of approximately 100 cells that exists from about

day 5 to day 10 after conception. The ESCs in a blastocyst are part of the inner cell mass. These cells can be removed from the blastocyst and put into culture, where they can be kept alive and encouraged to make identical copies of themselves (stem cell line). ESCs are pluripotent - they have the ability to become any type of cell in the body.

Induced Pluripotent Stem Cells (iPSs)

Takahashi *et al.*^[11] reported that pluripotent SCs can be generated from adult human fibroblasts, thereby establishing human iPS cells. These iPSs cells derived from the patients themselves could be utilized as the donor cell source. Although induced iPSs exhibit characteristics similar to those of ESCs^[12], the methodology for induction of hepatocytes from human iPSs and elimination of immature undifferentiated cells has not yet been established. Moreover, it remains unclear how hepatic stem/progenitor cells derived from iPSs should be transplanted so that recipients obtain successful liver repopulation and long-term engraftment^[13].

Hepatoblast

Both hepatocytes and cholangiocytes are derived from hepatoblasts in fetal liver^[14]. Human livers contain two pluripotent progenitors: hepatic SCs and hepatoblasts. The hepatic SCs uniquely express the combination of epithelial cell adhesion molecule (EpCAM), neural cell adhesion molecule (NCAM), cytokeratin (CK) 19, albumin +/-, and are negative for α -fetoprotein (α -FP). They are precursors to hepatoblasts, which differ from hepatic SCs in size, morphology, and in expressing the combination of EpCAM, intercellular cell adhesion molecule (ICAM-1), CK19, albumin, and α -FP. The hepatic SCs are located *in vivo* in stem cell niches: the ductal plates in fetal and neonatal livers and canals of Hering in pediatric and adult livers. The hepatoblasts are contiguous to the niches, decline in numbers with age, wax and wane in numbers with injury responses, and are proposed to be the liver's transit-amplifying cells. In adult livers, intermediates between hepatic SCs and hepatoblasts and between hepatoblasts and adult parenchyma are observed. Amplification of one or both pluripotent cell subpopulations can occur in diseases; for example, hepatic SCs amplification occurs in mild forms of liver failure, and hepatoblast amplification occurs in forms of cirrhosis. Liver is, therefore, similar to other tissues in that: regenerative processes in postnatal tissues parallel those occurring in development, and involve populations of SCs and progenitor cells that can be identified by anatomic, antigenic, and biochemical profiles^[15].

Fetal liver SCs, also named "hepatoblasts", appear when the hepatic endoderm has been specified and the liver bud is growing. Hepatoblasts are bi-potent, being able to give rise to both hepatocytes and bile duct cells, and co-express biliary and hepatocytic markers, such as albumin, α -FP, and CK19. Studies in diverse model organisms have revealed evolutionarily conserved inductive signals and transcription factor networks that elicit the differentiation of liver SCs^[16].

Murine hepatoblast cell lines have been established in research laboratories and their capacity to repopulate the liver upon transplantation in animal models has been extensively proved^[17]. In contrast to adult liver, ESCs and fetal liver SCs are thought to be highly proliferative, less immunogenic and more resistant to cryopreservation. Owing to the possibility of immune rejection and teratoma/teratocarcinoma formation in the recipients, their use is currently reserved to preclinical studies^[18].

The microenvironmental factors driving liver SCs themselves need to be defined prior to attempting any clinical application. The

identification of endogenous liver SCs and of the signals that govern their proliferation and differentiation to mature hepatocytes might lead to the development of clinically feasible methods to induce liver repopulation from these endogenous cells and/or to allow maturation of stem/progenitor cells to hepatocytes *in vitro* and *in vivo*^[10].

Annex SCs: Previous studies indicated that umbilical cord and umbilical cord blood, placenta and amniotic fluid are an easily accessible source of pluripotent SCs, which may be readily available for transplantation, or for further expansion and manipulation prior to cell therapies^[19,20]. These cells can be extensively expanded without loss of potency and have a broad differentiation potential, since they can generate progenies of all three germ layers. These pluripotent annex SCs can be forced to differentiate into hepatocyte-like cells *in vitro* and are capable of liver repopulation *in vivo*, upon transplantation in animal models^[21,22].

Adult SCs: Adult SCs are mostly multi-potent and are found in small amounts throughout body. They can become one of only a limited number of cell types. These cells haven't been isolated for all tissue types, are present in small quantities. Also they are difficult to isolate and purify, their numbers and quality decrease with age and they differentiate into a narrower range of cell types. Adult SCs include: hepatic progenitor SCs, hematopoietic SCs, and mesenchymal SCs.

Hepatic progenitor cells (HPCs): Oval cells are small proliferating cells with oval nuclei observed in rat livers following certain carcinogenic regimens^[23]. They are bi-potent (able to give rise to both cholangiocytes and hepatocytes) and co-express biliary and hepatocytic markers, such as CK19, α -FP, and albumin^[24]. In humans, the counterpart of oval cells is represented by the intermediate hepatobiliary cells, or ductular hepatocytes, or HPCs, which can be seen in several liver diseases, such as chronic cholestasis, submassive necrosis, alcoholic liver disease, focal nodular hyperplasia, and liver allograft failure^[25]. In such conditions, intermediates between hepatic SCs and hepatoblasts and between hepatoblasts and adult parenchyma are observed and amplification of one or both pluripotent cell subpopulations can occur^[15]. A frequent trigger of ductular reaction is the presence of chronic damage, resulting in hepatocyte senescence. Like oval cells, HPCs are bi-potent, co-express biliary and hepatocytic markers, and also express hematopoietic progenitor cell antigens^[26,27].

Hematopoietic SCs (HSCs): HSCs are responsible for the renewal of blood cells and can be also isolated from umbilical cord blood and peripheral blood. It is generally accepted that the most primitive and long-term human HSCs are characterized by the expression of CD133, Thy1 (CD90) and VEGFR2 and by a variable expression of CD34 and CD38^[28]. Bone marrow-resident HSCs can be mobilized into the peripheral blood under specific stimuli such as tissue injury or administration of Granulocyte colony-stimulating factor (G-CSF). G-CSF is a colony-stimulating factor hormone. It is a glycoprotein, growth factor and cytokine produced by a number of different tissues to stimulate the bone marrow to produce granulocytes and SCs^[29]. Mobilized HSCs can colonize extramedullary sites and participate to their regeneration, by promoting the immune response and/or by trans-differentiating into adult SCs within peripheral tissues^[19,29,30].

Mesenchymal SCs (MSCs): MSCs, also called stromal SCs,

mesenchymal progenitors, mesenchymal stromal cells, colony-forming unit-fibroblastic cells, are highly proliferating, adherent cells, that reside in a perivascular bone marrow niche and also in the wall of blood vessels within most organs^[31]. MSCs are able to differentiate into a variety of mesodermal cell lineages (osteoblasts, chondroblasts, adipocytes, myocytes, and cardiomyocytes), as well as non-mesodermal cells (such as hepatocytes and neurons), depending upon the micro-environment in which they reside^[32]. MSCs might become a more suitable source for SC-based therapies than HSCs, because of their immunological properties. MSCs are less immunogenic and can induce tolerance upon transplantation^[33]. Moreover, MSCs showed the highest potential for liver regeneration compared with other BM cell subpopulations in an animal model of hepatic injury^[34].

Hematopoietic SCs (HSCs) and mesenchymal stem cell (MSCs) are two main subtypes of bone marrow SCs. The diseased liver may recruit migratory SCs, particularly from the bone marrow, to generate hepatocyte-like cells either by trans-differentiation or cell fusion. Transplantation of bone marrow SCs has therapeutic effects of restoration of liver mass and function, alleviation of fibrosis and correction of inherited liver diseases^[35].

The comparisons of embryonic stem cells with adult stem cells were summarized in table 1^[36].

Table 1 comparison of embryonic stem cells with adult stem cells.

Features	Types of cells	
	Embryonic	Adult
Kind of cells they can generate	All cells in the body except extra-embryonic cell	A limited set of cells, usually in same system
Plasticity	Pluripotent	Multi-potent
Source	Early embryo (inner mass of blastocyst)	Adult tissue

Stem cell based therapy in hepatic diseases

Although SCs are a promising means for treatment of liver diseases, studies are still at the beginning of this era. Results of experimental animals and clinical trials are still preliminary. While all clinical trials to date have shown some improvement in liver function, it ought to be remembered that the natural history of cirrhosis tends to be variable. Thus, one would expect some patients to improve with time; particularly in compliant patients who can be followed up and remain abstinent from alcohol. HSCs transplantation and G-CSF infusion are effective in hematological diseases. They may succeed as well in chronic liver diseases. The major role for SCs therapy is as a bridge to transplantation or as a way of maintaining those patients who are not eligible for liver transplant. The long-term safety, tolerability, and efficacy of these SCs-based treatments, as well as their carcinogenic potential must be considered. New clinical trials are needed. Various clinical studies for liver disease have been reported, including hepatic administration of autologous CD34-positive cells induced by G-CSF, portal vein administration of CD133-positive mononuclear cells, and administration of autologous bone marrow derived mesenchymal SCs. Effectiveness of these approaches has been shown in some patients. Also, experimental studies have reported improved liver fibrosis and function with infusion of autologous bone marrow cells in a basic study with mice. The efficacy and safety of autologous bone marrow cell infusion (ABMI) therapy has also been reported by other institutions^[37]. On the basis of those results ABMI therapy for liver cirrhosis was ongoing.

Hepatocyte transplantation was attempted by Mito and Kusano in cirrhotic patients, but the ultimate clinical outcome was not altered significantly^[38]. Isolation of hepatic progenitors from a human source is a major challenge for the clinical application of this therapy.

Intrahepatic sources include cadaver livers that are considered of insufficient quality for organ transplantation. Hepatocytes isolated from aborted human fetuses are another potential source. Extra-hepatic sources include autologous bone marrow, umbilical cord blood, Wharton's jelly, and peripheral blood monocytes. Programmable cells of monocytic origin are capable of differentiating into neo-hepatocytes, which closely resemble primary human hepatocytes with respect to morphology, expression of hepatocyte markers, and specific metabolic functions^[39-41]. The ability to reprogram, expand, and differentiate peripheral blood monocytes in large quantities opens the real possibility of the clinical application of programmable cells of monocytic origin in tissue repair and organ regeneration^[42].

Results of recent clinical studies strongly suggest that liver function-improving effects can be achieved using infusion of bone marrow SCs for cirrhosis. New treatment methods using less-invasive bone marrow-derived cultured cells need to be developed^[40]. Kim *et al* reported significant improvement in serum albumin, quality of life, and the Child-Pugh Score in ten patients with advanced liver cirrhosis due to hepatitis B infection following autologous bone marrow infusion^[43]. Pai *et al* published the results of administering autologous expanded mobilized adult BM CD34+ cells via the hepatic artery in 9 patients with alcoholic liver cirrhosis. Significant decreases in serum bilirubin, ALT, and AST levels were observed, whilst the Child-Pugh Scores and radiological ascites improved in 7 and 5 patients, respectively^[44]. In contrast to the previous studies, a trial of 4 patients with decompensated cirrhosis treated with CD34+ stem cells via the hepatic artery was stopped prematurely due to one patient developing nephropathy and hepatorenal syndrome secondary to radiocontrast^[45]. New findings in adult stem cell biology are transforming our understanding of tissue repair raising hopes of successful regenerative hepatology. Although all clinical trials to date have shown some improvement in liver function and CD34+ cells have been used safely for BM transplantation for over 20 years, only randomized controlled clinical trials will be able to fully assess the potential clinical benefit of adult stem cell therapy for patients with liver insufficiency. Conversely, concerns have been raised about the adverse long-term effects of stem cell therapy. There is evidence to suggest that treatment with BMSCs may provide liver fibrogenic cells (hepatic stellate cells and myo-fibroblasts) which contribute to fibrosis and could have a deleterious effect on already decompensated cirrhotic livers^[46,47].

SCs delivery route: The optimal SCs delivery route should be easy to perform, less invasive and traumatic, minimum side effects, and with high cells survival rate^[48]. Liver SCs can be transplanted through several routes: Intraperitoneal and percutaneous intrahepatic artery catheterization in acute liver failure, and umbilical vein catheterization, percutaneous intrahepatic route, and portal vein or intrahepatic artery catheterization in metabolic liver diseases^[49]. Intrasplenic artery, hepatic artery and portal vein catheterization in chronic liver diseases. Attempts have been made to infuse cells from autologous bone marrow along with granulocyte stimulating factor. The preferred route is hepatic artery catheterization^[44]. Whatever the source or delivery route of SCs, how they can be manipulated for therapeutic interventions in a variety of hepatic diseases is of course of great interest in future studies.

Future trends

The selected methodology to purify stem cells is an important consideration in order to guarantee success regarding purity and viability that determines the positive outcome of the therapies. Currently, there is neither satisfactory technique nor strategy that positively addresses the issue raised in this review that allow the

full practical separation of stem cells at large scale. Therefore, there is a great need to focus research in the field on the development of a suitable separation and purification technology for stem cells to establish a manufacturing process, which is fundamental to avoid a delay in the widespread application of stem cells therapy. This therapy has been proposed as a promising treatment for diseases for which no cure is available^[50].

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- Peer reviewers:** Jian Wu, MD, PhD, Professor Department of Internal Medicine, Division of Gastroenterology & Hepatology, 2921 Stockton Blvd. Suite 1610, Sacramento, CA 95817, the United States; Minoru Tomizawa, MD, PhD, Department of Gastroenterology, National Hospital Organization Shimoshizu Hospital, 934-5 Shikawatashi, Yotsukaido City, Japan; Kenoki Ohuchida, MD, PhD, Assistant professor, Department of Surgery and Oncology, Kyusyu university, Fukuoka, 812-8582 Japan.