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Adult Stem Cell Therapy as Regenerative Medicine for End-Stage Liver Disease

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Abstract

The increased incidence of end-stage liver disease (ESLD) causes a major burden on the global health system and population health. Liver transplantation (LT) is one of the most effective treatments for ESLD patients, but its practice is extensively hampered by the scarcity of liver donors, the limited number of transplantation centers, the complexity of the procedure, and postoperative complication. In parallel, vast growing advances in cellular biology and biotechnology have opened new alternatives in clinics, including the transplantation of adult stem cells for chronic diseases such as ESLD. Numerous types of stem cells, such as mesenchymal stem cells, hematopoietic stem cells, endothelial progenitor cells, and other cells, obtained from bone marrow, umbilical cord, adipose tissue, or peripheral blood had been isolated and given to ESLD patients all over the world. Many clinical data had demonstrated promising results, indicating its potential. However, conclusive protocol and agreement on adult stem cell definition and transplantation method are still lacking, and thus further research must still be conducted.

Keywords

Adult stem cells · Cell transplantation · End-stage liver disease · Regenerative medicine

Abbreviations

BM	Bone marrow
EPC	Endothelial progenitor cells
ESC	Embryonic stem cells
ESLD	End-stage liver disease
HBV	Hepatitis B virus
HCC	Hepatitis C virus
HCC	Hepatocellular carcinoma
HLO	Human liver organoid
HSC	Hematopoietic stem cells
iPSC	Induced pluripotent stem cells
LPC	Liver progenitor cells
LT	Liver transplantation
MELD	Model for End-Stage Liver Disease
MSC	Mesenchymal stromal/stem cells
PHH	Primary human hepatocytes

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1 End-Stage Liver Disease

Liver disease is one of the major health problems in the world. It accounts for approximately two million deaths per year worldwide, one million due to complications of cirrhosis and one million to viral hepatitis (hepatitis B virus (HBV) and hepatitis C virus (HCV)) and hepatocellular carcinoma (HCC). Chronic liver disease is usually caused by prolonged excess alcohol consumption, metabolic disorders, and viral hepatitis infection (Asrani et al. 2019).

The number of end-stage liver disease (ESLD) cases is increasing resulting in a greater burden on the healthcare system (Fricker and Serper 2019). ESLD, often interchangeably called liver failure or decompensated cirrhosis, is the final stage of chronic liver disease and is associated with a high degree of mortality. The annual rates of liver disease progression to decompensated stage range from 4% for HCV to 6–10% for alcoholic cirrhosis and 10% for HBV (Asrani et al. 2019).

Liver cirrhosis is characterized by a silent, asymptomatic course that may be undetectable for years. This is usually referred to compensated cirrhosis. When the portal pressure is increased and liver function is significantly reduced, the clinical phenotype is observed. Decompensation is marked by the development of overt clinical signs, the most frequent of which are ascites, bleeding, encephalopathy, and jaundice (European Association for the Study of the Liver. 2018; Haep et al. 2021).

Liver transplantation (LT) is one (if not the only one) of the most effective treatments for any patients with ESLD. LT would extend life expectancy of the patients regardless of the natural history of underlying liver disease where LT is expected to improve the quality of life. However, in practice, LT is hampered by the shortage of donor organs, the limited number of liver transplantation facilities, and the high cost (Harries et al. 2019). Recently, the possibility of living donor liver transplantation (LDLT) can be another option. However, LDLT needs immense and complicated technical operations. And still, the donor shortage remains a concern (Au and Chan 2019; Choudhary et al. 2022).

Following LT, further, the liver recipient might suffer postoperative complications, transplant rejection, and long-term immunosuppression side effects (Feng and Bucuvalas 2017). Further, de novo malignancies are often detected in liver transplant patients undergoing daily immunosuppression regimens, one of the leading causes of late death. The incidence of de novo malignancies among transplant patients is predicted up to four times higher than in the healthy population (Herrero 2012; Manzia et al. 2019).

Since 2002, the Model for End-Stage Liver Disease (MELD) has been used to rank liver transplant candidates for ESLD (Kamath et al. 2001; Wiesner et al. 2003). It is considered an effective strategy for prioritizing candidates with a higher transplant survival benefit over those with lower survival benefit (Luo et al. 2018). This scoring system predicts liver disease severity based on serum creatinine, serum total bilirubin, and INR. It was previously shown to be useful in predicting mortality in patients with compensated and decompensated cirrhosis (Wiesner et al. 2003).

In brief, MELD score ranks patient to number 6 to >40 using the formula ($0.967*\log_e$(creatinine (mg/dL)) + $0.378 \times \log_e$(bilirubin (mg/dL)) + $1.120 \times \log_e$(INR) + 0.6431) × 10) and is suitable as a disease severity index to determine organ allocation priorities (Kamath et al. 2001). Regardless of various revisions and updates (MELD 3.0, MELD-Na, etc.) (Nagai et al. 2018; Kim et al. 2021), the change in MELD score is used as an indicator to measure the benefits of therapy following LT or other treatment regimens.

2 Liver Development and Regeneration

Liver is not only the largest internal organ in the body; it is also capable to replenish its mass by self-regeneration capacity. From a liver phenotypic point of view, it reflects the broad metabolic functions of hepatocytes as well as the liver's unique vascular anatomy, having an inflow

blood supply from both an arterial (hepatic artery) and venous (portal vein) sources (Haep et al. 2021).

Following liver injury, hepatocytes can proliferate to reinstate their morphological and physiological function. In the 1930s, liver regenerative ability in a murine model of partial hepatectomy (PH) had been evidenced. Following PH of around 70% of its total mass, the liver was recovered in about 1 week (Higgins 1931). Using thymidine tracking in the DNA, the restoration of liver mass and function was further demonstrated (Bucher and Swaffield 1964).

In the case of sustained damage such as fibrosis and impaired hepatocytes regeneration, the liver needs to activate its resident stem cells compartment. The canals of Hering and bile ductules in the human liver contain liver progenitor cells (LPC) that can differentiate toward the biliary and hepatocytic lineage (Theise et al. 1999; Libbrecht and Roskams 2002). The source of the LPC is still unclear. It has been variously demonstrated that adult mature hepatocytes can be reprogrammed into proliferative bipotent progenitor cells in response to chronic liver injury (Tarlow et al. 2014; Hu et al. 2018). A population of EpCAM+ cells has been identified within the canals of Hering and the bile ductules, serving as facultative bipotent progenitors capable of differentiating into hepatocytes and cholangiocytes (Safarikia et al. 2020).

During liver disease, the degeneration from healthy-functioning livers involves a dynamic process of hepatocyte damage leading to the reduction of hepatic function. As already known widely, the activation of stellate cells and the production of extracellular matrix (ECM) are the keystone of liver fibrosis. In the case of cirrhosis and ESLD, hepatocyte proliferation or liver regeneration is finished (Haep et al. 2021). Liver failure is also majorly influenced by the exposure to an inflammatory setting, a loss of cell-cell contact caused by cell death and ECM deposition, and changes in energy metabolism and transcriptional deprogramming of hepatocytes (e.g., HNF4α, HNF1, FOXA, HNF6, and C/EBP). Further, clinical manifestations in patients with ESLD are directly related to specific alternated metabolic pathways in failing hepatocytes (Haep et al. 2021).

ESLD is not only due to the lack of healthy hepatocytes but also to the disturbance of tissue architecture and the continuous deposition of inflammatory cells (Lorenzini et al. 2008). Thus, when ESLD occurs, it is hard for the liver to establish its capacity to regenerate.

3 Stem Cell Therapy

Cell therapy has been thought of as the source of liver regeneration (Fig. 1). For therapy applications, donor cells must act as fully functional differentiated cells, such as the expression of liver-specific markers and secretion of albumin and alpha-fetoprotein. Thus, careful protocol and cell characterization should be verified before the transplantation.

Freshly isolated primary human hepatocytes (PHH) are currently the benchmark cell type for cell therapy, but they are not readily available, dedifferentiate quickly, and rapidly die in culture (Hannoun et al. 2016). Several groups had reported methods to cryopreserve the PHH (Godoy et al. 2013; Sison-Young et al. 2017). Despite various optimization protocols, cryopreservation still has damaging effects on the viability and metabolic function (Hannoun et al. 2016). Further, a rather large number of cells (10–15% of liver mass) are needed to provide enough function (Fitzpatrick et al. 2009). So far, various studies had demonstrated the clinical application of hepatocyte transplantation in liver diseases (Lee et al. 2018). In chronic liver disease, however, there are some hassles with engraftment since the liver architecture is disrupted. It is one of the causes of the common failure in hepatocyte transplantation to date (Fitzpatrick et al. 2009).

Stem cells have the astonishing proliferative capacity, self-renewal ability, and differentiation properties. Due to their plasticity, stem cells have been proposed as a source for cell therapy. Embryonic stem cells (ESCs) are the most pluripotent cells that can become all cell types in the body. They are derived from the embryo, typically from the inner cell mass in the blastocyst.

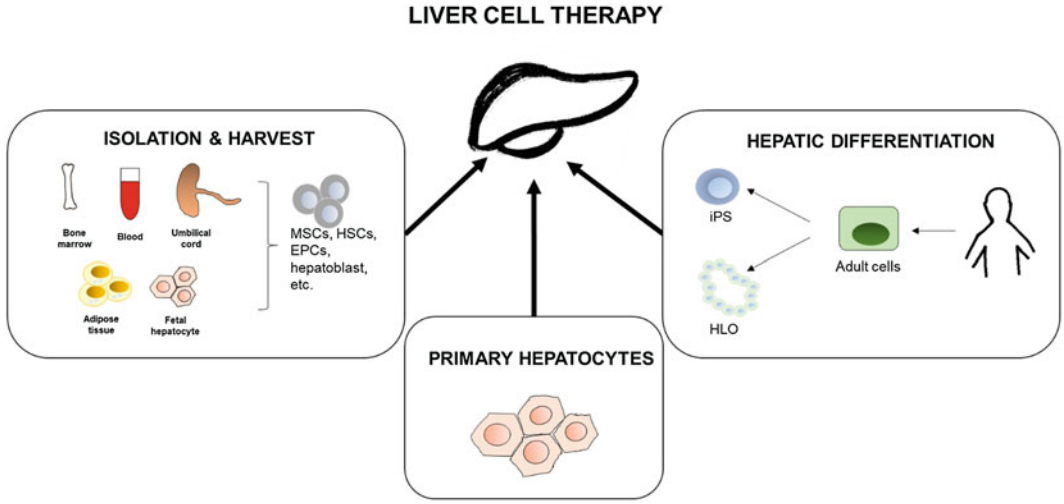

Fig. 1 Organ sources of cellular therapy for the liver

Due to its pluripotency, the human ESCs would be potent tools in regenerative medicine such as Parkinson's disease, spinal cord injury, myocardial infarction, and many more (Mountford 2008). ESCs have been demonstrated to have potential in cell therapy for liver disease. ESCs transplantation had been demonstrated to reduce liver fibrosis and to engraft the liver in rodents (Heo et al. 2006; Sharma et al. 2008; Moriya et al. 2008; Haideri et al. 2017). It is important to notice, however, that ESCs implantation may be tumorigenic where teratoma can occur (Fujikawa et al. 2005; Blum and Benvenisty 2008; Hentze et al. 2009; Stachelscheid et al. 2013). In the human study, it also has a significant ethical dilemma because it involves the destruction of an embryo to obtain the ESCs.

Adult stem cells (or somatic stem cells) can be found in a small number of undifferentiated cells in a specific area of tissue or organ in the body. Even though they are not as multipotent as the ESCs, the adult stem cells can easily be obtained and differentiated into various cells. More importantly, these cells can be ideal sources for autologous stem cell transplantation to replenish tissue damage in the same patient. The bone marrow (BM) compartment is the major source of committed progenitor (stem) cells that can develop into mesenchymal lineages and hematopoietic cells (Masson et al. 2004).

In the beginning, it was assumed that adult stem cells could differentiate only into their maturation lineages. For instance, bone marrow stem cells could only differentiate into blood cells. However, more studies demonstrated that adult stem cells are multipotent and they can differentiate into various cells. For example, bone marrow-derived stem cells could regenerate de novo myocardium (Orlic et al. 2001); skeletal (Gussoni et al. 1999), adipocytic, chondrocytic, or osteocytic lineages (Pittenger et al. 1999); microglial and perivascular cells in the brain (Corti et al. 2002; Hess et al. 2004); as well as the liver cells (Petersen et al. 1999). The injection of these cells ameliorated the outcome of diseases.

To date, there have been numerous clinical studies on adult stem cell therapy for the treatment of ESLD registered in the public database (https://clinicaltrials.gov/) even though many of these studies' results are still unavailable. As the primary outcome, usually, these studies measure the improvement of the MELD score and liver function as the success of the treatment.

3.1 Mesenchymal Stem Cells

The mesenchymal stromal/stem cells (MSC) is the most common stem cells used in clinical

therapy, in addition to being the most controversial. The term mesenchymal stem cells was firstly named in the late 1980s by Dr. Caplan for a cell type derived from bone marrow. These cells could be isolated and expanded in culture while maintaining their in vitro capacity to be induced to form a variety of mesodermal phenotypes and tissues (Caplan 1991). In regard to their multidifferentiation capacity and high self-renewal ability, MSC are a good option for promoting tissue regeneration and inhibiting fibrosis and, at the same time, lessening tissue inflammatory response (Xiang et al. 2022).

In the last three decades, however, the exponential growth of scientific articles had used this nomenclature across numerous isolated cells. In some cases, these cells are various tissue-specific cell types with the use of different cell-surface markers (Sipp et al. 2018), leading to confusion in the scientific community and clinical practice. A previous study demonstrated that "MSC" isolated from different anatomical sources (bone marrow, skeletal muscle, periosteum, and perinatal cord blood) actually differed widely in their transcriptomic signature and in vivo differentiation potential (Sacchetti et al. 2016).

Back in 2005, a working group of the International Society for Cellular Therapy (ISCT) acknowledged the MSC inconsistencies and ambiguities, and they recommended a new designation: multipotent mesenchymal stromal cells (Horwitz et al. 2005). The ISCT also proposed minimal criteria to define human MSC. First, MSC must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules. Third, MSC must differentiate into osteoblasts, adipocytes, and chondroblasts in vitro (Dominici et al. 2006). Another term of medicinal signaling cells (also abbreviated as MSC) was proposed by Dr. Caplan to more accurately reflect the fact the tissue origin or disease and secrete bioactive factors that are immunomodulatory and trophic (regenerative) medicine (Caplan 2010; Caplan 2017). Regardless of the nomenclature, the capacity of the MSC in the repair of liver tissues had been widely studied with various results.

Several sources of MSC had been used in various clinical trials for ESLD, with the most common sources being umbilical cord (UC) and bone marrow (BM; autologous or allogenic). Some also take advantage of adipose tissue-derived MSC, naively or following cell differentiation (Nhung et al. 2015). As for adipose tissues, sources are broad, and cells can be collected from the subcutaneous tissue, viscera, omentum, inguinal fat pads, peritoneal fat, and other sources (Hu et al. 2019).

As expected, the results of these studies are variable. The injection of autologous BM with CD44+ phenotype had resulted in short benefit in treated patients, regardless of the delivery method (hepatic or peripheral transfusion) (Kharaziha et al. 2009; Peng et al. 2011; Amin et al. 2013; Salama et al. 2014). In these studies, MSC-injected patients had improvement in their liver function and MELD and CP scores compared to control. A meta-analysis of five studies showed that bone marrow infusion in the treatment of decompensated cirrhosis improved liver function without serious side effects at least for the first year (Pan et al. 2014). However, at least in one of the studies, the long-term outcomes were not markedly improved with no significant difference in the incidence of hepatocellular carcinoma (HCC) or mortality between the two groups (Peng et al. 2011).

A recent report from a Japanese clinical trial (UMIN Clinical Trials Registry UMIN000022601) using freshly isolated autologous adipose tissue-derived stem cells in seven patients also showed promising results. Stem cell transplantation improved serum albumin in six out of seven patients and prothrombin activity in five out of seven patients. No trial-related adverse events, which were serious or nonserious, were observed (Sakai et al. 2020; Sakai et al. 2021).

For donor transplantation, the infusion of allogenic MSC from donors was also considered a safe procedure. In patients with liver failure, donor MSC significantly increased the survival rate by improving liver function (reduction of ascites volume, increase of albumin, decrease of

bilirubin, improvement of CP and MELD score) and decreasing the incidence of severe infections (Zhang et al. 2012; Lin et al. 2017; Schacher et al. 2021). In a longer study, upon allogeneic MSC infusion (obtained from donor BM, cord blood, and umbilical cord), MELD score improved at 6 months, 1 year, and 2 years of follow-up. No serious adverse events were observed during or after infusions of MSC in patients with decompensated cirrhosis as compared to control patients (Zhang et al. 2012; Liang et al. 2017). UC-derived MSC transfusion also increased liver function and survival rate in ACLF patients, either by intravenous infusion or hepatic arterial transfusion (Shi et al. 2012; Li et al. 2016).

However, in contrast, several studies showed no benefit of MSC transplantation. A previous study indicated the unsafety of the procedure, and even mortality, following cell transplantation. In a randomized, placebo-controlled trial, from 15 autologous MSC-injected patients, there were 3 deaths registered, while the rest of the patients did not show any improvement in liver function and CP or MELD score (Mohamadnejad et al. 2013). Another study had shown that in this study, even though it was considered safe and feasible, consecutive liver biopsy examinations suggested that MSC infusion via peripheral vessel could not reach the liver in a sufficient amount; thus there were no improvements in MELD scores and serum albumin (Kantarcıoğlu et al. 2015).

3.2 Hematopoietic Stem Cells

Hematopoietic stem cells (HSC) are the most accessible source of stem cells in the body. They give rise to all lineages of blood cell differentiation. In the beginning, it was thought that CD34 (CD34+ cells) is the HSC marker in mammals; however, then it was noticed that human CD34- also had self-renewing capability and acted as primitive HSC that could give rise to CD34+ cells (Zanjani et al. 1998; Wang et al. 2003; Sumide et al. 2018).

The differentiation capacity of HSC, especially to hepatic lineage, is still limited if not controversial. Previously, it was shown that HSC could become liver cells when co-cultured with injured liver separated by a barrier (Jang et al. 2004). In mouse model studies, the transplantation of human cord blood cells CD34+ was able to repopulate the liver (even though with a very low percentage) showing the contribution of HSC (Masson et al. 2004). However, this potentiality was challenged over time. A study showed that HSC expressed mRNAs of hepatic cell markers, but could not efficiently convert into hepatocytes in vitro even in the presence of cytokines or co-cultured hepatocytes (or tissue) (Lian et al. 2006). As mentioned by Thorgeirsson and Grisham, it seemed that the hematopoietic cells are only a minor contributor to hepatocyte formation under either physiological or pathological conditions. These cells, however, may provide cytokines and growth factors that promote hepatocyte functions by paracrine mechanisms (Thorgeirsson and Grisham 2006).

In the clinical study, the application of HSC transplantation in the ESLD had been another option, even though it is not as frequent as the MSC, in line with this limitation described above. One of the first studies comprised a rather small number (phase 1); autologous CD34 was injected into five patients with liver insufficiency. Patients were previously given subcutaneously granulocyte colony-stimulating factor (G-CSF) for 5 days to increase the number of harvested CD34+ cells from the circulation. Following portal vein or hepatic artery injection of these cells, four patients showed improvement in serum albumin (Gordon et al. 2006).

In another study which used the same method, 90 ESLD patients received G-CSF followed by autologous CD34+ and CD133+ HSC infusion in the portal vein. Up to 6 months of follow-up, around 50% had near normalization of liver enzymes and improvement in synthetic function, and 14% showed stable states, compared to control group (Salama et al. 2010). From the same group in another study, stem cell transplantation was done via portal vein infusion of 50% of HSC (CD34+/CD133+), and the other 50% were differentiated to MSC and infused systemically in a peripheral vein in the presence of growth

factors. This procedure had a low incidence of complications and it improved CP and MELD score and degree of ascites of the patients. When the infusion was done in two sessions, the sustained response was continued throughout the follow-up period of 12 months (Zekri et al. 2015).

Another group had shown that the infusion of cell population with CD133+ marker (stem/progenitor cell (SPC)) in ESLD patients was feasible and safe and improved liver function transiently. The recollection of SPC after G-CSF treatment was associated with increased levels of selected cytokines potentially facilitating SPC function (Catani et al. 2017).

Hematopoietic cell isolation and injection from BM also had been performed. In this study, autologous mononuclear (CD34/CD45+) from BM was infused via the peripheral vein in nine patients. Following the procedures, no major adverse effects were noticed. Infused patients had significantly improved CP scores at 1 and 6 months together with improvement in liver biopsy (Terai et al. 2006).

3.3 Endothelial Progenitor Cells

The endothelial progenitor cells (EPC) were discovered around two decades ago (Asahara et al. 1997). These cells were purified by magnetic bead selection with the surface markers antigens CD34+ and Flk1+; in vitro, these cells differentiated into endothelial cells (Asahara et al. 1997). As in MSC the nomenclature of EPC is still under discussion, where another term "endothelial colony-forming cells (ECFC)" is also used (Prasain et al. 2012; Keighron et al. 2018). This disagreement in consensus needs a more precise characterization of these cells based on a pre-defined cellular phenotype and function (Medina et al. 2017).

By using a nonhuman primate model, the localization of injected autologous EPC/endothelial cells (EC) can be traced. At 14 days postinjection via the portal vein, these cells were found scattered in the intercellular spaces of hepatocytes at the hepatic tissues, indicating successful migration and reconstitution in the liver structure as the functional EPC/EC (Qin et al. 2018). Another study examined the benefit of BM-EPC in a rat model of liver fibrosis/cirrhosis induced by carbon tetrachloride (Sakamoto et al. 2013; Lan et al. 2018). While EPC transplantation gave a beneficial result, combined transplantation of BM-EPC and BM-derived hepatocyte stem cells exhibited maximal treatment effect (Lan et al. 2018).

The transplantation of EPC in decompensated liver cirrhosis patients had been reported. In this phase 1–2 pilot clinical trial, autologous cells were harvested from the bone marrow of patients subjected to differentiation to EPC ex vivo. Following hepatic arterial administration in 11 patients, no treatment-related severe adverse events were observed. At 90 days post-transplantation, there was a significant improvement in MELD, and five of nine patients alive showed a decreased hepatic venous pressure gradient (D'Avola et al. 2017).

3.4 Fetal Human Hepatocytes

Fetal liver is becoming an available source of cells for the treatment of liver diseases. Group of Cardinale et al. defined fetal liver as the liver developed from 10 weeks of gestation, the timing when the hematopoietic progenitor cells migrate from the aorta-mesonephros-gonad region to colonize the liver (Giancotti et al. 2022). It contains hepatic stem/progenitor cells within the ductal plates and multipotent stem/progenitor cells within large intrahepatic bile ducts and extrahepatic bile ducts (Semeraro et al. 2013).

Still, limited information is available for the clinical application of fetal liver for ESLD. An Indian clinical study of fetal liver transplantation in 25 end-stage liver cirrhosis patients showed clinical improvement observed in terms of all clinical and biochemical parameters together with a decrease of MELD in 6 months' follow-up in all patients. These cells were obtained from fetal livers of spontaneous abortions from 16 to 20 weeks of gestation and showed positivity of EpCAM+ (Khan et al. 2010). A comparable result was obtained from a study in Italy. Following fetal liver transplantation in an ESLD patient,

the MELD score decreased from 15 to 11 at 3-month and 10 at 18-month follow-up with no signs of encephalopathy. These cells expressed highly significant amounts of proliferation markers compared to adult hepatocytes (Gridelli et al. 2012).

3.5 Hepatic Lineage Differentiation

Several studies had taken another additional step for the application of the MSC. Taking advantage of the multipotency ability, MSCs obtained either from BM, UC, or adipose tissues can be subjected to a hepatic lineage differentiation in vitro before the infusion into the patient/recipient. For example, adipose-derived MSC can be differentiated into hepatocytes in 14 days' culture condition with hepatogenic medium containing dexamethasone, insulin, hepatocyte growth factor (HGF), and epidermal growth factor (EGF), followed by activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway (Liang et al. 2009).

One of the first clinical studies using this approach in ESLD was reported in 2011. In this study, upon the isolation and the phenotyping of the autologous BM-MSC, MSC was stimulated into hepatic cells using in the presence of HGF for 7 days. The hepatic-committed lineage was then evaluated by morphological, immunophenotyping, and albumin production. Cells were then injected via the intrasplenic or intrahepatic route. The result showed that MSC-infused patients had significant improvement in ascites and serum albumin, CP, and MELD score over the control group. No difference was observed between intrahepatic and intrasplenic groups (Amer et al. 2011).

Another study used a two-step MSC differentiation into the hepatic lineage, using HGF and FGF, continued by oncostatin and dexamethasone. In this phase 2 trial, however, cells were injected intravenously. MSC-received patients showed partial improvement in liver function tests and MELD score at 3 and 6 months post-infusion. However, there was no significant difference regarding clinical and laboratory findings for MSCs transplantation of either undifferentiated or differentiated cells (El-Ansary et al. 2012).

4 Cell Reprogramming

In the last decades, advances in molecular and cellular biology technologies open exponential opportunities in the manipulation of cellular fate. One of the greatest breakthroughs of the century is the discovery that mature cells can be reprogrammed to become immature, even pluripotent cells, leading to a greatly appreciated shared Nobel Prize in Physiology or Medicine 2012 awarded to Sir John B Gurdon and Shinya Yamanaka (https://www.nobelprize.org/prizes/medicine/2012/summary/).

Back in the 1960s, John Gurdon was successful in transplanting nuclei from fully differentiated cells from the intestine of a tadpole into the cell nucleus of a frog's egg cell. The egg developed into a fully functional cloned tadpole. The transplanted nucleus promoted the formation of a differentiated intestinal cell and at the same time contained the genetic information necessary for the formation of all other types of differentiated somatic cell in a normal feeding tadpole (Gurdon 1962). This nuclear transfer technique was then widely publicized several decades later with the cloning of Dolly sheep, published in 1997 by Wilmut et al. (1997).

In 2006, by using four defined transcription factors Oct3/4, Sox2, c-Myc, and Klf4 (OSKM factors), Takahashi and Yamanaka showed that mouse fibroblasts could be reprogrammed into an embryonic stage, namely, the induced pluripotent stem cells (iPSC). These iPSC cells exhibited ESCs morphology and growth properties and ESCs marker genes. Furthermore, subcutaneous transplantation of iPSC cells into nude mice resulted in variety of tissues from all three germ layers (Takahashi and Yamanaka 2006). In the following year, this technique was then proven in a human cell. Human iPSC cells were similar to human ESC in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase

activity (Takahashi et al. 2007). Because of its ESC-like pluripotency, iPSC is a valuable tool in the basic research on the mechanisms of tissue formation, cell therapy, and patient-specific cell development.

4.1 Induced Pluripotent Stem Cells (iPSC)

First data on the iPSC differentiation to functional hepatocytes was reported in 2009 by Song et al. (Song et al. 2009). They used iPSC cell lines 3U1 and 3U2 subjected to hepatic differentiation protocol composed of four stages: endoderm induction (activin A), hepatic specification (FGF4, BMP2), hepatoblast expansion (HGF, KGF), and hepatic maturation (oncostatin M, dexamethasone, N2, B27, nonessential amino acids, and β-mercaptoethanol). The differentiated cells exhibited mature hepatocyte functions including albumin secretion, glycogen synthesis, urea production, and inducible cytochrome P450 activity (Song et al. 2009). This process takes around 21 days.

A more rapid protocol was then demonstrated. In about 12 days, iPSC could be directed into mature hepatocytes by using the protocol of endodermal induction (activin A, Wnt3a, HGF), hepatic lineage commitment (in the presence of nonessential amino acids, β-mercaptoethanol, DMSO), and hepatic (oncostatin M, dexamethasone, ITS) (Chen et al. 2012). The cells had similar gene expression profile to mature hepatocytes. Besides its functionality as mature hepatocytes including cytochrome P450 enzyme activity, secreted urea, uptake of low-density lipoprotein (LDL), and glycogen storage, these induced hepatocyte-like cells rescued lethal fulminant hepatic failure in a NOD-SCID mouse model (Chen et al. 2012).

The induction of iPSC into bipotent hepatic progenitor cells (HPC) gave rise to both mature hepatocytes and cholangiocytes (Yanagida et al. 2013). The induced-HPC from iPSC resulted in $CD13^{high}CD133+$ cells, positive markers of hepatoblast. Spheroid formation of the HPC could be induced into hepatocytes (dexamethasone, OSM) and cholangiocytes (EGF, HGF, R-spondin 1, Wnt-3a, A-83-01, and Y-27632) (Yanagida et al. 2013). The clinical application of iPSC was performed in several diseases such as degenerative and cardiovascular disease with various results (Martins et al. 2014; Bracha et al. 2017; Tsujimoto and Osafune 2021). However, for liver diseases, its application mostly is still conducted in a preclinical setting.

4.2 Human Liver Organoids (HLO)

Organoid biology is one of the fastest-growing interests in recent organ development and regeneration study. The capacity of isolated cells to self-assemble to form an entire organism was already reported in the early 1900s. When siliceous sponges are kept in confinement under proper conditions, they degenerate and gave rise to small masses of undifferentiated tissue which in turn grow and differentiate into perfect sponges (Wilson 1907).

Human liver organoids (HLO) derived from either adult stem/progenitors or pluripotent stem cells emulate the structure and cellular diversity of the human liver in vivo (Chang et al. 2021; Reza et al. 2021). Under a strict cell culture condition and the presence of correct growth factors (e.g., matrigel, TNFα), organoids can resemble a functional liver. A recent report even showed that from a single hepatocyte, organoids can be established and grown for multiple months while keeping its key morphological, functional, and gene expression features (Hu et al. 2018). However, when compared to the fetal culture, HLO derived from hepatocytes appeared to be more limited in their expansion times yet yielded organoids of very similar composition (Hu et al. 2018).

In the clinical application, HLO technology is not yet available, even though preclinical data in the animal model showed promising result. In a PH model in rat, the transplantation of HLO through portal vein is safer and more effective compared to monolayer cell transplantation, showing 70% replacement of the damaged liver (Tsuchida et al. 2019). Further, HLO in

combination with co-culture with other cell lines and advanced bioengineering tools (sheet layers, microfluidics, 3D scaffold) will increase the differentiation efficiency and enhance the functional maturity.

5 General Perspective

Stem cell therapy is a promising alternative for the treatment of ESLD, especially when the availability of donor liver for LT is scarce. Thriving development of technology in stem cell isolation and maintenance, characterization, and in vitro differentiation to hepatic cells is growing fast, thus allowing an improved method in clinical application.

In ESLD, however, at least until now, stem cell therapy application is still rather far from ideal. The biology of stem cells is still needed to be explored. Clinicians and basic scientists must know whether the transplanted cells are multipotent and self-renewable or the cells' phenotype (Fig. 2), both in donor cells and in the recipient patient, including the protocol of administration, patient's status, safety, and efficacy. Further, vast differences in the source of the cells, type of the cells, transplantation protocol, and criteria of recipients render technical hitches. The administration of stem cell injection (quantity and mode of delivery) may vary between laboratories based on each protocol and experience. Several studies were conducted to definite numbers of stem cells for the injection, while others calculate the body weight of the recipient. Similarly, several studies preferred intrahepatic administration while others via intrasplenic or peripheral vein. Therefore, so far, there is no definite indication or international consensus regarding the protocol of adult stem cells in ESLD patients.

Apart from a scientific perspective, the clinical application of cell therapy is related also to the vast speed of the internet spread. Advances in information technology significantly increase the global transfer of knowledge, including in the search for stem cell therapy in one click. As can be seen in cell therapy for regenerative medicine, the so-called stem cell tourism (Berger et al. 2016; Sipp 2017) is also a problem in hepatology and gastroenterology (Hermerén 2014). This problem requires prompt action for the regulation of cell therapy, from scientists, clinicians, professional associations, and government or authorities. Stem cell therapy for ESLD had shown some promising results, but more research and the definition of a better protocol are still significantly needed.

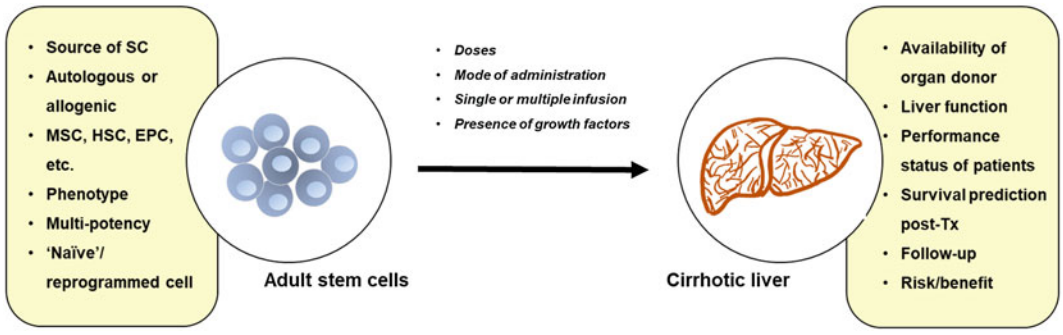

Fig. 2 Important factors for adult stem cell therapy for ESLD. Stem cell therapy would need to consider aspects both in the donor cells (source, types, phenotypes, potency) and in the recipient (liver status, patients' performance, risk/benefit), together with the mode of administration (site, presence of growth factor, doses) and correct protocol

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