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Review Article

Stem cell transplantation for treating liver diseases: progress and remaining challenges

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Abstract: With the development of regenerative medicine, various stem cells are increasingly considered for treating liver diseases. Various stem cells have been reported to play an essential role in liver recovery, and studies have verified the preliminary effectiveness and safety of these therapies. Stem cell-based therapies will emerge as an effective treatment strategy for liver diseases. Thus, the research progress and challenges to the related stem cells were reviewed, namely the classification of stem cells, cell culture, transplantation, cell tracing in the body, therapies for various liver diseases.

Keywords: Stem cell, liver diseases, transplantation, therapy

Introduction

Liver disease is a significant cause of death and disability. Stem cells, which are characterized by their potential for self-renewal, high proliferation, and multi-directional differentiation [1], have been used for treating an increasing number of diseases, including central nervous system diseases, macular degeneration, diabetes, and tumors [2, 3]. At present, accumulating evidence has revealed that the immunomodulation and differentiation characteristics of stem cells play an important role in liver regeneration and repairment [4-8]. Although most studies are limited to cellular and animal research, and clinical transformation still needs multiple trials, with the enrichment and deepening of research technology and content, stem cells are expected to transform the treatment of liver disease, especially end-stage liver disease. This article reviews the recent research progress on the classification of related stem cells, cell culture and transplantation, cell tracking, and treatment of liver diseases.

Stem cells for treating liver diseases

Current studies have shown that many kinds of stem cells ameliorate liver injury and may be used to treat liver diseases, including mesenchymal stem cells (MSCs), liver stem cells (LSCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and peripheral blood stem cells (PBSCs) (**Table 1**).

Characteristics of mesenchymal stem cells (MSCs)

MSCs are pluripotent stem cells isolated from bone marrow, adipose tissue, umbilical cord blood, placenta, and other tissues. Mesenchymal stem cells belong to adult stem cells. MSCs can treat many diseases because of their wide range of sources, low immunogenicity, and the ability of self-proliferation and differentiation [9, 10]. There are many studies on bone marrow mesenchymal stem cells (BM-BMSCs), adipose mesenchymal stem cells (AD-MSCs), and umbilical cord mesenchymal stem cells (UC-MSCs) in the basic and clinical aspects of liver disease [11-13]. MSCs can differentiate into various adult mature cells under specific induction conditions in vivo and in vitro. Many studies have indicated that MSCs can differentiate into liver-like cells under the action of growth factors, cytokines, hepatocytes, or non-parenchymal hepatocytes [14, 15], and participate in the immune regulation,

Table 1. Advantages and disadvantages of stem cell types used for liver diseases

Cell type	Advantages	Disadvantages
MSCs	• Easy access from multiple tissues	• Limited cell quantity
	• Rapid in vitro expansion	• Limited differentiation potential
	• Low ethical concerns	• Undefined in situ phenotype
	• Transplantation of autologous and allogenic cells due to low immunogenicity	• Difficult to generate mature hepatocytes on a large scale
	• Beneficial immunomodulation	• Inconsistent therapeutic results
	• Low risk of tumorigenicity	
	• Proof of safety in clinical trials	
LSCs	• Possible generation of cell lines	• Access from the invasive hepatic biopsy or partial liver resection
	• Bidirectional differentiation into hepatocytes or cholangiocytes	• Undefined in situ phenotype
	• Low ethical concerns	• Insufficient cell characterization
	• Low risk of tumorigenicity	• Lack of standardized generation procedure
	• Transplantation of autologous and allogenic cells due to low immunogenicity	• Lack of adequate clinical trials
	• Beneficial immunomodulation	
	• Proof of safety in animal experiment	
	• Proof of safety in a phase IIa clinical trial	
iPSCs	• Easy access from multiple tissues	• Difficult to generate mature hepatocytes in large quantities
	• Pluripotent differentiation potential	• Genomic instability
	• Low ethical concerns	• Risk of tumorigenicity
	• Suitable for autologous transplantation	• Risk of immunologic rejection
	• Unlimited quantity	• Lack of standardized generation procedure
ESCs	• Pluripotent differentiation potential	• Difficult to generate mature hepatocytes in large quantities
	• Unlimited quantity	• Ethical concerns
	• Easy generation of cell lines	• Risk of tumorigenicity
	• Allows generation of off-the-shelf cell products	• Genomic instability
	• ESCs-derived hepatocytes integrate into the host liver tissue	• Lack of availability
		• Risk of immunologic rejection
PBSCs	• Defined in situ phenotype	• Difficult access from peripheral blood
	• Low ethical concerns	• Low cell quantity
	• Suitable for autologous transplantation	• Limited differentiation potential
	• Rapid expansion after colony-stimulating factor mobilization	• Lack of adequate clinical trials
	• Low risk of tumorigenicity	

cell proliferation, and injury repair in liver diseases [16]. MSCs are rich in sources, easily obtainable and cultured, have low immunogenicity. Based on these advantages, MSCs are expected to become an ideal source of seed cells for stem cell research in liver diseases.

Characteristics of liver stem cells (LSCs)

LSCs are adult stem cells found in the liver, mainly including hepatic oval cells (HOC) and small hepatocyte-like progenitor cells (SHPC). An oval shape, small cell body, large nucleus, and small cytoplasm characterize HOC, which can differentiate into hepatocytes or bile duct epithelial cells in both directions [17]. The morphology of SHPC is between that of stem cells and bile duct cells. Usually, an adult HOC differentiates into SHPC and then into mature hepatocytes. Most scholars believe that LSCs are located around the portal vein and in the Hering duct of the terminal bile duct [18]. Recently, a study has identified a population of proliferating and self-renewing cells adjacent to the central vein in the liver lobule. These cells can differentiate into hepatocytes and replace all hepatocytes along the liver lobule during homeostatic renewal [19]. Liver stem cells are ideal for treating liver diseases because of their directional differentiation into hepatocytes or cholangiocytes, and lake of ethical concerns. Still, there are rare reports about human liver stem cell lines at home and abroad [20-22]. After years of exploration, our research group has successfully developed and isolated human liver stem cell line (named: HYX1) from the adult liver. Human liver stem cells can be maintained for 50 generations, and two mouse liver stem cell lines (named: YE, R5) can be passed for more than 100 generations. At present, all these cells have been applied in experimental animal research [23-26].

Characteristics of induced pluripotent stem cells (iPSCs)

iPSCs are stem cells with stem cell characteristics obtained from adult cells reprogrammed by viral vectors. They have similar functions to ESCs but, at the same time, bypass ethical disputes and immune rejection of ESCs [27]. Some scholars consider that iPSCs have the characteristics of reprogramming, high self-renewal, and multi-directional differentiation, which increase the risk of mutation and tumorigenesis. However, a recent study through a comparative analysis of iPSCs and subclonal replicated cells found that most mutations in iPSCs do not occur in the process of reprogramming or iPSC production, suggesting that iPSCs can be safely used in basic and clinical research. Recently, a new programming technique called pedigree reprogramming was developed based on classical iPSC reprogramming, which bypasses iPSCs to realize the transformation of cell types within or between lineages [28]. Through this technique, Wang Yunfang's team reprogrammed the digestive tract epithelial cells into Human-induced endodermal progenitor cells (hiEndoPCs) using small molecular compounds rather than transcription factors. hiEndoPCs can differentiate into mature hepatocytes in vitro and integrate into the liver of experimental mice, which provides a safer and more effective source of seed cells for treating liver diseases, especially end-stage liver diseases [29].

Characteristics of embryonic stem cells (ESCs)

ESCs obtained from the endoderm of fertilized eggs, are highly undifferentiated and are the most proliferative pluripotent stem cells [30]. Two groups from Kyoto University and the National Center for Child Health and Development (NCCHD) established a novel derivation/cultivation system of ESCs. Twelve ESCs are now available for industrially and clinically applicable regenerative medical products [31]. Under appropriate induction and culture conditions in vitro, ESCs can differentiate into mature hepatocytes. Mouse ESCs were transplanted into the mouse model of liver injury. A large number of transplanted cells were observed in the mouse liver and showed a state of proliferation [32]. The unlimited proliferation ability and differentiation potential of ESCs have high basic and clinical research value in liver diseases. Still, the ethical and legal disputes, immune rejection, and tumorigenesis risk of ESCs limit its research and application for treating clinical diseases [27].

Characteristics of peripheral blood stem cells (PBSCs)

PBSCs are the only $CD34^+$ cells in peripheral blood. Under normal conditions, the number of PBSCs is insignificant, but it increases rapidly after colony-stimulating factor mobilization. After collection, PBSCs can be used for treating

diseases, especially blood diseases. Recently, it was shown that PBSCs can also be used in the treatment of liver injury [33-35]. However, research on the specific mechanism and stability of PBSCs for treating liver diseases is still rare. Whether PBSCs are an ideal cell source remains to be further verified.

Methods for cell culture and transplantation

In the related research of stem cells for treating liver disease, stem cells in different cultured states can be transplanted to the experimental animal models or human body through different ways to play a corresponding role. At present, stem cells in vitro mainly proliferate and differentiate under two-dimensional (2D) culture conditions. Although 2D systems have the advantages of easy operation and low cost, the growth state, morphology, structure, and function of the cells are significantly different from growth in vivo. Thus, the in vitro results cannot replicate in vivo experimental results, and ideally, the cells should be cultured in a three-dimensional space.

In recent years, three-dimensional (3D) culture technology has been widely studied in stem cell culture, including 3D culture techniques that require scaffold and those that do not require it [36-40]. At present, examples of the common 3D cell scaffolds include collagen, hydrogel, and microfiber. With the development of 3D biological printing and related technology, new types of 3D scaffolds emerge one after another, providing a more effective culture system for stem cell research. Non-scaffold 3D culture technology operates mainly through physical methods that keep the cells in a state of suspension, including microcarrier, hanging drop, ultra-low attachment surface, and micro molding. Recently, a study used microfluidic technology to culture hepatocytes and fibroblasts in a water/water/oil double emulsification as a template to construct a miniature 3D liver model in a droplet [41]. This culture model may also have some reference value in stem cell research. Studies have shown that 3D culture can better optimize the production of stem cell exosomes compared with the 2D culture [42, 43].

At present, the main ways to transplant stem cells into animals or human bodies are injections through a peripheral vein (such as rat tail vein, fundus vein plexus), portal vein, hepatic artery, and direct injection into the abdominal cavity, liver, and spleen. A meta-analysis of 23 controlled studies showed that a single MSCs injection was more effective than multiple injections, and that MSCs administration was more effective via the hepatic artery than the peripheral vein [44].

Besides, there are some novel ways of culture and transplantation. Japanese researchers cultured human induced pluripotent stem cell-derived hepatocyte-like cells (iPS-HLCs) on biomaterials to form cell sheets, and then attached the cell sheets to the liver surface of mice with liver failure to affect anti-liver damage. Simultaneously, compared with intrasplenic transplantation, this method reduces the risk of accidental transplantation of organs other than the liver [45]. Some studies induced the formation of liver bud by co-culturing human iPSCs, interstitial progenitor cells, and endothelial progenitor cells in vitro, and then ectopic transplantation into the abdominal cavity of immunodeficient mice to play a certain role in liver metabolism [46]. Moreover, stem cells are implanted into the acellular liver, and their matrix skeleton and vascular network structure are used to help support the differentiation and maturation of stem cells and the survival of mature cells [47]. Summing up the above studies, it was found that different culture methods, different periods, different times, and different ways of transplantation will produce different results. Choosing a suitable culture and transplantation scheme and studying a better one to preserve or even improve the function of stem cells is also one of the current stem cell research tasks.

Transplanted stem cells tracking

To better understand the implantation, distribution, survival, migration, differentiation, and function of stem cells, stem cell tracking methods are particularly relevant. These include fluorescent dye labeling, nucleic acid labeling, and reporter gene transfection markers, which all belong to the pathological tracking. At the same time, optical imaging, radionuclide imaging, and magnetic resonance imaging can be traced in vivo [48].

The following are three of the more common methods of cell tracking: 1. Cell membrane fluorescent dye labeling, such as red fluorescent

dye PKH26, can stably bind to the lipid region of the cell membrane, does not affect the biological activity and proliferation of the labeled cells, and can efficiently track the transplanted cells. Still, the fluorescent dye can be diluted and quenched with the proliferation and differentiation of stem cells, so it is only suitable for short-term cell-tracking research. 2. Lentivirus green fluorescent protein transfection enables stem cells to express green fluorescent protein efficiently and stably through gene modification mediated by a lentiviral vector, which can better trace the transplanted stem cells. Still, it may affect the biological characteristics of cells after transfection. The specific application needs to be compared and monitored. 3. A magnetic resonance imaging technique of superparamagnetic iron oxide nanoparticles labeling transplanted cells is a relatively novel technique. Through the combination of cell labeling and nuclear magnetic resonance imaging in vitro, it can effectively realize noninvasive in vivo tracking of transplanted stem cells [49]. Besides, according to the needs of the study, various pathways of cell tracking can be combined to achieve long-term cell tracking as well as for interrogating the cellular fate and tissue distribution of transferred cells [50, 51].

Stem cells and treatment of liver diseases

Liver failure

Liver failure is a clinical syndrome of severe liver dysfunction or decompensation caused by various reasons, and the mortality is extremely high. At present, liver transplantation is the only effective way in the end. However, as liver transplantation is limited by liver source, cost, and immune rejection after transplantation, there is an urgent need to find a new and effective treatment.

Stem cells provide a new direction for treating liver failure because of their self-renewal and differentiation potential. Recently, many related studies have been reported [6, 45, 52-54]. Italian researchers reported for the first time that human hepatic stem cells were used to treat a mouse model of fulminant hepatic failure induced by LPS/D-galactose. Human hepatic stem cells could alleviate hepatocyte apoptosis and promote hepatocyte proliferation in model mice, thus playing a protective role [20]. A study in 2017 showed that MSCs could rescue acute hepatic failure by polarizing M2 macrophages and altering levels of anti-inflammatory and pro-inflammatory factors [55]. Our group could significantly reduce ConA-induced acute liver injury in mice by intraperitoneal transplantation of adult human liver-derived stem cells, and reveal the best time and time for transplantation treatment [23, 25]. A domestic study successfully treated a large animal (pig) model of fulminant liver failure by intrahepatic transplantation of human BM-MSCs, during which new techniques such as large-scale antibody chip, whole gene sequencing, and protein spectrum analysis were used to explain the interaction between human BM-MSCs and host. The results showed that stem cell implantation could significantly inhibit the storm of lethal cytokines in fulminant liver failure. Paracrine suppresses the secretion of inflammatory mediators, regulates the immune response, alleviates liver injury, and finally promotes liver regeneration and repair, and reveals that delta-like ligand 4 (DLL4) molecules play an essential role in liver tissue repair after stem cell transplantation [56].

There is an increasing number of studies on stem cell therapy in patients with acute-on-chronic liver failure (ACLF) [57, 58]. Recently, a randomized controlled trial proved that infusion of MSCs into HBV-ACLF patients can reduce mortality by improving liver function and reducing complications such as infection [59]. A phase I study showed that $CD34^+$ cells from peripheral blood (PBSCs) after granulocyte colony-stimulating factor (G-CSF) mobilization could improve serum bilirubin and albumin in patients with chronic liver failure after this PBSCs infusion, and no specific side effects related to the procedure were observed [34]. Belgian biotechnology company Promethera presented the results of its phase IIa clinical trial of liver stem cells (LSCs) at the European Association For The Study Of The Liver (EASL) annual meeting in 2019. LSCs were infused intravenously in 12 patients with acute-on-chronic liver failure (ACLF) and 7 patients with acute decompensated liver disease (AD). Preliminary data show that LSCs can improve liver function with no obvious side effects.

To sum up the above studies, the main possible mechanisms of stem cell transplantation in improving liver failure include the differentia-

tion and proliferation into hepatocytes to play a substitute role, fusion with host hepatocytes, immune regulation, and promote residual hepatocyte proliferation and tissue repair.

Besides, stem cell derivatives such as microvesicles and exosomes are considered to be a new type of acellular immunotherapy through cell-to-cell communication, which is also a current focus of liver disease research [22, 60-62]. Filled with a selection of nucleic acids, lipids, and proteins, stem cell derivatives are used to exchange information between cells through ligand-to-receptor binding. The exact mechanism behind the therapeutic effect of stem cell derivatives remains largely obscure. However, current research shows that stem cell derivatives could inhibit the storm of inflammatory factors, suppress apoptosis and promote tissue restoration, to ameliorate liver failure. For example, studies have determined that adipose tissue-derived MSC-derived exosome-encapsulated microRNA-17 can improve acute liver failure by targeting thioredoxin-interacting protein (TXNIP) and inhibiting the activation of inflammatory bodies in hepatic macrophages [63].

The efficacy and safety of stem cells and their secreted microvesicles and exosomes in improving liver failure seem clear. However, there is still a lack of large-scale, long-term follow-up clinical trials, which need to be implemented.

Liver cirrhosis

The incidence of liver cirrhosis in China is increasing year by year, the effect of medical treatment of decompensated patients is poor. In recent years, stem cell transplantation has also achieved initial results for treating liver cirrhosis [64-68]. In a multicenter, randomized controlled study, 72 patients with alcoholic liver cirrhosis were treated with autologous arterial infusion of BM-MSCs. It was observed that BM-MSCs could improve liver fibrosis and liver function at the histological level, and proved to be safe [69]. In a long-term analysis of patients with decompensated liver cirrhosis, autologous transplants of PBSCs significantly improved long-term survival compared with a control group, and did not increase the risk of hepatocellular carcinoma [35]. Recently, a PRISMA-compliant meta-analysis based on the Chinese population found that Autologous bone marrow stem cell transplantation via the hepatic artery was safe and effective in treating hepatitis B virus-related cirrhosis without causing severe adverse events [70]. The possible mechanism was that stem cell transplantation could stimulate hepatocyte proliferation, repair damaged liver tissue, reduce tissue collagen deposition, inhibit liver fibrosis, reduce complications, and improve survival time [71]. However, a randomized controlled study suggests that peripheral vein autologous bone marrow MSC transplantation does not have a beneficial effect on patients with liver cirrhosis [72]. A multicenter, open, randomized controlled phase 2 trial in three UK hospitals found that infusion of granulocyte colony-stimulating factor and bone marrow $CD133^+$ hematopoietic stem cells in patients with liver cirrhosis did not improve liver function or the degree of liver fibrosis, and may be associated with an increased frequency of adverse events [73]. Therefore, the efficacy and long-term safety of stem cells for treating patients with liver cirrhosis need to be further discussed and verified.

Hepatocellular carcinoma (HCC)

There is an increasing number of studies on the treatment of hepatocellular carcinoma with stem cells [74, 75]. A Japanese study indicated that interferon-β (IFN-β) has an anti-cancer effect. Still, because of its rapid inactivation in vitro and poor tissue penetration, the drug cannot be widely used. iPS cell-derived myeloid lineage cells bone marrow cells (iPS-ML) derived from human pluripotent stem cells can be used to express IFN-β. After intraperitoneal injection of iPS-ML into a mouse model of liver cancer, it alleviates tumor progression and prolongs the survival time of mice. iPS-ML can be used as a drug delivery system and may have therapeutic value for patients with liver cancer [76]. Stem cell derivatives such as microvesicles and exocrine for treating liver cancer are also the focus of research. The microvesicles (MVs) derived from adult liver stem cells can transmit selected miRNA to inhibit the growth of hepatocellular carcinoma and stimulate apoptosis [21]. The exosomes of adipose mesenchymal stem cells modified by miR-122 and miR-199a can increase the chemosensitivity of hepatocellular carcinoma [77, 78]. The conditioned medium of adipose tissue-derived stem cells cultured in 3D inhibits the migration and invasion of hepa-

tocellular carcinoma cells by inhibiting epithelial-mesenchymal transformation [79]. These studies provide new ideas for treating liver cancer and are enlightening for the treatment of liver cancer.

Nonalcoholic fatty liver disease (NAFLD)

In the past 20 years, the incidence of NAFLD has increased rapidly, which is seriously harmful to human health. In addition to diet control, exercise, and reducing blood lipids, there is still a lack of specific drugs at home and abroad. Recent experimental studies have shown that stem cell transplantation can improve nonalcoholic fatty liver. AD-MSCs isolated from adipose tissue of rats were transplanted through the portal vein into NAFLD rats induced by a high-fat diet. Compared with the control group, liver histopathology of model rats showed remission of liver injury and a decrease of lipid accumulation. Serum proinflammatory factors such as tumor necrosis factor-α and interleukin-6 are down-regulated, that is, by improving liver function, promoting lipid metabolism, and reducing oxidative stress to help reverse NAFLD, to play a role in liver protection [15, 80]. Other studies have shown that exosomes of adipose-derived stem cells can improve insulin sensitivity, reduce obesity and reduce liver steatosis in obese mice, mainly by polarizing M2 macrophages and promoting browning in white adipose tissue [81]. It is suggested that stem cells and their derivatives may be a new potential therapeutic strategy for NAFLD.

Autoimmune liver disease (ALD)

The prevalence rate of ALD in China is increasing year by year, the clinical treatment is limited, and a certain number of patients have an inadequate response to internal medicine-specific treatment drugs. Looking for new autoimmune liver disease treatment methods has always been one of the research directions. A study has found that UC-MSCs transplantation can relieve fatigue and pruritus in patients with primary biliary cirrhosis (PBC) who do not respond to Ursodeoxycholic Acid (UDCA) therapy [82]. In 10 PBC patients who did not respond to UDCA therapy, after allogeneic bone marrow MSCs treatment, the number of $CD8^+$ T cells in peripheral lymphocytes decreased, whereas the $CD4^+CD25^+Foxp3^+$ T cells increased, furthermore, the corresponding indexes of liver function improved, and the treatment process was safe [83]. MSCs can play an immunosuppressive role and improve liver injury in mice with autoimmune hepatitis by activating the programmed death-1 (PD-1) pathway [84]. The exosomes derived from BM-BMSCs have a protective effect on liver injury in autoimmune hepatitis, and this mechanism may be related to the regulation of NLRP3 and caspase-1 by miR-223 in exosomes [85]. These studies suggest that stem cells and their derivatives can regulate the immune response and improve the degree of liver injury in ALD.

Challenges

Although stem cell transplantation might become a potentially efficacious tool to treat liver diseases and large numbers of in vivo and in vitro trials have been performed, there are still some vital challenges to be resolved before stem cells are widely used in the clinic.

The identification and differentiation of stem cells are some of the challenges ahead. After the cells are separated from the tissue, to induce the cells to differentiate into the desired cell types is another obstacle. To overcome this problem, researchers need to have a good understanding of the regulation of stem cells and determine the appropriate conditions for culture and induced differentiation.

Cell transplantation requires a sufficient number of cells to be effective, therefore, generating functional and transplantable hepatocytes at large scales is a major challenge that must be confronted. A great deal of accumulative evidence has suggested that 3D culture systems may better mimic the in vivo microenvironment and facilitate the derivation of hepatic lineages. Recently, researchers have established a large-scale suspension system that uses human endoderm stem cells (hEnSCs), which makes it possible to prepare high purity hepatocytes or cholangiocytes at large scales [86].

Although the immunogenicity of stem cells is very low, allotransplantation can still trigger the immune response. How to further reduce the immunogenicity is also a problem that researchers need to explore. Besides, stem cells may lead to tumor growth, and the genomic stability of stem cells used in cell therapy is a critical issue. In vitro cultured pluripotent stem cells and reprogrammed cells are prone to genomic abnormalities. It has been reported that

approximately 10% of pluripotent stem cells have at least one large chromosome aberration in a short time after induction [87]. Genomic instability is one of the characteristics of cancer, and scientists need to find a balance between promoting the cell growth and preventing cell from overgrowth. More in-depth research is urgently needed to understand diseases and endogenous repair mechanisms, and their interaction with stem cells.

Although a variety of induction systems have been developed, it is still challenging to choose the most convenient, safest, and cost-effective scheme in the field of liver disease. After years of exploration, our research group has successfully developed and isolated a human liver stem cell line (named: HYX1) from the adult liver, and can be maintained for 50 generations. HYX1 has been large-scale expanded and cryopreserved in vitro, allowing them to be used in cases where stem cells are immediately needed in an emergency. Our study showed that HYX1 could alleviate ConA-induced acute liver failure in mice by modulating myeloid-derived suppressor cells and $CD4^+$ T cells [25]. Since HYX1 can differentiate into hepatocytes or cholangiocytes, and large number of frozen cells, and has low ethical concerns, low risk of tumorigenicity, and beneficial immunomodulation, HYX1-based therapy could be a promising therapeutic method for patients with liver diseases. However, the research on human-derived liver stem cells is still rare at home and abroad, the road to developing an effective HYX1-based therapy for liver disease is still paved with challenges.

Besides, the best way of transplantation has not yet been established. In theory, direct administration of stem cells into the portal vein or hepatic artery may be more effective. But the invasive approaches may lead to hemorrhage and thrombosis and hypertension in the hepatic artery hinders the effective entry of cells into the liver. In patients with liver cirrhosis, because of the reversal of portal blood flow, the transplanted cells may enter directly into the spleen rather than the liver. Choosing the best method of transplantation is still a problem to be solved in the future. Therefore, further high-quality randomized clinical studies are still needed to improve the safety and efficacy of stem cells in the treatment of liver diseases.

At the same time, a patient response prediction system needs to be developed to determine which patients will benefit the most from stem cell therapy. Personalized treatment focuses on the specific characteristics of the patient to provide the highest quality treatment while reducing the risk of side effects and the cost of ineffective interventions. Therefore, improving the standardization of research designs and establishing global multi-center clinical trials will greatly improve the clinical translational applications of stem cells in liver diseases.

Conclusion and prospects

Stem cells have many properties, including immunomodulation, differentiation into hepatocytes, and repairment of damaged tissues. At present, it has been preliminarily proved that stem cells are effective and safe. However, the long-term efficacy and safety, the regulatory mechanism of stem cell development and differentiation, and the best scheme underlying the treatment of liver diseases are not clear, and there are still many challenges and problems to be solved. It is believed that with the continuous breakthroughs in stem cell-related fields, such as stem cell isolation, gene editing, stem cell culture, transplantation, tracking, construction of a bioartificial liver, and tissue-engineered liver, stem cell transplantation will eventually be widely used in the treatment of clinical liver diseases. Exosome-based therapies avoid the potential tumorigenicity, rejection of cells, emboli formation, and undesired differentiation of stem cell transplantation. An in-depth study of stem cell exosomes is helpful to explain the accurate mechanism of stem cell therapy. It's a good direction to be expected in both research and application in liver diseases. The main description of this review is shown in **Figure 1**.

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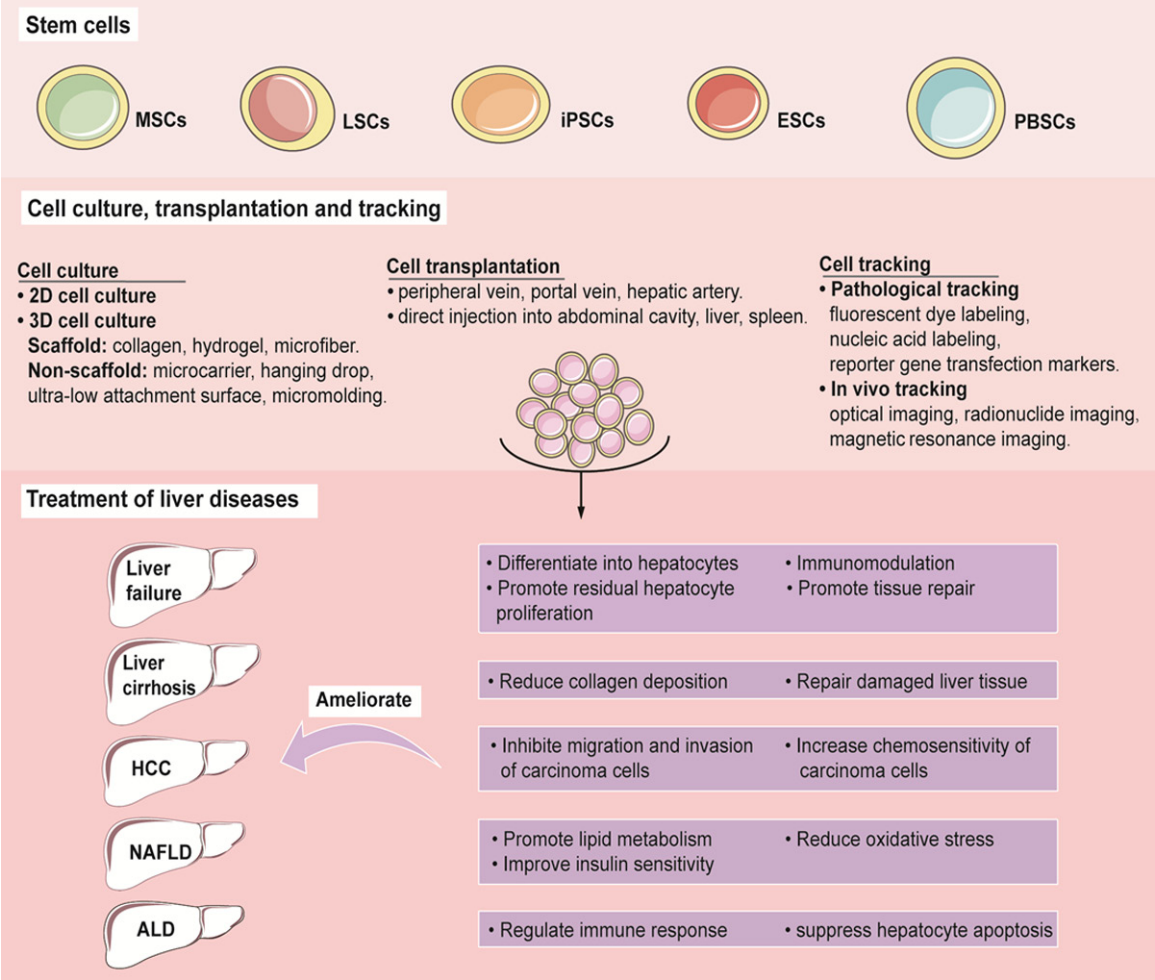

Figure 1. Schematic diagram of stem cells in the treatment of common liver disease. We summarize the types of stem cells, cell culture, transplantation and tracking, the possible mechanisms of stem cell transplantation for treating common liver diseases. 2D: two-dimensional, 3D: three-dimensional, ALD: autoimmune liver disease, ESCs: embryonic stem cells, HCC: hepatocellular carcinoma, iPSCs: induced pluripotent stem cells, LSCs: liver stem cells, MSCs: mesenchymal stem cells, NAFLD: nonalcoholic fatty liver disease, PBSCs: peripheral blood stem cells.

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Disclosure of conflict of interest

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