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Multiple Dimensions of using Mesenchymal Stem Cells for Treating Liver Diseases: From Bench to Beside

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Abstract

Liver diseases impose a huge burden worldwide. Although hepatocyte transplantation has long been considered as a potential strategy for treating liver diseases, its clinical implementation has created some obvious limitations. As an alternative strategy, cell therapy, particularly mesenchymal stem cell (MSC) transplantation, is widely used in treating different liver diseases, including acute liver disease, acute-on-chronic liver failure, hepatitis B/C virus, autoimmune hepatitis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, alcoholic liver disease, liver fibrosis, liver cirrhosis, and hepatocellular carcinoma. Here, we summarize the status of MSC transplantation in treating liver diseases, focusing on the therapeutic mechanisms, including differentiation into hepatocyte-like cells, immunomodulating function with a variety of immune cells, paracrine effects via the secretion of various cytokines and extracellular vesicles, and facilitation of homing and engraftment. Some improved perspectives and current challenges are also addressed. In summary, MSCs have great potential in the treatment of liver diseases based on their multi-faceted characteristics, and more accurate mechanisms and novel therapeutic strategies stemming from MSCs will facilitate clinical practice.

Keywords Cellular therapy · Clinical research · Liver diseases · Mesenchymal stem cells · Regenerative medicine

Introduction

Liver diseases encompass a wide spectrum of pathological consequences, and have become one of the predominant causes of death worldwide [1, 2]. Currently, liver cancer is reported as the second most common cause of morbidity in digestive system diseases and the fourth leading cause of cancer death [3, 4], with an incidence rate increasing by approximately 4% each year [5, 6]. To minimize the risk of liver diseases, many strategies have been developed, mainly including surgery, partial hepatectomy, radiation therapy, chemotherapy, transarterial chemoembolization, interventional therapy, orthotopic liver transplantation (OLT), nutritional control, management of thrombocytopenia, and immunotherapy [7–12]. Amongst them, OLT is considered to be the most effective approach to treating liver diseases, particularly chronic liver diseases [13]. However, due to the shortage of donors, possible immune rejection, complications of surgery, and high cost, OLT is not a favorable choice for a large proportion of patients.

Hepatocyte transplantation was first conducted in mice in 1976 [14]. This was a seminal event in the history of

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cell-based therapy for liver diseases. Ever since Fox et al. successfully treated Crigler–Najjar syndrome by hepatocyte transplantation in one USA patient in 1998 [15], hepatocyte transplantation has been seen as a potential clinical strategy for treating liver diseases [16]. Although hepatocyte transplantation has been performed for over 20 years, its clinical implementation has shown some obvious limitations, including immune rejection, reduced engraftment, low repopulation, and insufficient survival rate of hepatocytes [17]. Therefore, more applicable cell therapies have been explored, and stem cell transplantation, particularly that of mesenchymal stem cells (MSCs) appears to be a promising strategy [18–20].

MSCs have multifaceted therapeutic effects in the treatment of liver diseases [21–23]. Generally, MSCs are considered to exert a therapeutic function on liver repair by supplying exogenous hepatocytes, regulating intrahepatic cells via crosstalk, prompting the differentiation of endogenous progenitor cells into hepatocyte-like cells (HLCs), reducing hepatocyte apoptosis, and restraining the inflammatory response of the liver microenvironment [24–27].

In this review, we comprehensively summarize the current progress, therapeutic potential, and underlying mechanisms by which MSCs are used in treating liver diseases. In addition, some innovative therapeutic strategies are proposed for facilitating MSC-based therapy in liver diseases.

Characterization of MSCs

According to the International Society for Cellular Therapy, MSCs should meet a minimum of three criterions, including adhering to plastic cell culture flasks under standard culture conditions; expressing cluster of differentiation (CD)-73, CD90, and CD105, and be negative for CD11b/CD14, CD19/CD79a, CD34, CD45, and human leukocyte antigen II expression; having the ability to differentiate into osteoblasts, adipocytes, and chondrocytes of the mesoderm lineage [28, 29]. MSCs were first identified from the bone marrow (BM) and then later from many other tissues [30, 31]. Currently, MSCs can be isolated from almost all tissues in an organism, including BM [32], adipose tissue (AD) [33], umbilical cord (UC) [34], peripheral blood [35], placenta [36], endometrium [37], amniotic membrane [38], amniotic fluid [39], fetal tissue [40], dental pulp [41], hair follicles [42], skin [43], lungs [44], spleens [45], pancreas [46], kidneys [47], thymus [48], urine [49], liver [50], intestines [51], muscles [52], menstrual blood [53], synovial fluid [54], synovial membranes [55], etc. A schematic diagram is presented regarding different sources of human MSCs, including adult tissues and birth-associated tissues, in Fig. 1. Although different sources of human MSCs were

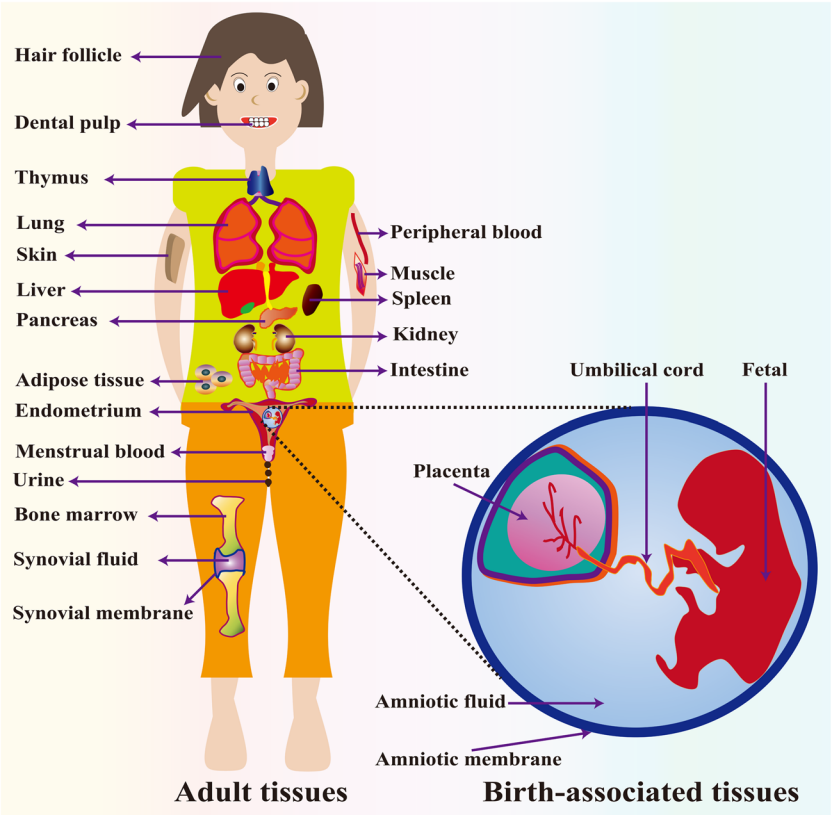

Fig. 1 Therapeutic potential of MSCs from human adult tissues and birth-associated tissues. Multifaceted MSCs can be obtained from different sources in an organism, including bone marrow, adipose tissue, umbilical cord, peripheral blood, placenta, endometrium, amniotic membrane, amniotic fluid, fetal, dental pulp, hair follicle, skin, lung, spleen, pancreas, kidney, thymus, urine, liver, intestine, muscle, menstrual blood, synovial fluid, and synovial membrane

effective for treating liver diseases, human UC-MSCs and BM-MSCs are most commonly used for clinical trials or clinical practice (Table 1). Different sources of MSCs have some commonalities and different characteristics, for example, stage-specific embryonic antigen-4 is expressed in MSCs from BM, placenta, dental pulp, menstrual blood, and synovial membrane, but it is not detected in AD-MSCs or UC-MSCs [56, 57]. CD49d is detected in AD-MSCs, but not in BM-MSCs [58]. Interestingly, menstrual blood MSCs can express the embryonic stem cells marker octamer-binding transcription factor 4, while others are not detected [59].

Mechanisms by which MSCs Treat Liver Diseases

MSCs exert therapeutic effects in liver diseases through different mechanisms, including differentiation into HLCs, supplementing exogenous hepatocytes, immune-modulation by interaction with various immune cells, paracrine effects via a series of growth factors, cytokines, and extracellular vesicles (EVs) with phospholipid bilayer membranes secreted by MSCs, and homing and engraftment function targeting injured liver cells [25, 60–62]. A detailed schematic diagram demonstrating the mechanisms by which MSCs exert effects in treating liver diseases are shown in Fig. 2.

Differentiation into HLCs

MSCs can differentiate into a variety of tissues, including cardiomyocytic, respiratory epithelial, neural, cartilaginous, myocytic, endothelial, renal, pancreatic, hepatic, adipocytic, and osteogenic tissues [63–65]. The idea of using differentiated HLCs to treat liver diseases depends on an assumption that supplementation with exogenous HLCs can improve and recover liver function. Many methods have been established to induce HLCs using MSCs in vitro. MSCs can differentiate into HLCs by stimulation with hepatocyte growth factor (HGF), epidermal growth factor (EGF), fibroblast growth factor (FGF)-1, hepatocyte nuclear factor (HNF)-1α, HNF-3γ, HNF-4α, FGF-4, GATA-binding protein 4, insulin-transferrin selenium (ITS), oncostatin M (OSM), dimethyl sulfoxide (DMSO), and dexamethasone [39, 66–70]. Differentiated HLCs express hepatocyte-specific markers, including alpha-fetoprotein, albumin (ALB), cytokeratin 18 (CK-18), connexin 32, cytochrome p450 (CYP)-1A1, CYP3A4, CYP7A1, type IV dipeptidase (CD26), glucose-6-phosphatase, HepPar, HGF receptor, and HNF-4α [68, 71–74]. Additionally, these cells have been verified to store glycogen, take up indocyanine green, express carbamyl phosphate synthase, remove ammonia/urea, and take up low-density lipoprotein [66, 75–77].

Immunomodulation

Although the immunomodulation of MSCs has not yet been fully elucidated, it is likely to regulate both innate and adaptive immune responses through interaction with immune cells [78, 79]. MSCs are considered to have immune privilege because they do not express MHC II, with low expression of MHC I molecules and co-stimulatory antigens CD80/CD86 [80]. MSCs regulated the immune system by blocking maturation of T cells and dendritic cells (DCs), inhibiting the proliferation of natural killer cells, and promoting the proliferation of regulatory T cells (Tregs) to mediate cell–cell interaction [81]. MSCs have the ability to induce an increase in Tregs and inhibit $CD4^+$/$CD8^+$ T cells in injured areas [82]. MSCs are involved in the induction of toll-like receptor 4 damage-induced DC activation, which results in the inhibition of DCs to regulate $CD4^+$ T cells [83]. MSCs can block B cell proliferation by regulating the cell cycle and inhibiting differentiation of B cells [84, 85]. MSCs can also induce the production of regulatory B cells through soluble factors and EVs [86]. In addition, MSCs can improve immunomodulatory effects by regulating the polarization of macrophages [87]. Additionally, MSCs inhibit the activation and degranulation of mast cells [88, 89].

Paracrine Effect

Paracrine effects form a vital part of the treatment effects of MSCs in various diseases [90, 91]. Soluble factors secreted by MSCs play an important role in improving liver regeneration and protecting liver apoptosis [92, 93]. Microencapsulated MSCs could reduce inflammation in a murine liver fibrosis model by secreting interleukin (IL)-1Ra, IL-10, and matrix metalloproteinase 9 (MMP-9), improving liver fibrosis [94]. Sobrevals et al. found that MSCs secreted insulin-like growth factor-binding protein-2 (IGF-BP2), IL-6, IL-1Ra, and monocyte chemoattractant protein-1 (MCP-1), which led to improvement of liver injury in a liver fibrosis model [95]. Tsai et al. found that the expression levels of HGF and mesenchymal epithelial transition factor-phosphorylated type (MET-P) were increased in human UC-MSCs used for treating liver fibrosis [96]. Wang et al. showed that HGF and BM-MSCs induced hepatic stellate cell (HSC) apoptosis by inhibiting the TLR4/NF-κB signaling pathway [97]. Parekkadan et al. demonstrated that IL-10 and tumor necrosis factor-alpha (TNF-α) secreted by MSCs significantly inhibited HSC proliferation [98]. Additionally, Pan et al. demonstrated that MSC-derived FGF-2

Table 1 Clinical trials using MSCs in treating liver diseases

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT00476060	Mesenchymal Stem Cell Transplantation in Decompensated Cirrhosis	BM-MSCs	Cirrhosis	University of Tehran, Iran	Phase 2	27	May 21, 2007	Completed	MELD score, quality of life, liver volume, histological improvement (In a subset of patients with evidences of clinical and biochemical improvement, follow up liver biopsy will be performed at the end of follow up). [Time Frame: One year]
NCT00956891	Therapeutic Effects of Liver Failure Patients Caused by Chronic Hepatitis B After Autologous mscs Transplantation	BM-MSCs	Liver failure	Sun Yat-sen University, China	N/A	158	Aug 11, 2009	Completed	Short-term therapeutic effects of transplantation Long-term outcomes of transplantation
NCT00976287	Autologous Bone Marrow Mesenchymal Stem Cells Transplantation Via Hepatic Artery in Patients With Liver Cirrhosis	BM-MSCs	Liver cirrhosis	Sun Yat-sen University|Third Affiliated Hospital, Sun Yat-Sen University, China	Phase 2	50	Sep 14, 2009	Unknown	The levels of serum alanine aminotransferase (ALT), total bilirubin (TB), prothrombin time (PT), albumin (ALB)
NCT00993941	Bone Mesenchymal Stem Cell (BMSC) Transplantation in Liver Cirrhosis Via Portal Vein	BM-MSCs	Liver cirrhosis	Sun Yat-sen University, China	Phase 2	60	Oct 14, 2009	Unknown	The levels of serum alanine aminotransferase (ALT), total bilirubin (TB),prothrombin time (PT), albumin (ALB), prealbumin(PA), precollagen III(PCIII), collagenIV(IV-C), laminin(LN), hyaluronidase(HN), liver histological improvement

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT01218464	Safety and Efficacy of Human Mesenchymal Stem Cells for Treatment of Liver Failure	UC-MSCs	Liver failure	Beijing 302 Hospital, China	Phase 1/2	70	Oct 11, 2010	Unknown	The levels of serum Total Protein and Albumin The levels of serum Total Bilirubin and Direct Bilirubin The levels of serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Cholinesterase (CHE)
NCT01220492	Umbilical Cord Mesenchymal Stem Cells for Patients With Liver Cirrhosis	UC-MSCs	Liver cirrhosis	Beijing 302 Hospital, China	Phase 1/2	266	Oct 14, 2010	Completed	Survival time incidence of HCC events The levels of serum albumin
NCT01224327	Umbilical Cord Mesenchymal Stem Cells Infusion Via Hepatic Artery in Cirrhosis Patients	UC-MSCs	Liver cirrhosis	Qingdao University, China	Phase 1/2	50	Oct 20, 2010	Unknown	The result of liver function and liver histological improvement The disappearance or reduction of abdominal dropsy The clinical symptom improve(including food appetite,debilitation,abdominal distension,edema of lower limbs)
NCT01233102	Mesenchymal stem cells treat liver cirrhosis	UC-MSCs	Liver cirrhosis	Yufang Shi|Soochow University|Chinese Academy of Sciences, China	Phase 1/2	200	Nov 3, 2010	Suspended	The level of serum alanine aminotransferase (ALT) The level of serum total bilirubin (TB) The level of serum prothrombin time (PT)
NCT01256125	Allogene mscs Transplantation in Patients With Chronic Liver Diseases Through Peripheral Vein	N/A	Chronic liver diseases	Sun Yat-sen University, China	N/A	60	Dec 8, 2010	Unknown	Short-term therapeutic effects at 1–8 weeks after Allogene MSCs transplantation through peripheral vein Long-term follow-up of Allogene MSCs transplantation through peripheral vein

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT01256138	Allogene mscs Transplantation in Liver for Patients With Chronic Liver Diseases Through Portal Vein by Ultrasound Guiding	N/A	Chronic liver diseases patients	Sun Yat-sen University, China	N/A	60	Dec 8, 2010	Unknown	Short-term therapeutic effects at 1–8 weeks after Allogene MSCs transplantation
NCT01342250	Human Umbilical Cord Mesenchymal Stem Cells Transplantation for Patients With Decompensated Liver Cirrhosis	UC-MSCs	Liver cirrhosis	Shenzhen Beike Bio-Technology Co., Ltd.|No.85 Hospital, Changning, Shanghai, China	Phase 1/2	20	Apr 27, 2011	Completed	Overall Survival (OS) Liver function improvement The size of liver and the width of portal venous
NCT01429038	Mesenchymal Stem Cells After Renal or Liver Transplantation	BM-MSCs	Liver failure	University of Liege, Belgium	Phase 1/2	40	Sep 5, 2011	Completed	Infusional toxicity Incidence of infections (bacterial, viral, fungal, parasitic) and cancers Patient and graft survivals
NCT01454336	Transplantation of Autologous Mesenchymal Stem Cell in Decompensate Cirrhotic Patients With Pioglitazone	BM-MSCs	Liver fibrosis	Royan Institute, Iran	Phase 1	3	Oct 19, 2011	Completed	ALT AST Serum Albumin

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT01483248	Human Menstrual Blood-derived Mesenchymal Stem Cells for Patients With Liver Cirrhosis	MenSCs	Liver cirrhosis	S-Evans Biosciences Co., Ltd.|Zhejiang University|Zhejiang General Hospital of Armed Police|First Affiliated Hospital of Zhejiang University|Wuhan General Hospital of Guangzhou Military Command, China	Phase 1/2	50	Dec 1, 2011	Unknown	Overall Survival Liver function improvement Complications
NCT01499459	Autologous Mesenchymal Stem Cell Transplantation in Liver Cirrhosis	BM-MSCs	Liver cirrhosis	Saglik Bilimleri Universitesi Gulhane Tip Fakultesi, Portugal	N/A	25	Dec 26, 2011	Unknown	clinical improvement liver regeneration
NCT01573923	Safety and Efficacy Study of Umbilical Mesenchymal Stem Cells for Liver Cirrhosis	UC-MSCs	Liver cirrhosis	Alliancells Bioscience Corporation Limited, China	Phase 1/2	320	Apr 10, 2012	Unknown	Survival timeSerum markers regarding liver and kidney functionSerum markers regarding lipid and sugar profile
NCT01661842	Umbilical Cord Mesenchymal Stem Cells for Patients With Autoimmune Hepatitis	UC-MSCs	Autoimmune hepatitis	Beijing 302 Hospital, China	Phase 1/2	100	Aug 10, 2012	Unknown	Liver Histology change Serum alanine aminotransferase (ALT) Serum AST
NCT01690247	Human Mesenchymal Stem Cells Induce Liver Transplant Tolerance	UC-MSCs	Evidence of Liver Transplantation	Beijing 302 Hospital, China	Phase 1	50	Sep 21,2012	Unknown	Incidence rate of acute rejection and early liver function recovery Patient and graft survival, and prevalence of adverse events

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT01711073	Mobilization of Stem Cells With G-CSF and Mozobil in Patients With End Stage Liver Disease	N/A	End stage liver disease	Proteonomix, Inc.|University of Medicine and Dentistry of New Jersey|Numoda, USA	Phase 1	15	Oct 22, 2012	Unknown	Toxicity as measured by bone pain, hematologic parameters, GI measures and renal parameters Effects of Mobilization
NCT01724398	Umbilical Cord Mesenchymal Stem Cells Transplantation Combined With Plasma Exchange for Patients With Liver Failure	UC-MSCs	Liver failure	Third Affiliated Hospital, Sun Yat-Sen University, China	Phase 1/2	120	Nov 9, 2012	Unknown	Survival rate and time Improve biochemical indexes [alanine aminotransferase (ALT), albumin (ALB), total bilirubin (TBIL), prothrombin time (PT), INR and so on] The clinical symptom improvement [including appetite, debilitation, abdominal distension, edema of lower limbs, et al.]
NCT01728727	Safety and Efficacy of Human Umbilical Cord Derived Mesenchymal Stem Cells for Treatment of HBV-related Liver Cirrhosis	UC-MSCs	Liver cirrhosis/end stage liver disease	Air Force Military Medical University, China|The Second Affiliated Hospital of Chongqing Medical University|Eastern Hepatobiliary Surgery Hospital|Chinese Academy of Medical Sciences|Fudan University, China	Phase 1/2	240	Nov 20, 2012	Unknown	one year survival rate MELD score Child Pugh Score
NCT01741090	The Effectiveness and Safety for Mesenchymal Stem Cell for Alcoholic Liver Cirrhosis	BM-MSCs	Alcoholic liver cirrhosis	Yonsei University, Korea	Phase 2	12	Dec 4, 2012	Unknown	The improvement of Liver Histologic grade The evaluation of hepatic dendritic cells activity by immunohistochemistry Liver fibrosis quantitative analysis using Hydroxyproline contents in liver tissue

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT01844063	Safety and Efficacy of Diverse Mesenchymal Stem Cells Transplantation for Liver Failure	BM-MSCs/ UC-MSCs	Liver failure	Third Affiliated Hospital, Sun Yat-Sen University, China	Phase 1/2	210	May 1, 2013	Unknown	survival rate Liver function Marker of liver cancer
NCT02260375	MSC Therapy in Liver Transplantation	BM-MSCs	Liver Transplant Rejection	Monia Lorini, Mario Negri Institute for Pharmacological Research, Italy	Phase 1	20	Oct 9, 2014	Recruiting	Number of adverse events Liver tissue mRNA level of Transferrin receptor CD71 (TFRC) and Hepcidin antimicrobial peptide (HAMP) genes Circulating naive and memory T cell conts (CD45RA/CD45RO) (flow cytometry analysis) T-cell function in mixed lymphocyte reaction
NCT02652351	Human Umbilical Cord-Mesenchymal Stem Cells for Hepatic Cirrhosis	UC-MSCs	Hepatic cirrhosis	Shenzhen Hornetcorn Bio-technology Company, LTD|The Second Affiliated Hospital of University of South, China	Phase 1	20	Jan 11, 2016	Unknown	Severity of adverse events Hepatic function Liver fibrosis index
NCT02705742	Mesenchymal Stem Cells Transplantation for Liver Cirrhosis Due to HCV Hepatitis	AD-MSCs	Liver cirrhosis	Saglik Bilimleri Universitesi Gulhane Tip Fakultesi, Turkey	Phase 1/2	5	Mar 10, 2016	Unknown	All cause mortality

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT02706132	Therapeutic Strategy and the Role of Mesenchymal Stromal Cells for ABO Incompatible Liver Transplantation	N/A	Liver Transplantation	Third Affiliated Hospital, Sun Yat-Sen University, China	Phase 1/2	15	Mar 11, 2016	Unknown	Efficacy: one year graft survival rate the rate of acute rejection the rate of ischemic-type biliary lesions safety: rate of (serious) adverse events in the study population
NCT02786017	Injectable Collagen Scaffold™ Combined With HUC-mscs Transplantation for Patients With Decompensated Cirrhosis	UC-MSCs	Decompensated cirrhosis	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School, China	Phase 1/2	40	May 30, 2016	Unknown	Improvement of liver function measured by change in the model for end-stage liver disease (MELD) score Improvement of liver function measured by change in Child–Pugh score Change in clinical laboratory parameters of liver function
NCT02812121	UC-MSC Infusion for HBV-Related Acute-on-Chronic Liver Failure	UC-MSCs	Liver failure	Sun Yat-sen University, China	Phase 2	261	Jun 24, 2016	Unknown	The incidence of adverse reactions after umbilical cord blood derived mesenchymal stem cells (UC-MSC) infusions The survival time of patients after UC-MSC infusions The influence on levels of ALT (U/L) and AST (U/L) after UC-MSC infusions
NCT02943889	Stem Cell Transplantation in Cirrhotic Patients	BM-MSCs	Liver cirrhosis	Assiut University, Egypt	Phase 1/2	40	Oct 25, 2016	Unknown	Improvement of liver function in form of improvement in child score Postpone or to overcome liver transplantation complications

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT02957552	Safety and Tolerance of Immunomodulating Therapy With Donor-specific MSC in Pediatric Living-Donor Liver Transplantation	BM-MSCs	Pediatric Liver Transplantation	University Hospital Tuebingen, Germany	Phase 1	7	Nov 6, 2016	Unknown	Number of participants with MYSTEP-score grade 3 and grade 2 (toxicity of MSC infusion) Number of participants with occurrence of any severe adverse events (SAE) Graft function after liver transplantation—Number of participants with abnormal liver tests
NCT02997878	Selected Mesenchymal Stromal Cells to Reduce Inflammation in Patients With PSC and AIH (Merlin)	UC-MSCs	Cholangitis, Sclerosing Hepatitis, Autoimmune	University of Birmingham|European Union|NHS Blood and Transplant, United Kingdom	Phase 1/2	56	Dec 20, 2016	Recruiting	Dose finding and incidence of treatment emergent adverse events (safety and tolerability) in all PSC and AIH Patients Incidence of treatment emergent adverse events (safety and tolerability) for PSC and AIH patients treated at the Highest Safe Dose (HSD) only Activity and Safety at the Highest Safe Dose (HSD) of ORBCEL-C in PSC patients, by measure of change in Alkaline Phosphatase (ALP)
NCT03209986	Trial of Mesenchymal Stem Cell Transplantation in Decompensated Liver Cirrhosis	N/A	Liver cirrhosis	Xijing Hospital of Digestive Diseases, China	N/A	200	Jul 6, 2017	Unknown	Efficacy: Change of liver functions as assessed by MELD score Safety: Adverse events as assessed according to CTCAE 4.03 Survival Benefit: Survival Rate at different time points
NCT03254758	A Study of ADR-001 in Patients With Liver Cirrhosis	AD-MSCs	Decompensated Liver Cirrhosis	Rohto Pharmaceutical Co., Ltd., Japan	Phase 1/2	21	Aug 18, 2017	Completed	Safety profile of ADR-001 including the incidence of adverse events (Phase 1) Improvement rate of Child–Pugh score (Phase 2) Change of liver function evaluated by Child–Pugh score (Phase 1)

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT03460795	Safety and Efficacy Study of Co-transfering of Mesenchymal Stem Cell and Regulatory T Cells in Treating End-stage Liver Disease	N/A	Liver cirrhosis	Nanjing Medical University, China	Phase 1/2	30	Mar 9, 2018	Not yet recruiting	Albumin (ALB) Alanine aminotransferase (ALT) Prealbumin (PA)
NCT03529136	Clinical Trial of Umbilical Cord Mesenchymal Stem Cell Transfusion in Decompensated Liver Cirrhosis	UC-MSCs	Decompensated liver cirrhosis	Shandong Qilu Stem Cells Engineering Co., Ltd.|Shanghai Public Health Clinical Center|First Affiliated Hospital of Fujian Medical University|Yantai Hospital for Infectious Diseases|The Second Affiliated Hospital of Chongqing Medical University|Jinan Hospital for Infectious Diseases, China	Phase 2	252	May 18, 2018	Unknown	Overall survival
NCT03626090	Mesenchymal Stem Cell Therapy for Liver Cirrhosis	BM-MSCs	Liver cirrhosis	Stem Med Pte. Ltd.|Parkway Cancer Centre|Asian American Liver Centre|Desmond Wai Liver & Gastrointestinal Diseases Centre, Singapore	Phase 1/2	20	Aug 10, 2018	Unknown	Clinical Examination MR Elastography The level of serum alanine aminotransferase (ALT)
NCT03668171	Mesenchymal Stem Cell Transplantation for Acute-on-chronic Liver Failure	N/A	Acute-on-chronic liver failure	Xijing Hospital of Digestive Diseases, China	N/A	200	Sep 12, 2018	Unknown	Efficacy: 12 week mortality rate Clinical remission rate at week 12

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT03838250	Study to Evaluate Hepatic Artery Injection of Autologous Human Bone Marrow-Derived mscs in Patients With Alcoholic LC	BM-MSCs	Alcoholic liver cirrhosis	Pharmicell Co., Ltd., USA	Phase 1	10	Feb 12, 2019	Unknown	Incidence of Serious Adverse Events Number of patients with Hepatocellular carcinoma (primary liver cancer) development Incidence of Adverse Events
NCT03863002	Safety and Efficacy of Mesenchymal Stem Cell Transplantation for Acute-on-Chronic Liver Failure	N/A	Liver Failure/Acute on Chronic	Tianjin Weikai Bioeng., Ltd.|Tianjin Nankai Hospital, China	Phase 1/2	45	Mar 5, 2019	Unknown	survival rate Adverse reactions White blood cell
NCT03945487	Mesenchymal Stem Cells Treatment for Decompensated Liver Cirrhosis	UC-MSCs	Decompensated liver cirrhosis	Beijing 302 Hospital, China	Phase 2	200	May 10, 2019	Recruiting	Liver function The incidence of serious complications The incidence of adverse events
NCT04243681	Combination of Autologous MSC and HSC Infusion in Patients With Decompensated Cirrhosis	N/A	Cirrhosis/liver	Asian Institute of Gastroenterology, India	Phase 4	5	Jan 28, 2020	Completed	To assess the safety of combination of hematopoetic and mesenchymal stem cell in patients of liver cirrhosis Change in MELD (Model for End stage Liver disease) score Change in Child Pugh score Change in the percentage of CD 34 cells in liver

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT04357600	Umbilical Cord Mesenchymal Stem Cell for Liver Cirrhosis Patient Caused by Hepatitis B	UC-MSCs	Liver cirrhosis	PT. Prodia Stem Cell Indonesia, Indonesia	Phase 1/2	12	Apr 22, 2020	Recruiting	Child Pugh Score Examination of liver function MELD Score
NCT04822922	Safety of UC-MSC Transfusion for ACLF Patients	UC-MSCs	Acute-On-Chronic Liver Failure	Shanghai Jiao Tong University School of Medicine, China	Phase 2	16	Mar 30, 2021	Not yet recruiting	Safety of treatment Short-time efficacy of treatment
NCT05080465	Long Term Follow up Mesenchymal Stem Cell Therapy for Patients Virus-related Liver Cirrhosis	BM-MSCs	Liver Cirrhosis	Ukraine Association of Biobank, Ukraine	Phase 3	700	Oct 15, 2021	Active, not recruiting	MR Elastography The level of serum alanine aminotransferase (ALT) Clinical Examination
NCT05106972	Umbilical Cord Mesenchymal Stem Cell Transplantation for Decompensated Hepatitis B Cirrhosis	UC-MSCs	Liver Cirrhosis	Asia Stem Cell Regenerative Pharmaceutical Co., Ltd., China	N/A	30	Nov 4, 2021	Recruiting	Number of Participants with abnormal Total bilirubin Number of Participants with abnormal albumin Ishak Inflammation Rating System
NCT05121870	Treatment With Human Umbilical Cord-derived Mesenchymal Stem Cells for Decompensated Cirrhosis	UC-MSCs	Decompensated Cirrhosis	Beijing 302 Hospital|Shanghai Changzheng Hospital|LanZhou University|Renmin Hospital of Wuhan University| Chinese PLA General Hospital|VCANBIO CELL & GENE ENGINEERING CORP.,LTD, China	Phase 2	240	Nov 16, 2021	Recruiting	Change in Model for End-Stage Liver Disease (MELD) score from baseline to 24th week Change in MELD score from baseline to 48 weeks Incidence of each complication associated with decompensated cirrhosis

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT05224960	Human Umbilical Cord-derived Mesenchymal Stem Cells for Decompensated Cirrhosis (MSC-DLC-2)	UC-MSCs	Decompensated Cirrhosis	Beijing 302 Hospital, Chinese PLA General Hospital|Shanghai Changzheng Hospital|LanZhou University|Renmin Hospital of Wuhan University|Jin Yin-tan Hospital|Hainan Hospital of Chinese PLA General Hospital|VCANBIO CELL & GENE ENGINEERING,CORP.,LTD, China	Phase 2	240	Feb 4, 2022	Not yet recruiting	Change in Model for End-Stage Liver Disease (MELD) score from baseline to 24th week Change in MELD score from baseline to 96 weeks Incidence of each complication associated with decompensated cirrhosis
NCT05227846	Human Umbilical Cord-derived Mesenchymal Stem Cells for Decompensated Cirrhosis (MSC-DLC-1)	UC-MSCs	Decompensated Cirrhosis	Beijing 302 Hospital|VCANBIO CELL & GENE ENGINEERING Corporation, LTD, China	Phase 1	12	Feb 7, 2022	Recruiting	Incidence of Adverse Events Incidence of Adverse Events Change in Model for End-Stage Liver Disease (MELD) score from baseline to 28th day Change in Model for End-Stage Liver Disease (MELD) score from baseline to 3 days, 7 days, 14 days, 3 months, 6 months, 9 months, 12 months, 15 months, 18 months, 21 months, and 24 months
NCT05331872	Umbilical Cord-derived Mesenchymal Stem Cell Infusion in the Management of Adult Liver Cirrhosis	UC-MSCs	Liver Cirrhosis	Vinmec Research Institute of Stem Cell and Gene Technology, Vietnam	Phase 1	20	Apr 18, 2022	Recruiting	Number of Adverse events and serious adverse events (AEs or SAEs) Change of liver function through the Model for End Stage Liver Disease (MELD) score Change in health-related quality of life using Chronic Liver Disease Questionnaire—(CLDQ)

Table 1 (continued)

NCT Number	Title	Interventions	Conditions	Sponsor/Collaborators	Phases	Enrollment	First Posted date	Status	Results/outcomes
NCT05507762	Study of Human Umbilical Cord Mesenchymal Stem Cell in Patients With Cirrhosis Due to Hepatitis B (Compensation Stage)	UC-MSCs	Cirrhosis Due to Hepatitis B	Renmin Hospital of Wuhan University, China	Phase 1/2	20	August 19, 2022	Recruiting	FibroScan IV-C HA

was a key factor in down-regulation of hepatocyte delta-like 1 to restrain HSC activation [99]. Moreover, MSCs can release cytokines, such as IGF-1, CXCL12/stromal cell-derived factor-1 (SDF-1), and vascular endothelial growth factor (VEGF), to inhibit hepatocyte apoptosis in injured liver [100]. Lin et al. demonstrated that nerve growth factor (NGF), secreted by MSCs, inhibited HSC proliferation and promoted HSC apoptosis [101]. Our team also found that paracrine cytokines, including IL-6, IL-8, HGF, MCP-1, growth-related oncogene (GRO), and osteoprotegerin (OPG), produced by menstrual blood-derived MSCs, significantly inhibited the activation and proliferation of HSCs in mice with CCl_4-induced liver fibrosis [102]. Recently, Wang et al. found that MSC-secreted IL-4 ameliorated acute liver injury (ALI) in mice [103]. Thus, MSCs can play a vital role in treating liver diseases, via their paracrine effects. The soluble cytokines known to be involved are IL-1Ra, IL-4, IL-6, IL-8, IL-10, MMP-9, IGF-BP2, MCP-1, HGF, TNF-α, Met-P, FGF-2, IGF-1, SDF-1, VEGF, NGF, GRO, and OPG.

Elaboration of innovative treatments via cell-free therapies with a new type of "next-generation drug delivery systems" is receiving much attention [104]. Other than soluble cytokines, a growing number of studies have shown that EVs secreted by MSCs can serve as a novel type of cell-free strategy for the treatment of liver diseases, due to their paracrine function, that can be applied without concerns about immune rejection and ethical issues [105, 106]. EVs are endogenous nanoparticles that play a very important role in cell-to-cell communication [107]. Cell-derived EVs are membrane-bound vesicles containing various biomolecules and a considerable portion of the active components (including miRNAs, mRNAs, proteins, and lipids) [108]. Exosomes are small EVs (30–100 nm in diameter), released by various types of cells, which contain proteins, liposomes, and nucleic acid variants [109]. MSC-derived EVs play a vital role in improving liver diseases, including let-7a-5p [110], miR-618 [111], miRNA-223-3p [112], beclin-1 (BECN1) [113], Y-RNA-1 [114], and IL-10 [115]. Although recent advances in the use of EVs from MSCs for treating various liver diseases have been reported, the mechanisms regarding MSCs-EVs require further elucidation.

Homing and Engraftment

MSCs have the ability to migrate into injured liver sites, where they are integrated through endothelial cells to enhance their therapeutic role [116]. A damaged liver microenvironment can express multiple receptors and ligands, such as CXC-chemokine receptor (CXCR)-4, CXCR-7, and SDF-1, to facilitate MSC migration [117, 118]. In addition, chemokines are released from inflammatory sites to form a

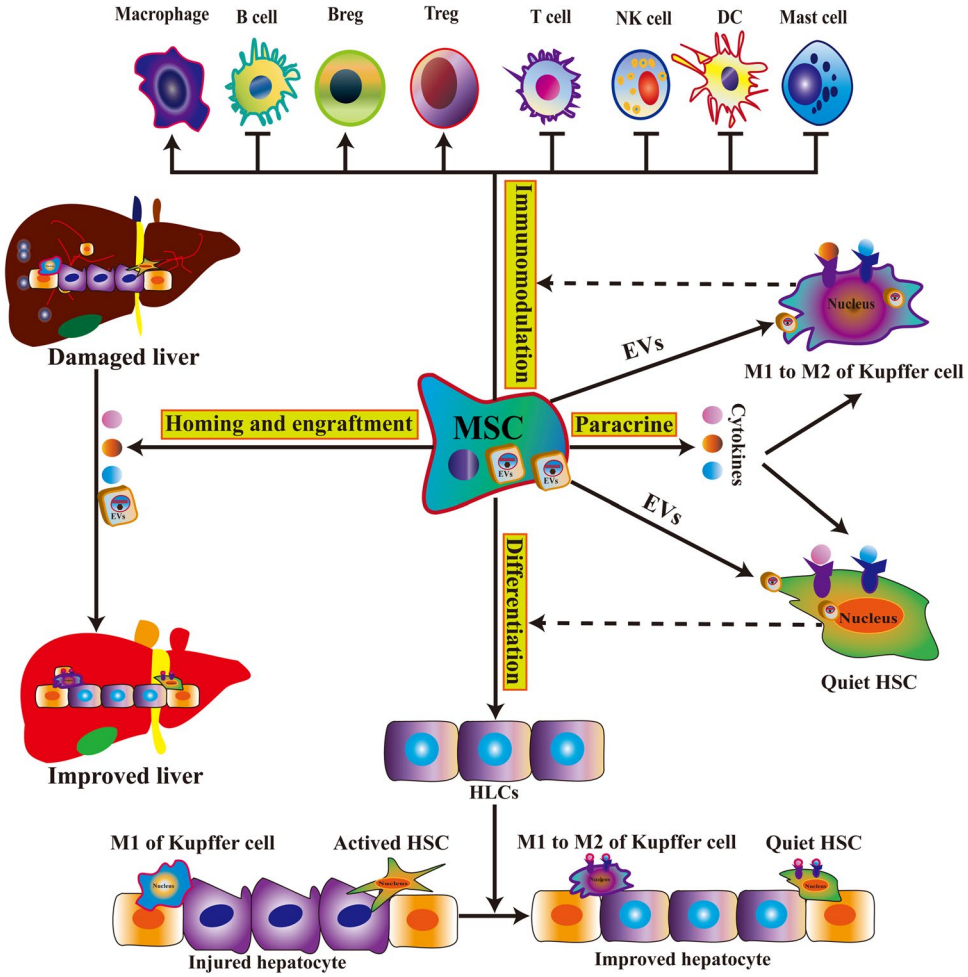

Fig. 2 Multifaceted MSCs exert therapeutic effects for liver diseases mainly through the following mechanisms: differentiation into HLCs possessing hepatocyte function, immunomodulation by interacting with various immune cells, paracrine effect by producing a range of cytokines and EVs to target Kupffer cells and HSCs, and homing and engraftment targeting injured liver cells. MSCs interact with immune cells by inhibiting B cell, T cell, NK cell, mast cell, and DC proliferation; promoting Treg, Breg, and macrophage growth. Abbreviations: HLCs, hepatocyte-like cells; EVs, extracellular vesicles; HSCs, hepatic stellate cells; B cell, B lymphocyte cell; T cell, T lymphocyte cell; NK, natural killer; DC, dendritic cell; Treg, regulatory T cell; Breg, regulatory B cell

gradient to ensure effective access of MSCs [119]. Moreover, MSCs also express integrins (such as integrin α4β1), selectins (such as CD44), and CC chemokine receptors (such as CC chemokine receptor-2), which are involved in MSC migration [120–122]. Homing and engraftment allow MSCs to exert a therapeutic effect targeting injured liver sites as well as continuously delivering signaling molecules to targeted areas.

Therapeutic Roles of MSCs in Various Liver Diseases

The roles of MSCs have been widely explored in various diseases, both in basic research and in clinical medicine [123–127]. MSCs also exert their functions for treating liver diseases [18, 21, 128]. Common liver diseases include ALI, acute-on-chronic liver failure (ACLF), hepatitis B/C virus (HBV/HCV), autoimmune hepatitis (AIH), nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), alcoholic liver disease (ALD), liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC). Based on progression, liver diseases can be divided into ALI, ACLF, and chronic liver disease.

MSCs for Treating ALI

ALI is a life-threatening clinical syndrome involving rapid hepatocyte necrosis [129]. Although the incidence of ALI is low, it has a high mortality due to its rapid onset [92]. Recently, Wang and Chen summarized the underlying mechanism of MSCs relevant for the treatment of ALI, and discussed some methods for MSC transplantation in ALI [130]. Currently, various MSC sources can be applied to treat ALI in experimental models, including pig, rat, mouse, and human sources. In the D-galactosamine (D-Gal)-induced porcine ALI model, transplantation of porcine BM-MSCs not only effectively improved the pig's liver function, but also prolonged its survival [131]. In addition, BM-MSCs transplanted via the hepatic artery, portal vein or tail vein can immigrate into damaged liver tissue of D-Gal-induced ALI rats [132]. Furthermore, Long et al. found that rat BM-MSCs improved blood biochemical indexes by decreasing

the levels of IL-18 and caspase-1 [133]. Amiri et al. demonstrated that autophagy-inhibited BM-MSCs possessed regenerative potential through a paracrine effect in CCl_4-induced ALI mice [134]. In addition, mouse BM-MSCs play a significant role in improving liver function in mice with both CCl_4- and α-galactosylceramide (α-GalCer)-induced ALI. It was further shown that BM-MSCs have the capacity to alter the levels of natural killer T cell (NKT) regulatory factor 17 and inhibit hepatotoxicity of NKT in an indoleamine 2,3-dioxygenase (IDO)-dependent manner [135]. Gazdic et al. showed that murine BM-MSCs attenuated α-GalCer-induced ALI in mice by restraining the cytotoxicity of liver NKT and enhancing the ability of Tregs to secrete IL-10 [136, 137]. Mouse BM-MSCs play a therapeutic role in inhibiting hepatocyte apoptosis in D-Gal-induced ALI mice via secretion of IL-10 [138]. Furthermore, Liang et al. found that a nanoparticle containing a beneficial regenerative factor from human BM-MSCs could improve liver function and enhance blood stability in CCl_4-induced ALI mice [139]. Deng et al. showed that transplanted mouse AD-MSCs improved liver function in CCl_4-induced and thioacetamide-induced ALI mice, and transplanted cells differentiated into HLCs by increasing the expression of ALB and CK-18 [140]. Transplantation of human UC-MSCs reduced liver necrosis and promoted endogenous liver regeneration mainly via paracrine effects in acetaminophen- or CCl_4-induced ALI mice [141–144]. In addition, Zhou et al. found that both UC-MSCs and HLCs differentiated from UC-MSCs significantly increased the survival rate of Gal/LPS-induced ALI mice [145]. Cao et al. showed that human placental MSCs had a therapeutic effect on D-gal-induced ALI in Chinese experimental miniature pigs by differentiating into HLCs [146]. Zhang et al. concluded that human UC-MSCs improved the survival rate and liver function of D-GalN/LPS-induced ALI rats by stimulating regeneration of the endogenous liver and inhibiting hepatocellular apoptosis [147]. Zagoura et al. reported that human AF-MSCs demonstrated a therapeutic effect on CCl_4-induced ALI mice via paracrine factors (IL-10, IL-1Ra, IL-13, and IL-27) [39]. Both menstrual blood-derived MSCs and their derived exosomes attenuated ALI in mice by inhibiting the secretion of inflammatory cytokines [148, 149].

MSCs for Treating ACLF

ACLF is a syndrome with liver-related disease characterized by acute decompensation of chronic liver disease, multiple organ failure, and high short-term mortality [150]. With the rapid development of stem cell transplantation and its use in clinical trials, multiple studies have shown that treatment of ACLF with MSCs is well-tolerated and safe. This has therefore become a hot research topic. Shi et al. discovered that UC-MSCs improved liver function and survival rates during a 48-week or 72-week follow-up in HBV-related ACLF patients [151]. Lin et al. further found that BM-MSCs notably improved clinical laboratory indexes in patients with HBV-related ACLF [152]. He et al. found that UC-MSC injection can effectively improve liver function and fibrosis in rats with ACLF by blocking NOTCH/STAT1/STAT3 pathway signaling [153]. Although these clinical trials have shown that MSCs can reduce the probability of serious infection or death, perhaps through immune regulation, the underlying mechanism requires elucidation.

MSC for Treating Chronic Liver Disease

MSC for Treating HBV/HCV

HBV/HCV imposes a large economic burden worldwide, particularly in China [154]. Over 500 million people have chronic HBV/HCV infection, subsequently increasing the risk of HCC significantly in this population [155, 156]. In the past few decades, host immune responses have been associated with the clearance of HBV/HCV infection [157]. However, to date, no effective method for completely curing HBV/HCV infection exists. Thus, different strategies are currently being explored, including immunotherapy [158], exploring new animal models [159], and reversing T cell exhaustion [160]. Rong et al. showed that MSCs inhibit HBV metastasis through homing and engraftment [161]. In addition, Peng et al. found that autologous BM-MSCs notably improved liver function (as assessed using alanine aminotransferase [ALT], ALB, total bilirubin [TBIL], and prothrombin time [PT]) in both short-term efficacy and long-term prognosis of patients with HBV [32]. Wang et al. found that AD-MSCs and HLCs differentiated from AD-MSCs were not sensitive to HBV infection in vitro, suggesting that AD-MSC transplantation is an alternative treatment strategy for HBV [162]. Human UC-MSCs improved liver function (as assessed by ALT, ALB, TBIL, PT, and international normalized ratio) in patients with HBV-related liver failure and liver cirrhosis [163, 164]. Salama et al. showed that autologous BM-MSCs had a supporting function in the treatment of HCV-induced liver disease by improving liver synthesis and liver fibrosis [165].

MSCs for Treating AIH

AIH occurs in almost all races and regions, and particularly affects women [166]. Most AIH patients need long-term immunosuppressive therapy, but some patients cannot bear long-term immune suppression. Therefore, novel treatment options are sought for AIH. Chen et al. found that BM-MSC transplantation could significantly improve AIH in mice by

inhibiting IL-17 and up-regulating programmed cell-death ligand [167]. Furthermore, MSC-derived exosomes have been used to treat AIH by targeting microRNA-223-3p in an experimental mouse model [112]. Menstrual blood-derived MSCs played a crucial role in ConA-induced AIH mice by lowering the level of pro-inflammatory cytokines, including TNF-α, IL-17A, IL-12p70, IL-6, IL-2, IL-1b, and interferon γ (IFN-γ), to inhibit macrophage activation and M1 polarization [168].

MSCs for Treating NAFLD/NASH

NAFLD is the most common chronic liver disease in developed countries, affecting around a quarter of the global population [169, 170]. NASH is a serious form of NAFLD. Pathogenically, NAFLD/NASH is associated with overeating and metabolic disorder [7, 171]. Lee et al. proved that human BM-MSCs improved obesity-associated NAFLD/NASH [172]. Seki et al. found that human AD-MSCs ameliorated liver function by decreasing the ratio of CD8+/CD4+ cells and by activating T helper cells in NASH model mice [33]. Moreover, HLCs differentiated from human BM-MSCs had a therapeutic effect on a NASH mouse model [173]. Menstrual MSC-derived HGF could effectively promote hepatic glycogen storage and attenuate lipid accumulation in NAFLD, which was mediated by downregulation of RNF186 in the liver [174]. Furthermore, murine BM-MSCs have been shown to reduce liver lipid peroxidation, steatosis, and hepatic lobular inflammation by suppressing lymphocyte activation in methionine–choline-deficient (MCD)-induced NASH mice [175].

MSCs for Treating ALD

Alcoholism causes marked global morbidity and mortality worldwide. Excessive and long-term alcohol abuse leads to complications, including NAFLD/NASH, HBV/HCV, liver fibrosis, liver cirrhosis, and HCC [176, 177]. The pathological hallmark of ALD is alcohol use and the effect of its toxic metabolites on various liver cell types, formation of fat deposits in hepatocytes, and subsequent development of a series of lesions [178]. In recent years, MSCs have been explored for treatment in ALD [179]. Jang et al. showed that autologous BM-MSCs improved liver function in patients with alcoholic cirrhosis, with no side effects during treatment [180]. Lantheir et al. showed that autologous BM-MSCs promoted liver regeneration in ALD by targeting macrophages in the inflammatory response [181].

MSCs for Treating Liver Fibrosis

Liver fibrosis is characterized by accumulation of extracellular matrix (ECM) and is the ultimate common pathway for chronic liver diseases [93]. Although a comprehensive understanding of the molecular mechanism underlying liver fibrosis has been sought for over 40 years, success in liver fibrosis treatment has been much harder to achieve than expected [182, 183]. At present, no specific drug is approved for the treatment of liver fibrosis. The ECM replaces the damaged liver area, forming a fibrotic lesion. The main source of the ECM is myofibroblasts, a major precursor cell population. These cells express myosin, collagen, alpha-smooth muscle actin and transforming growth factor-beta 1 (TGF-β1) [80]. Chang et al. demonstrated that human BM-MSCs improved liver function in CCl_4-induced liver fibrosis in rats [184]. Similarly, Miryounesi et al. found that multiple transplantations of human BM-MSCs improved liver function in mice with CCl_4-induced liver fibrosis [185]. In addition, Ali and Masoud showed that mouse BM-MSCs reduced liver fibrosis and significantly increased ALB levels in CCl_4-injured mice [186]. Pan et al. further confirmed FGF2 involvement in the repair of liver function [99]. Fiore et al. found that IGF-I-overexpressing mouse BM-MSCs improved liver fibrosis by inhibiting antiviral immune responses in thioacetamide-induced fibrotic mice [187]. In addition, Milosavljevic et al. found that mouse BM-MSCs attenuated liver fibrosis in CCl_4-injured mice by reducing Th17 cell infiltration in an IDO-dependent manner [188]. Both intravenous and intrasplenic injections of BM-MSCs achieved similar therapeutic effects by reducing the expression of IL-1β, IL-6, and interferon-γ in rats with CCl_4-induced fibrosis [189]. Furthermore, Nasir et al. showed that a combination of IL-6 and mouse BM-MSCs enhanced a reduction in CCl_4-induced liver fibrosis by improving the liver microenvironment in mice, in contrast to administration of mouse BM-MSCs administration [190]. Du et al. demonstrated that human BM-MSCs modified with MMP-1 could inhibit CCl_4-induced fibrotic rats [191]. Wang et al. found that rat AD-MSCs have therapeutic effects by improving the microcirculation in CCl_4-induced fibrotic liver rats [192]. Compared with AD-MSCs, MiR-122-modified AD-MSCs enhanced the therapeutic effect of CCl_4-induced liver fibrosis in mice, and AD-MSCs reduced collagen deposition through miR-122 secretion by exosomes [193]. Jung et al. demonstrated that human UC-MSCs ameliorated CCl_4-induced liver fibrosis in rat models [194]. It has been reported that HGF-overexpressing human UC-MSCs exerted a positive effect in CCl_4-induced liver fibrosis by promoting liver regeneration [195]. Furthermore, Jung et al. provided evidence that human placenta-derived MSCs improved CCl_4-injured rats via autophagy-upregulated hypoxia-inducible factor-1α (HIF-1α) [196]. Recently, our group also showed that menstrual blood-derived MSCs improved liver fibrosis by secreting IL-6, IL-8, HGF, MCP-1, GRO, and OPG to inhibit the activation of HSCs in CCl_4-induced fibrotic mice [102].

MSC for Treating Liver Cirrhosis

Liver cirrhosis is widespread worldwide and has different etiologies [197]. Liver cirrhosis occurs after a prolonged period of inflammation that results in the replacement of healthy liver parenchyma by fibrotic tissue and regenerative nodules, and the disease progresses from an asymptomatic phase (compensated cirrhosis) to a symptomatic phase (anogenesis compensated cirrhosis), with complications often leading to hospitalization, impaired quality of life, and high mortality [198]. Su et al. found that overexpressing Smad7 in MSCs obviously improved liver function scores, and reduced biomarkers of fibrosis and inflammatory markers in CCl_4-induced experimental liver cirrhosis [199]. Liu et al. showed that hair follicle-derived MSCs with high ECM-1 significantly improved liver function and the damage of liver cirrhosis by targeting HSCs both in vivo and in vitro [200]. Shi et al. reported that infusion of UC-MSCs could improve liver function in patients with decompensated liver cirrhosis (DLC) [201]. Their clinical study recruited 219 patients with HBV-related DLC and found that UC-MSC treatment not only had good tolerance and safety, but could also significantly improve the long-term survival rate and liver function of patients with HBV-related DLC.

MSCs for Treating HCC

HCC is the major cause of global cancer deaths in the world, with a mortality rate steadily increasing annually [202]. Clinical studies have shown that HCC significantly increases the risk of liver-specific death in patients [203, 204]. MSCs are able to home to tumor cells [120] and can exert an anti-cancer function [205]. Studies have shown that human BM-MSCs reduced the tumorigenicity in mice by co-culturing with human HCC cells. The WNT signaling pathway is a major mediator inhibiting the proliferation of HCC cells [206]. Chen et al. demonstrated that IL-12-producing BM-MSCs can delay HCC metastasis in mice [207]. Li et al. indicated that human BM-MSCs significantly suppressed the invasiveness and metastasis of HCC in mice by decreasing TGF-β1 expression [208]. Furthermore, overexpression of pigment epithelial-derived factor in human BM-MSCs significantly enhanced their anti-tumor function in HCC in a mouse model, in which these cells preferentially migrated to HCC cells to inhibit tumor neoplasia [209]. It has been reported that mouse BM-MSCs transfected with a soluble VEGF receptor 1 could restrain tumor growth in HCC mice [210]. Tang et al. showed that human UC-MSCs inhibited the growth of HepG2 cells (an HCC cell line) and promoted HepG2 apoptosis [211]. In addition, Wu et al. found that overexpression of HNF-4α by human UC-MSCs significantly inhibited the growth and metastasis of HCC, by down-regulating the WNT/β-catenin signaling pathway [212]. Yulyana et al. found that human fetal MSC-derived conditioned medium inhibited HCC cell proliferation via production of paracrine factors [40]. The autocrine motility factor produced by HCC could induce migration of different MSC types (including BM-MSCs, AD-MSCs, and UC-MSCs) towards HCC cells [213]. Although many studies have suggested the positive role of MSCs in HCC, MSCs can also result in tumor growth and tumor metastasis [214]. Therefore, the role of MSCs in treatment of tumors (for example, HCC) remains controversial, and more comprehensive assessment is necessary.

Improved Strategies to MSCs in Treating Liver Diseases

Although MSCs have shown a promising role in the treatment of liver diseases, some strategies can be manipulated to improve the function and microenvironment of MSCs. Improved strategies mainly include clustered, regularly interspaced, short palindromic repeats (CRISPR)/CAS9, preconditioning, cell-free approaches, single-cell RNA sequencing, and organoids.

CRISPR/CAS9

At present, gene editing has great potential in the fields of functional genomics, transgenic animals, gene therapy, and translational medicine. It has been widely used in basic research and clinical applications worldwide [215, 216]. Gene editing is based on programmable and highly specific nucleases that produce site-specific cleavage, and then induce DNA repair in cells, thereby achieving coding changes for specific genes within cells [217]. CRISPR/CAS9 is a recently discovered genome-editing technology that is widely applied in gene modification, gene therapy, and translational medicine [218–220]. Xue et al. used hydrodynamic injection technology to mutate CRISPR plasmid DNA directly and introduce single-stranded RNA expressing CAS9 into hepatocytes, providing new ideas for the treatment of liver diseases [221]. With advances in stem cell therapy, CRISPR/CAS9-based gene editing is widely used in complex genetic manipulation to enhance the abilities of stem cells by reprogramming in various disease models [222]. The application potential of CRISPR/CAS9 in current stem cell research, and the future development direction of CRISPR/CAS9 technology combined with stem cells in translational medicine and regenerative medicine has been garnering attention [220, 223, 224]. Recently, Babazadeh et al. constructed CXCL12/SDF-1 knock-out within BM-MSCs using the CRISPR/CAS9 system, which can regulate polarization of macrophages to affect tumorigenesis [225].

CRISPR/CAS9 technology is undoubtedly a promising and highly specific gene modification method for MSCs, which can promote the clinical application of MSCs for the treatment of liver diseases in future.

Cell-free Approaches

Cell-free approaches are widely used for liver disease treatment, such as EV isolation or gene modifications. As mentioned above, EVs are likely to serve as carriers of intercellular information, as well as specific drug-targeting carriers and sources of diagnostic and prognostic markers. However, studies to elucidate these effects may be confused by the existence of different EV subtypes with different biogenic mechanisms, organelle origins, and composition. Therefore, further description and isolation of the range of EV subpopulations from specific sources are needed to determine the true function and diagnostic/therapeutic use of EVs [226]. Commonly used isolation strategies, with approximate yield and scalability, include differential centrifugation, density-gradient centrifugation–gel-permeation chromatography, affinity capture, microfluidic devices, synthetic polymer precipitation, and membrane filtration. From this long list of approaches, it is evident that various methods are used to purify EVs, but no gold standard way currently exists that can allow research to be compared between laboratories [226]. In order to evaluate better exosome therapy, various common methods of isolating exosomes can be compared, thereby optimizing the basis of cell-free therapeutic strategies, ensuring the quality and stability of exosomes for better treatment of liver diseases [106, 107]. Gene modifications have also been used to enhance the therapeutic effect of MSC-EVs. These genes include lysosome-associated membrane protein (*LAMP*), *GDNF*, and the tetraspanin superfamily *CD63/CD9/CD81* [104, 227]. Further gene modification await exploitation.

Preconditioning

Increasingly, researchers have noticed that pre-treatment of MSCs could improve the potential for liver disease treatment by increasing cell homing, improving their capacity to differentiate into hepatocytes, enhancing their paracrine effects, and by immunoregulation [228]. These stimulant for preconditioning of MSCs mainly includes hypoxia [229, 230], chemicals (such as zeaxanthin dipalmitate, rapamycin, and melatonin) [231–233], cytokines (such as HGF, ECM-1, follistatin-like 1, NOTCH, and VEGF) [22, 153, 200, 234, 235], inflammatory factors (such as IDO, IL-6, prostaglandin E2, TNF-α, IFN-γ, and TGF-β1) [190, 236, 237].

Single-cell RNA Sequencing

Single-cell RNA sequencing can allow high-throughput, high-resolution transcription analysis, and quantitative detection of single cells in tissues [238]. Compared to traditional sequencing methods, this method provides a new dimension of transcriptome information, which can distinguish the total number of cells and the cell population in liver tissues [239]. Single-cell RNA sequencing is a new method for biological research into tissue-specific groups of cells, transcription kinetics, and intergenic regulation [240, 241]. Halpern et al. obtained a partition map of all liver-related genes with high spatial resolution [242]. Additionally, Zheng et al. analyzed the T cell populations of liver cancer patients through single-cell sequencing and found some notable T cell subtypes and clonal expansion of infiltrating lymphocytes, which provided insight into immune cell changes in liver cancer patients [243]. The Ramachandran group from the Inflammation Research Center of the University of Edinburgh analyzed more than 100,000 human single-cell transcriptomes, and obtained liver nonparenchymal cells (NPCs) types and their molecular identification in healthy and cirrhotic human liver [244]. This study analyzed single cell populations with unprecedented resolution, elucidated the fibrotic niche of liver cirrhosis, and provided directions for research into cellular interactions and molecular mechanisms related to liver NPCs. Recently, Wang's team used liver tissue and blood samples from patients with HBV-related cirrhosis to perform single-cell sequencing, and revealed the peripheral immune characteristics associated with HBV infection in the liver [245]. With the rapid development of MSCs in recent years, MSCs can now be extracted from a variety of tissues and organs. Combined with state-of-the-art genome editing tools, organic compounds can be further designed to mimic disease-related genetic and epigenetic states. Therefore, single-cell RNA sequences can directly detect the transcriptome information of specific cell types in MSCs. We can use this information to target specific genes and proteins directly to treat different liver diseases.

Organoids

Currently, organoids show great potential in both basic research and clinical application [246, 247]. Tissue samples produce similar phenotypic characteristics under experimental conditions as those of the tissue source, including organ-like diameter, cell composition, three-dimensional structure, and gene expression. The organoid culture system can also be applied to various studies, including studies