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REVIEW

Improvement of mesenchymal stromal cells and their derivatives for treating acute liver failure

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Abstract

After the death of large numbers of cells in liver tissue is triggered by various hepatotoxic factors, intimidating and life-threatening acute liver failure (ALF) can develop with high mortality and expensive costs. Although liver transplantation and hepatocyte transplantation have become substitutes for improving liver regeneration, their applications are inhibited by scarce tissue and cell resources. Therefore, the transplantation of mesenchymal stromal cells (MSCs) and their derivatives including hepatocyte-like cells (HLCs), conditioned medium (CM), and exosomes (Ex) can help alleviate liver injury in ALF individuals or animal models via engraftment into liver tissue, hepatogenic differentiation, the promotion of host hepatocyte proliferation, the secretion of anti-inflammatory factors and antioxidants, and the enhancement of liver regeneration in vivo. In addition, biomaterial scaffolds protect MSCs against a harsh microenvironment in vitro and in vivo, in addition to providing physical and directional support for liver regeneration. In this review, we aimed to discuss the underlying mechanisms and therapeutic effects of MSCs and their derivatives on rescuing ALF animal models according to current studies. Further breakthroughs are required to establish safer, more stable, and more effective stem cell–based therapy in regenerative medicine for repairing liver injury, thus reducing the morbidity and mortality of ALF in the near future.

Keywords Acute liver failure · Mesenchymal stromal cell · Hepatocyte-like cell · Conditioned medium · Exosomes

Abbreviations

ALF	Acute liver failure
MSC	Mesenchymal stromal cell
iPSCs	Induced pluripotent stem cells
ESCs	Embryonic stem cells
HLCs	Hepatocyte-like cells
CCl_4	Carbon tetrachloride
CM	Conditioned medium
Ex	Exosomes
HE	Hepatic encephalopathy
INR	International normalized ratio
ACLF	Acute-on-chronic liver failure
APAP	Acetaminophen
NKT	Natural killer T
NK	Natural killer
IFN-γ	Interferon-gamma
TNF-α	Tumor necrosis factor alpha
ConA	Concanavalin A
α-GalCer	Alpha-galactosylceramide
LPS	Lipopolysaccharide
DCs	Dendritic cells
Tregs	T regulatory cells
TLR4	Toll-like receptor 4
PMNs	Polymorphonuclear neutrophils
IDO	Indoleamine 2,3-dioxygenase
TGF	Transforming growth factor
PGE2	Prostaglandin E2
ATP	Adenosine triphosphate
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
TBIL	Total bilirubin
HO-1	Heme oxygenase-1

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AFP	Alpha fetal protein
CK	Cytokeratin
VEGF	Vascular endothelial growth factor
HMGB1	High mobility group box 1 protein
EpCAM	Epithelial cell adhesion molecule
GSH	Glutathione
Nrf2	NF-E2-related factor 2
SOD	Superoxide dismutase
HGF	Hepatocyte growth factor
UCMSCs	Umbilical cord–derived MSCs
IL-1Ra	IL-1 receptor antagonist
BMMSCs	Bone marrow–derived MSCs
ConA	Concanavalin A
UCB-MSCs	Umbilical cord blood–derived MSCs
ADMSCs	Adipose-derived MSCs
iPSC-MSCs	iPSC-derived MSCs
PCNA	Proliferating cell nuclear antigen
SDF	Stromal-derived factor
CXCR4	Chemokine CXC receptor 4
ZD	Zeaxanthin dipalmitate
H_2O_2	Hydrogen dioxide
miR-210	MicroRNA-210
CAT	Catalase
AF-MSCs	Amniotic fluid–derived MSCs
HPL	Hepatic progenitor-like
STAT3	Signal transducer and activator of transcription 3
NKTregs	Natural killer T regulatory cells
NKT17	IL-17-producing natural killer T
MSC-H-CM	CM derived from MSCs cocultured with hepatocytes
D-GalN	D-galactosamine
H-CM	CM derived from hepatocytes
NCM	Nonconditioned medium
TAA	Thioacetamide
ICAM	Intercellular cell adhesion molecule
GPX1	Glutathione peroxidase-1
PG	Prostaglandin
PLGA	Poly (lactic acid-glycolic acid)
RSF	Regenerated silk fibroin
LADs	Liver assist devices

Introduction

Various hepatotoxic factors including hepatitis viruses, drugs, immunologic injury, and other factors can induce the death of a large number of cells in liver tissue, thus resulting in intimidating and life-threatening acute liver failure (ALF). The annual prevalence of ALF is approximately one to six cases per million individuals worldwide, accompanied by high mortality and expensive costs [1, 2]. Notwithstanding the multiple treatments used to prevent ALF-related complications and decelerate the rate of progression in ALF, liver transplantation serves as the most effective strategy; however, its application is restricted by scarce liver donors, high costs, and organ transplant rejection [3]. Thus, hepatocyte transplantation could become a substitute for improving liver regeneration; however, there is a lack of high-quality primary hepatocytes in vitro because they are difficult to expand and it is easy for them to lose their hepatic characteristics in vitro [4, 5].

To compensate for the shortage of liver transplantation and hepatocyte transplantation, mesenchymal stromal cell (MSC)–based therapy has emerged as a new and effective strategy in patients with ALF. In general, MSCs can be isolated and purified from various tissues, such as bone marrow, umbilical cord, adipose, umbilical cord blood, amniotic fluid, and menstrual blood [6–11]. Currently, other cell resources such as induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) have become new sources of MSCs [12, 13]. MSCs are self-renewing and multipotent and can generate abundant somatic cells including adipocytes, osteocytes, chondrocytes, and hepatocyte-like cells (HLCs) [14]. After transplantation in vivo, MSCs can not only protect organisms from inflammatory injury and apoptosis but also play a critical role in immunosuppression, angiogenesis, and paracrine-mediated tissue repair [15].

MSCs respond differently according to the injured or healthy state of the liver, as shown by allogenic MSCs engrafted into injured sites significantly reducing the mortality of carbon tetrachloride (CCl_4)–induced ALF mice, while fewer MSCs engraft into the normal liver [16]. Furthermore, the engrafted MSCs rarely undergo hepatogenic differentiation in injured liver sites, and these engrafted MSCs are efficiently cleared from the liver after injection for 1 month [17]. Moreover, the poor engraftment of MSCs in the liver is a consequence of the immune rejection of transplanted MSCs [18]. Baertschiger et al. [19] found that intrahepatic injection but not intrasplenic injection guarantees stable engraftment of MSCs in the liver, while these engrafted cells are mainly differentiated into myofibroblasts. Another study indicated that this obstacle should be overcome by the differentiation of MSCs into HLCs in vitro before transplantation in vivo [20]. Current evidence has shown that not only MSCs but also MSC derivatives such as HLCs and conditioned medium (CM) or exosomes (Ex) can be applied to regenerative medicine for repairing liver injuries [9, 11, 21]. In this review, we aimed to discuss the therapeutic mechanisms of MSCs and its derivatives in ALF at the molecular cell level; we then comprehensively analyzed the effects of MSCs and MSC derivative–based therapy in rescuing ALF according to recent studies.

Definition and causes of ALF

Wlodzimirow et al. [22] highlighted that there were 41 different definitions of ALF used in 87 separate studies without a definitive consensus. The definitions varied according to the grade of hepatic encephalopathy (HE), the time interval between the onset of symptoms and HE, the severity of coagulation disorders, and the type of preexisting liver disease. It is urgent to define consensus criteria to facilitate more effective management of ALF. The majority of investigators have accepted that an international normalized ratio (INR) > 1.5 and any grade of HE within 26 weeks of the onset of illness in a patient without a history of liver disease represents an incident of ALF [23]. Patients with acute-on-chronic liver failure (ACLF) include patients with chronic liver diseases that have developed into ALF. Although patients with ACLF have a lower incidence of coagulopathy and HE, it is associated with a high short-term mortality and immense health care expenditure [24]. Herein, we considered patients with ALF after the exclusion of ACLF patients with chronic liver disease but the inclusion of patients with hyperacute liver failure in which encephalopathy had an onset interval of 7 days or less [25].

The causes of ALF remain unknown, and novel viruses or toxins may induce ALF. Infections with hepatitis A and E are responsible for inducing the majority of cases of ALF in developing countries [26, 27], and hepatitis B infection is also a common cause in Asian and Mediterranean countries [28]. Other viruses including Epstein–Barr virus, cytomegalovirus, herpes simplex virus, and parvoviruses can trigger the initiation of ALF [29]. Drug-induced liver injury, particularly acetaminophen (APAP)-induced injury, is the most familiar cause of ALF in the USA [30]. Although APAP-induced hepatotoxicity typically occurs in a dose-dependent manner, some idiosyncratic individuals develop ALF independent of the dose. In addition, ischemic or hypoxic conditions induced by severe sepsis in other large organs (heart or lung) will consequently induce acute liver injury accompanied by extremely high levels of serum aminotransferases [31, 32], and the prognosis depends on both the severity of the primary disease and the subsequent severity of the liver injury. Other causes, including neoplastic infiltration, acute Budd–Chiari syndrome, heatstroke, and poisonous substance ingestion, can also induce ALF [33]. Therefore, we believe that the main causes of ALF can be divided into several categories, including infection, toxins/drugs, abnormal perfusion, metabolic disorders, autoimmune disorders, and neoplastic infiltration. All of these factors will initiate cell death in the liver tissue and cause liver injury for the generation of ALF in animals and humans.

The immunopathogenesis of ALF

Recently, multiple studies found that immune dysfunction exists in ALF, and the gradually exaggerated inflammatory response plays a key role in the pathogenesis and outcome of ALF. Patients with ALF were found to have low levels of C3 and C5 [34] and impaired neutrophil function by impairing the phagocytic capacity [35, 36]. Acute liver injury initiated the activation of Kupffer cells and natural killer T (NKT) lymphocytes [37] and then effectively recruited neutrophils, lymphocytes, and macrophages into injured sites and caused a reduction of nitric oxide (NO) production and massive liver necrosis [38, 39]. Moreover, ALF patients demonstrated higher levels of intrahepatic eosinophils, C-reactive protein, and interleukin (IL)-6 accompanied by lower levels of IL-5 in the liver and peripheral blood than healthy controls [40, 41]. Other studies indicated the number of CD8+ interferon-gamma (IFN-γ) + T lymphocytes was significantly increased [42] and a series of cytokines, such as IL-10, tumor necrosis factor alpha (TNF-α), and IL-12, were significantly upregulated in ALF [43, 44]. As a result of the immune dysregulation, patients with ALF demonstrate increased susceptibility to infection, which is associated with the development of further complications [45].

Multiple hepatotoxic factors such as concanavalin A (ConA), alpha-galactosylceramide (α-GalCer,) and lipopolysaccharide (LPS) induce immune dysfunction and result in ALF; thus, the detailed mechanisms are discussed as follows. The ConA-induced liver injury model is currently considered a model of autoimmune hepatitis, viral hepatitis, and related ALF; however, the immunology of this model is complex and only partially understood. After treatment with ConA in vivo, it is able to bind to sinusoidal endothelial cells and recruit CD4+ T lymphocytes, which thus results in the injury of endothelial cells [46]; ConA subsequently binds to Kupffer cells and promotes the release of TNF-α [47]. In ConA-induced ALF models, the activated conventional dendritic cells (DCs) are promoted to increase the expression of IL-12, and the activated NKT cells are promoted to secrete IFN-γ [48]. In addition, ConA significantly triggers neutrophil infiltration and the accumulation of macrophages in the liver, which thus lead to liver cell apoptosis and hepatocellular damage [49]. α-GalCer (a specific ligand for invariant Valpha14 NKT cells)-induced liver injury resembled acute autoimmune hepatitis and was mediated by NKT cells and autoantibody-producing B-1 cells [50]. It was reported that α-GalCer promoted the secretion of IFN-γ for the recruitment of IL-10-producing T regulatory cells (Tregs) and CXCR3+ Tregs in the liver [51]. The administration of α-GalCer also upregulated the levels of FasL and TRAIL, receptors responsible for NKT cell–mediated apoptosis and cytotoxicity, leading to liver injury [52]. Although hepatic Kupffer cells serve as a vital factor for TNF-α secretion in ConA-induced hepatitis, they are nonessential in α-GalCer-induced liver

injury. In addition, α-GalCer significantly upregulated the levels of TNF-α, IFN-γ, IL-2, IL-4, and IL-6 in the liver and plasma [53]. LPS is an innate immune-activating stimulus that binds to toll-like receptor 4 (TLR4) to activate macrophages and promote the secretion of CXC chemokines and inflammatory cells [54, 55]. Evidence showed that LPS stimulated Kupffer cells to release higher levels of TNF-α, IL-1, and IL-6 [56], and the liver also responds to LPS with the production of reactive oxygen intermediates [57]. Moreover, LPS stimulation enhanced the release of NLRP3 inflammasome and TLR4 and thus enhanced the release of caspase-1 in Kupffer cells [58]. LPS-induced hepatic polymorphonuclear neutrophil (PMN) accumulation and the secretion of cytokine-induced neutrophil chemoattractant-1 thus preceded the onset of hepatic parenchymal cell injury and subsequent sinusoidal endothelial cell injury [59]. It is worth noting that other factors also trigger immune dysfunction in vivo; however, the detailed process should be further clarified after the collection of sufficient evidence.

Autophagy, apoptosis, and necrosis in the development of ALF

Programmed cell death including autophagy, apoptosis, and necrosis can lead to irreversible liver injury. The liver is a large and special organ in which autophagy often occurs, and several selective types of autophagy, including mitophagy and lipophagy, also occur in both cultured hepatocytes and liver tissue [60, 61]. Autophagy can serve as a protective pathway or a devastating pathway for the acceleration of hepatic apoptosis via the modulation of mitochondrial recycling. Apoptosis can be initiated by oxidative stress–related intrinsic pathway, including DNA damage or p53 activation, or a caspase-related extrinsic pathway that begins with the activation of TNF-α and FasL [62]. Apoptosis is a process that involves minimal inflammation because cellular shrinkage and implosion lead to silent cell death in the liver tissue, while necrosis induces cell swelling and eventual rupture, which triggers a clear inflammatory response after the depletion of adenosine triphosphate (ATP) [63]. Consequently, ATP depletion and cellular swelling lead to the formation of membrane blebs, followed by mitochondrial depolarization, lysosomal breakdown, and rapid ion changes, thus recycling the components in response to pathological changes and resulting in cell membrane rupture [64]. Membrane rupture subsequently leads to irreversible cell death and secondary inflammation, while quiescent hepatocytes can be resurrected after cellular membrane rupture in liver ischemia/reperfusion injury [62]. These molecular changes have the potential to induce a wave of systemic inflammation and the accumulation of circulating harmful cytokines, and the cell death rate becomes substantially higher than the rate of hepatocyte regeneration. In this setting, damaged liver tissue shows synthetic dysfunction for the secretion of albumin and urea and glycogen storage and impaired detoxification abilities, such as cytochrome P450 activity and metabolic disturbances (Fig. 1).

MSC transplantation and the underlying mechanisms

MSCs from various tissues can effectively improve the outcome of ALF animal models via paracrine pathways, immune protective effects, upregulation of hepatocyte proliferation, and maintenance of hepatic metabolic homeostasis (Table 1).

MSCs significantly reduced the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), ammonia, and inflammatory cytokines in ALF rats via the upregulation of heme oxygenase-1 (HO-1). The upregulation of HO-1 consequently reduced PMN infiltration to further promote liver regeneration [6]. To protect against the immune response in the pathogenesis of ALF, MSCs effectively inhibited cytotoxic T lymphocytes and NK cells via various intercellular contact and paracrine factors, including indoleamine 2,3-dioxygenase (IDO), transforming growth factor (TGF)-β and prostaglandin E2 (PGE2) [3]. The immune protective effect of MSCs can be achieved by enhancing the number of Treg cells and M2-type macrophages and reducing the number of Th1 and Th17 cells in ALF models [75]. MSC transplantation can effectively improve the liver functions of ALF rats via reducing the release of inflammatory cytokines (TNF-α, IFN-γ, IL-1β, IL-*6*, and IL-10) and chemokines (CXCL1 and CXCL2) [76, 77]. In addition to paracrine mechanisms, intravenously injected MSCs can engraft into the injured sites and then attenuate lymphocyte proliferation and systemically reduce the number of activated NKT cells in vivo [78]. Moreover, MSC transplantation is also able to inhibit the activation and cytotoxicity of DCs and B cells [79, 80] and reduce the number of peripheral blood and liver neutrophils [76]. Engrafted MSCs subsequently eliminated hepatocyte necrosis, promoted liver regeneration, and prolonged the survival time of ALF models via differentiation into HLCs and the secretion of albumin, alpha fetal protein (AFP), and cytokeratin (CK)-18 after implanting into liver tissue [7, 8, 65]. In addition to maintaining normal liver enzymes and synthetic function, MSCs also participate in the promotion of revascularization via vascular endothelial growth factor (VEGF)– mediated pathways [66]. In addition, MSC transplantation significantly decreased the serum and liver levels of high mobility group box 1 protein (HMGB1), upregulated the level of epithelial cell adhesion molecule (EpCAM), and activated M2 polarization as demonstrated by the upregulation of CD163, IL-10, IL-4, and arginase-1 in ALF rats [67, 68]. However, Yuan et al. [69] argued that MSC transplantation substantially downregulated the serum and hepatic levels of CD163 and IL-10 during the early stages

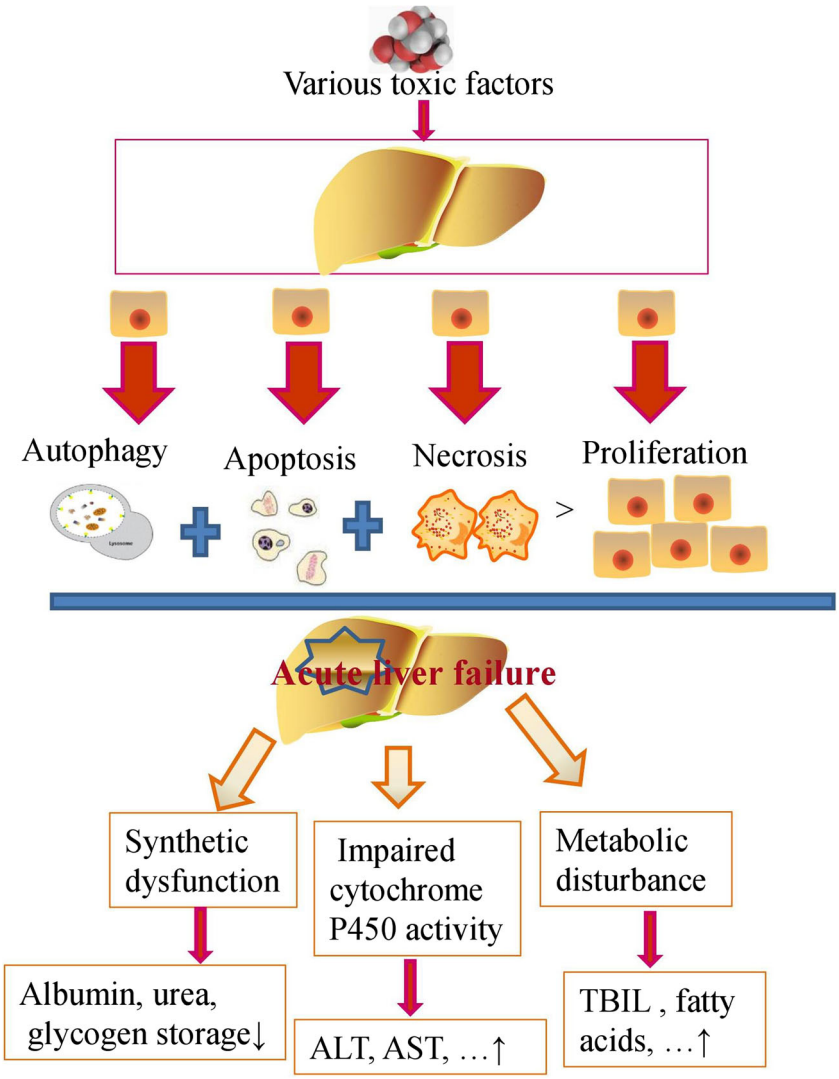

Fig. 1 Cell death–induced liver dysfunction in the development of ALF

of MSC transplantation and determined the serum levels of CD163 and IL-10 as the prognosis indicators in the progress of ALF in animals and humans after MSC administration. Intriguingly, Shi et al. [70] showed that MSCs rescued ALF pigs and stabilized ALF within 7 days as demonstrated by the normalization of liver enzymes and inhibition of life-threatening cytokine storms in ALF pigs. The profiling analysis indicated a delta-like ligand 4 activated Notch pathway after MSC transplantation, and delta-like ligand 4 has been validated as a vital factor for improving the survival rates of ALF pigs and ALF rats. Furthermore, the metabolic trajectory, including conjugated bile acids, phosphatidylcholines, lysophosphatidylcholines, fatty acids, amino acids, and sphingomyelin, returned to the original level at week 3 after MSC transplantation [71]. APAP overdoses rapidly deplete glutathione (GSH) and cause oxidative stress-induced injury in humans and animals, and MSC transplantation increases the survival rate of APAP-induced ALF mice by upregulating the antioxidant response and weakening cytochrome P450 activity to reduce the nitrotyrosine level and upregulate the NF-E2-related factor 2 (Nrf2) level in vivo [72]. Moreover, MSCs prolong the survival rate of APAP-induced ALF mice via the upregulation of superoxide dismutase (SOD), GSH, and hepatocyte growth factor (HGF), and downregulation of the inflammatory factors TNF-α and IL-6 [73].

Yun et al. compared the safety of MSCs at a gradient concentration in vivo and found that implanted MSCs did not alter the body weight, food/water consumption, clinical symptoms, urinalysis, hematology, clinical chemistry, organ weight, or histopathology at any density, and MSCs in vivo were cleared away in mice at week 13. After long-term observation for 26 weeks, the MSCs triggered the downregulation of hepatic necrosis and lobular neutrophilic infiltration in the injured liver but did not exert tumorigenicity [74]. The long-term investigation indicated that MSC transplantation at various concentrations is safe for the acute phase of ALF and long-term survival of ALF animal models.

Table 1 MSCs from various tissues can effectively improve the outcome of ALF animal models via multiple mechanisms in vivo

Route	Dose	Study group	Control group	Inducer	Animal	Effects	Mechanism	Ref
Tail vein	1×10^6	BMMSCs	BMMSC + ZnPP	D-Gal/LPS	Rats	(ALT, AST, TBIL, ammonia, inflammatory cytokines, hepatocyte apoptosis) ↓; hepatocyte proliferation ↑	HO-1 ↑; PMN infiltration ↓	[6]
Tail vein	5×10^5	UCMSCs	DMEM	GalN/LPS	Mice	Hepatic necrosis ↓; (liver regeneration, survival rate) ↑	(Migration, hepatogenic differentiation) ↑	[7]
Tail vein	1.5×10^6	ADMSC- derived hepatocytes	N/A	CCl4	Mice	(Liver functions, purine metabolism) ↑	Vacuolar degeneration ↓; (mitochondria, endoplasmic reticulum) ↑	[65]
Tail vein	1.4×10^7 /kg	BMMSCs	Saline	D-Gal/LPS	Rats	Hepatocyte apoptosis ↓; (revascularization, hepatocyte proliferation) ↑	(Caspase-1, IL-18) ↓; VEGF ↑	[66]
Tail vein	1×10^7	BMMSCs	Saline	D-Gal/LPS	Rats	(Liver function, liver pathology) ↑	(Serum HMGB1, liver HMGB1) ↓	[67]
Tail vein	5.5×10^5	BMMSCs	PBS	D-Gal	Rats	Survival rate ↑	Apoptotic hepatocytes ↓;(hepatocyte regeneration, EpCAM, M2 polarization) ↑	[68]
Portal and tail vein	1.4×10^7 cells/kg	BMMSCs	Saline	D-Gal/LPS	Rats	Liver function ↑; pathological changes ↓	(CD163, IL-10) ↓	[69]
Portal vein	3×10^6/kg	BMMSCs	NS	D-Gal	Pigs	(Survival rate, survival time) ↑	Cytokine storms ↓; (migration, hepatogenic differentiation) ↑	[70]
Portal vein	3×10^7	BMMSCs	Saline	D-Gal	Pigs	(Biochemical changes, histological changes) ↓	Metabolic homeostasis ↑	[71]
Tail vein	1×10^6	ADMSCs	PBS	APAP	Mice	Survival rate ↑	Nitrotyrosine ↓; (Nrf2, antioxidant response) ↑	[72]
Intravenous	5×10^5	UCMSCs	NS	APAP	Mice	(ALT, AST, TBIL) ↓; survival rate ↑	(GSH, SOD, HGF) ↑; (TNF-α, IL-6) ↓	[73]
Tail vein	5×10^5, 2.5×10^6, 1.25×10^7	UCMSCs	N/A	CCl_4	Mice	Liver function ↑; no tumorigenicity	(Hepatic necrosis, lobular neutrophilic infiltration) ↓	[74]

Table 2 The comparison of MSCs and other cell types for rescuing ALF animal models

Route	Dose	MSC type	Cell type	Inducer	Animal	Effects	Mechanisms	Ref
Tail vein	2×10^6	iPSC-MSCs	BMMSCs	CCl_4	Mice	(LDH, TBIL, lipid peroxidation) ↓; survival rate ↑	(Hepatocyte proliferation, HGF-mediated pathway) ↑	[12]
Splenic	4×10^7/kg	UCMSCs	Adult human hepatocytes	CCl_4	Mice	(Endogenous liver regeneration, survival rate) ↑	(IL-1β, TNF-α, IL-6, IL-10, IL-1RA) ↓	[86]
Splenic	1×10^6	BMMSCs	Adult hepatocytes, fetal liver cells, induced hepatic stem cells	ConA	Mice	Liver damage ↓; survival rate ↑	(TNF-α, IFN-γ, FasL) ↓; IL-10 ↑	[87]
Tail vein	1×10^6	ADMSCs	BMMSCs	CCl_4	Mice	(Liver histopathology, liver functions, survival rate) ↑	N/A	[88]

Choice of MSC source

It was shown that ALF did not alter the stem cell characteristics or cell activities of MSCs, and the levels of liver-specific genes and hepatogenic potency were increased in ALF-derived MSCs [81, 82]. Autologous MSCs isolated from ALF patients may highly reduce the rejection rate, while the cell preparatory period is too long for ALF patients with poor liver functions. Furthermore, MSCs have a high immune privilege and relative safety when used in allogenic hosts [83]. Although allogenic MSCs are more practical for cell transplantation in ALF patients because they can be isolated from healthy individuals and can be proliferated at any time, they also carry obstacles for safe transplantation. Allogenic MSCs are permissive for cytomegalovirus and herpes simplex virus infections in vitro and carry the risk of viral transmission to the recipient [84]. Park et al. demonstrated that autologous MSC transplantation significantly improved the outcomes of five patients with liver failure via decreasing the serum albumin levels and liver stiffness and improving the liver volume, subjective healthiness, and quality of life. Thus, they indicated that autologous MSC transplantation may serve as a bridge to liver transplantation in patients with liver failure [85]. Moreover, the outstanding therapeutic effects of allogenic MSCs for treating ALF without a clear rejection incidence indicate the promising wide application of allogenic MSCs in further studies.

As MSC transplantation has gradually replaced primary hepatocyte transplantation because of its abundance and anti-inflammatory effects, numerous studies have compared the transplantation efficacy of MSCs and cells from other sources in vivo in ALF models (Table 2). Although undifferentiated umbilical cord–derived MSCs (UCMSCs) have weaker liver-specific functions than primary hepatocytes, UCMSCs clearly improve the viability and recovery of damaged hepatocytes more than primary hepatocytes in vitro. Moreover, the transplantation of primary hepatocytes produced higher numbers of HepPar1−/albumin-positive cells than the transplantation of UCMSCs into the recipient liver, while the administration of UCMSCs more effectively rescued ALF mice and stimulated endogenous liver regeneration via the downregulation of inflammatory factors, including IL-1β, TNF-α, IL-6, IL-10, and the IL-1 receptor antagonist (IL-1Ra), rather than hepatogenic differentiation to compensate for the lost liver function [86]. Sun et al. [87] demonstrated that only the transplantation of bone marrow–derived MSCs (BMMSCs) recovered liver damage and rescued ConA-treated ALF mice via inhibiting the expression of TNF-α, IFN-γ, and FasL but increasing the IL-10 level compared with adult hepatocytes, fetal liver cells, and induced hepatic stem cells.

There are other comparisons of MSCs from different sources to guide the selection of the optimal MSC source. Umbilical cord blood–derived MSCs (UCB-MSCs) expanded

Table 3 Transplantation route for improving the therapeutic effects of MSCs in ALF animals

Study route	Dose	MSC type	Control route	Inducer	Animal	Effects	Mechanisms	Ref
Intrahepatic	$3–5 \times 10^6$	UCMSCs	Tail vein	CCl_4	Rats	(CK8, CK18, AFP, liver repair) ↑; (ALT, AST) ↓	Transdifferentiation of MSCs ↑	[89]
Hepatic artery, portal vein, tail vein	1.4×10^7/kg	BMMSCs	Intraperitoneal	D-GalN/LPS	Rats	(Liver enzymes, liver damage) ↓	(PCNA, HGF, homing of MSCs) ↑; caspase-3 ↓	[90]
Tail vein	$3–5 \times 10^6$	UCMSCs	Liver lobes	CCl_4	Mice	Comparable efficacy in repairing liver functions and enhancing liver regeneration	Hepatogenic differentiation ↑	[91]
Tail vein	5×10^6	ADMSCs	Intrasplenic	TAA	Mice	(Survival benefit, liver regeneration) ↑	(Engraftment, HLCs) ↑	[92]
Tail vein	1.5×10^6	ADMSCs	Portal vein, liver parenchyma	CCl_4	Mice	Biochemical changes ↓	Scattered ADSCs in the liver with clustered ADSCs alongside the portal vein or the injection site	[93]
Portal vein	1.4×10^7/kg	BMMSCs	Tail vein	D-GalN/LPS	Rats	Liver function ↑	(SDF-1α, VEGF) ↑	[94]
Portal vein	1×10^8	PDMSCs	Jugular vein	D-Gal	Pigs	(Liver functions, survival rate) ↑	(Liver inflammation, hepatocyte denaturation, hepatocyte necrosis) ↓; (hepatogenic differentiation, liver regeneration) ↑	[95]
Intraportal	3×10^7	BMMSCs	Peripheral vein	D-Gal	Pigs	Survival time ↑	Hepatogenic differentiation ↑	[96]
Intraportal	1×10^7	BMMSCs	Hepatic artery, peripheral vein, intrahepatic	D-Gal	Swine	(Liver injury, histopathological score, apoptosis rate of hepatocytes) ↓; survival time ↑	caspase-3 ↓; (survivin, AKT, phospho-AKT, ERK, phospho-ERK) ↑	[97]

weakly and thus could not be used for application in transplantation, while both adipose-derived MSCs (ADMSCs) and BMMSCs expanded in vitro can be applied to repair CCl_4-induced injury of ALF mice via hepatogenic differentiation in vivo [9]. Zare et al. [88] showed that the liver functions demonstrated by ALT and AST were more significantly improved in response to ADMSCs than BMMSCs, although there were no significant differences in the survival rate and liver histopathology of ALF mice. Moreover, both iPSC-derived MSCs (iPSC-MSCs) and BMMSCs significantly decreased lipid peroxidation and increased the survival rate of ALF animals via the HGF-mediated pathway; iPSC-MSCs significantly augmented their proliferative ability and compensated for the viable cell count for transplantation [12]. In summary, other MSCs overcome the disadvantages of BMMSCs by having abundant tissue sources, enhanced proliferative capacity, and a reduced operation wound.

Transplantation routes

MSCs can be injected into organisms via an intravenous route, intrahepatic route, intraperitoneal route, hepatic artery route, and splenic route; however, the selection of the optimal transplantation route remains unclear (Table 3).

Zheng et al. [89] showed that transplantation of MSCs via the intrahepatic route and tail vein route had similar effects on improving hepatic synthesis (secretion of CK8, CK18, and AFP), decreasing liver enzymes (ALT and AST) and promoting liver repair following ALF in animal models. Transplantation via the intraperitoneal route exerted no therapeutic effect because the MSCs could not migrate into the injured liver; alternatively, the other three routes (portal vein, hepatic artery, and vena caudalis) promoted the homing of MSCs to the damaged liver tissue and decreased liver damage in ALF rats via increasing the expression of proliferating cell nuclear antigen (PCNA) and HGF, while decreasing the caspase-3 level [90]. Sun et al. concluded that the selection of blood vessels for transplantation does not affect the therapeutic outcome [90].

However, the majority of studies recommend intravenous routes for MSC transplantation in ALF animal models. Some authors recommend transplantation via the tail vein, while other authors recommend transplantation via the portal vein. Feng et al. [91] demonstrated that the administration of MSCs via the tail vein and directly into the liver lobe showed comparable efficacy in repairing liver functions and enhancing liver regeneration in ALF mice, and they considered that injection via the tail vein is more convenient than the intrahepatic route since transplantation via the hepatic artery was not more beneficial for the transdifferentiation of MSCs. Moreover, transplantation via the tail vein provided an additional survival benefit to rescue ALF than transplantation via

an intrasplenic route since all of the implanted MSCs integrated into the liver parenchyma and underwent hepatogenic differentiation into HLCs at the injured site for liver regeneration [92]. Furthermore, another study highlighted that the tail vein route showed the most prominent effects on reducing the levels of biochemical parameters including ALT, AST, and ammonia in ALF mice compared with the use of the portal vein and liver parenchymal delivery [93]. In contrast, other authors report that transplanting MSCs via the portal vein can result in a large amount of engraftment of MSCs and stronger anti-inflammatory effects. For example, the transplantation of MSCs via the portal vein and tail vein both decreased the serum levels of liver enzymes and inhibited inflammation, hepatic degeneration, and necrosis in ALF rats; however, the protein levels of stromal-derived factor (SDF)-1α and VEGF were significantly higher in the portal vein group than in the tail vein group [94]. Cao et al. [95] determined that portal vein MSC transplantation enhanced hepatogenic differentiation, anti-inflammation, and liver regeneration while inhibiting hepatocyte denaturation and hepatocyte necrosis in ALF pigs; however, the transplantation of MSCs via the jugular vein did not demonstrate benefits. Li et al. [96] reported that the injection of MSCs via a peripheral vein did not rescue ALF pigs, while most of the ALF pigs survived for a long time over 6 months after transplantation of MSCs via the portal vein. Thirty percent of the hepatocytes in hepatic lobules and the liver parenchyma of the surviving pigs were derived from humans at week 10. More recently, Sang et al. [97] concluded that intraportal injection was the best route for repairing liver injury in swine with ALF compared with hepatic intra-arterial injection, peripheral intravenous injection, and intrahepatic injection, as demonstrated by the longest survival time, least liver injury, lowest histopathological score, and lowest apoptosis rate of hepatocytes via decreasing the expression of caspase-3 and elevating the expression of survivin, AKT, phospho-AKT, ERK, and phospho-ERK during the initial stage of ALF. According to the current evidence, intrahepatic injection serves as the optimal route to improve the outcome of ALF for MSC transplantation, although it is not sufficiently convenient compared with the peripheral vein route.

Modification of MSCs or recipients

To improve the transplantation efficacy, cotreatment and preconditioning of MSCs and/or the recipients have been widely applied for promoting liver regeneration in ALF models (Table 4).

Cotreatment and pretreatment/preconditioning

Jin et al. [98] demonstrated that cotreatment with SDF-1 enhanced the migrative capacity of MSCs, improved the hepatic secretion of albumin and decreased the serum aminotransferase levels in ALF mice. As the vital role of IL-10 is always highlighted in rescuing ALF animal models, Wang et al. [108] demonstrated that the administration of IL-10 and MSCs ameliorated the upregulation of ALT, AST, TBIL, ammonia, and inflammatory cytokines, while blockage of IL-10 abolished the beneficial effects of MSCs.

Preconditioning with serum from donor ALF rats clearly improved the migrative ability of MSCs into the portal area and liver parenchyma via increasing the chemokine CXC receptor 4 (CXCR4) level in ALF rats [99]. Preconditioning with zeaxanthin dipalmitate (ZD) clearly upregulated the cell survival rate and hepatocyte differentiation and abolished ROS-induced injury in MSCs treated with LPS and hydrogen dioxide (H_2O_2) in vitro via the activation of the PKC/Raf-1/MAPK/NF-κB pathway and upregulation of microRNA-210 (miR-210). In addition, ZD-pretreated MSCs demonstrated the best effects on improving hepatocyte proliferation and ameliorating liver injury via the acceleration of the host regenerative progress [100]. In addition, pretreatment with edaravone upregulated the ROS production, the GSH/oxidized glutathione (GSSG) ratio, and the expression of catalase (CAT) and SOD-1 in MSCs via the regulation of the MAPK-PKC-Nrf2 pathway. Transplantation of edaravone-pretreated MSCs effectively rescued the death of ALF mice via improving their homing ability, enhancing their proliferative capacity, decreasing apoptosis, and upregulating the secretion of HGF in MSCs [101].

In addition to the pretreatment of MSCs, the pretreatment of recipients will consequently activate or inhibit specific pathways for enhancing the repair capacity of MSCs in vivo. Preconditioning of recipients with anti-PMN effectively improved liver function and the survival rate of ALF rats after MSC transplantation by diminishing the number of neutrophils and decreasing the release of TNF-α, IL-1β, CXCL1, and CXCL2 while increasing the IL-10 level [76]. Preconditioning of recipients with IL-1β siRNA before CCl_4 injection significantly improved the liver regeneration and survival rates of ALF mice compared with monotherapy by MSC transplantation via the downregulation of inflammatory factors, including CXCL1, IL-1β, and IL-6, and the upregulation of anti-inflammatory factors, including IL-10, VEGF, and HGF [102]. The optimal dose and safety of cotreatment and preconditioning should be further investigated to improve the MSC efficacy in vivo.

Gene modification of MSCs

In addition to external cotreatment and pretreatments with serum or pharmacokinetics, recent studies have investigated gene targeting strategies of MSCs to increase liver regeneration. Implantation of MSCs that overexpressed IL-1Ra significantly alleviated the progression of liver failure and decreased

Table 4 Cotreatment and pretreatment of MSCs for improving the therapeutic effects of MSCs in ALF models

Route	Dose	MSC modification	Control group	Inducer	Animal	Effects	Mechanisms	Ref
Tail vein	1×10^6	BMMSCs and SDF-1	BMMSCs	CCl_4 and 2-- -- acetylaminofluore-ne	Mice	Survival time ↑	(Migrative capacity, albumin) ↑; aminotransferases ↓	[98]
Jugular vein	3×10^6	Pretreatment with serum from rats with ALF in UCMSCs	UCMSCs	D-GalN	Rats	Migration into portal area and liver parenchyma ↑	CXCR4 ↑	[99]
Tail vein	2×10^6	ZD-pretreated ADMSCs	Knockdown of miR-210 in ZD-pretreated ADMSCs; ADMSCs	D-Gal/LPS	Mice	Hepatocyte proliferation ↑; liver injury ↓	Host regeneration ↑	[100]
Tail vein	2×10^6	Edaravone- pretreated UCMSCs	UCMSCs, DEM-pretreated UCMSCs	D-Gal/LPS	Mice	(Liver function, liver regeneration) ↑; death of ALF mice ↓	(HGF, homing ability, proliferative capacity) ↑; apoptosis ↓	[101]
Tail vein	1×10^7	Pretreatment with anti-PMN on recipients	BMMSCs/ anti-PMN	D-GalN/LPS	Rats	(Liver function, survival time) ↑	(Neutrophils, TNF-α, IL-1β, CXCL1, CXCL2) ↓; IL-10 ↑	[76]
Tail vein	1×10^6	IL-1β siRNA and BMMSCs	BMMSCs	CCl_4	Mice	(Liver regeneration, survival rates) ↑	(CXCL1, IL-1β, IL-6) ↓; (IL-10, VEGF, HGF) ↑	[102]
Portal vein	1×10^6	Overexpression of IL-1Ra in AF-MSCs	AF-MSCs	D-Gal	Rats	(Liver failure, mortality) ↓	(Anti-inflammation, anti-apoptosis) ↓; (engraftment, proliferation) ↑	[103]
Tail vein	1.0×10^7/kg	Overexpression of c-Met in BMMSCs	BMMSCs	D-Gal/LPS	Rats	(Liver function, survival rates) ↑	(Hepatic activity index, homing ability) ↓	[104]
Tail vein	1×10^6	Overexpression of CXCR4 in BMMSCs	BMMSCs	CCl_4	Mice	Survival time ↑	(HGF, VEGF, colonization, proliferation, migrative capacity) ↑	[105]
Tail vein	1×10^6	Overexpression of HGF in UCMSCs	UCMSCs	APAP	Mice	Liver injury ↓; survival time ↑	(GSH, γ-GCS, SOD, CAT, migration) ↑; apoptosis ↓	[106]
Tail vein	$1–1.3 \times 10^6$	Inhibition of autophagy in BMMSCs	BMMSCs	CCl_4	Mice	(Liver enzymes, necrosis) ↓; normal histology	Autophagy ↓	[107]

the mortality of rats with ALF more than the control MSC group as the IL-1Ra-MSCs demonstrated enhanced proliferative ability and engraftment in injured tissues [103]. The overexpression of c-Met improved the migrative capacity of MSCs in a HGF-dependent manner in vitro, and these modified MSCs showed better engraftment in the injured site accompanied by improved liver functions and higher survival rates of ALF rats [104]. Similarly, the overexpression of CXCR4 significantly increased the release of HGF and VEGF in MSCs, which thus improved the migrative capacity and colonization of MSCs, leading to a longer lifetime of ALF mice [105]. HGF-MSCs maintained redox homeostasis, reduced liver injury, and prolonged the survival of ALF mice via increments in serum GSH, γ-glutamylcysteine synthetase, SOD, and CAT. Moreover, they also inhibited hepatocyte apoptosis via the upregulation of Bcl2 and downregulation of Bax and TNF-α [106].

In addition to gene modification by the overexpression of anti-inflammatory factors, chemotactic factors and growth factors, Amiri et al. showed that the inhibition of autophagy improved the regenerative capacity of MSCs as demonstrated by reduced liver enzymes and necrosis scores in ALF rats compared with the scores in control MSC rats. Intriguingly, ALF mice that received autophagy inhibited MSCs demonstrated normal histology without necrosis, while ALF mice that received unmodified MSCs demonstrated mild necrosis [107]. Accordingly, the knockdown of inflammatory factors or apoptosis-related genes may become a hot topic to improve the therapeutic effects of MSCs in ALF animal models.

Hepatogenic differentiation and HLC transplantation

After incubation with specific combinations of growth factors in vitro, MSCs can be differentiated into HLCs with hepatocyte functions. MSCs changed the morphology and expression of hepatocyte-specific genes and acquired liver-specific functions in response to hepatogenic differentiation medium. However, HLCs typically exhibit liver-specific functions, including secretion of albumin and urea, uptake of low-density lipoprotein and indocyanine green, glycogen storage, and cytochrome P450 activity for 2–3 weeks in vitro [9, 109] but lose these beneficial functions after a prolonged culture time [110]. Multiple studies have further investigated the therapeutic effects of hepatogenic MSCs with liver-specific functions for repairing liver injury in ALF models (Table 5).

Transplantation of HLCs significantly improved the liver function of CCl_4-treated mice via the secretion of TGF-β1, IL-6, and IL-10 [111]. Transplantation of HLCs before liver resection decreased the extensive lipid accumulation in hepatocytes and maintained the balance of amino acids, acylcarnitines, sphingolipids, and glycerophospholipids, thus promoting hepatocyte survival and inhibiting hepatocyte apoptosis in partial hepatectomy-induced ALF animal models [112]. Culturing on Matrigel that contained HGF and fibroblast growth factor-4 efficiently promoted the hepatogenic differentiation of MSCs, and intrasplenic injection of these HLCs prevented liver injury in 90% of hepatectomized rats [113].

An issue regarding the efficacy of HLCs compared with MSCs is that HLCs rapidly lose their liver functions and are sentenced to apoptosis after confronting a harsh environment. Transplantation of HLCs differentiated from amniotic fluid–derived MSCs (AF-MSCs) did not exert a recovery effect on ALF mice because they failed to enter the injured liver section, while transplantation of hepatic progenitor–like (HPL) cells, which are derived from AF-MSCs, underwent hepatogenesis for 1 week and showed a better effect in reducing liver injury [10]. Wang et al. [114] showed that HLCs expressed lower levels of HGF and had impaired immunosuppression compared with undifferentiated MSCs; thus, HLCs showed inferior potency to repair the injury in an ALF mouse model. However, other authors reject these points of views. Li et al. demonstrated that undifferentiated MSCs and HLCs exert similar effects on liver regeneration in ALF rats, and both groups decreased the levels of transaminases and TBIL 7 days after transplantation compared with the control group [115]. Undifferentiated MSCs and HLCs also exhibited similar abilities in homing into the injured liver tissue and rescued nearly all ALF mice after tail vein injection; while they rarely differentiated into human hepatocytes in the mouse liver, they stimulated the proliferation of mouse hepatocytes [109]. In addition, ADMSCs and BMMSCs displayed similar effects on repairing injuries compared with HLCs from both types of MSCs, although the gene expression profile of HLCs from ADMSCs was more close to a normal hepatogenic differentiation profile [9]. In our opinion, HLCs are more sensitive to harsh environments in vitro and in vivo; thus, MSCs without differentiation can benefit ALF animal models more than HLCs.

MSC-derived CM and Ex

MSC-CM and MSC-Ex, which contain many soluble factors, have been reported to exert therapeutic effects on ALF by inhibiting hepatocyte apoptosis, reducing panlobular leukocytic infiltrates and improving liver regeneration in recent years (Table 6).

MSC-CM

MSC-CM and MSC treatment comparably increased the liver function, reduced the serum levels of IFN-γ, IL-1β, and IL-6, and upregulated the serum IL-10 levels in ALF models.

Table 5 Evaluation of the therapeutic effects of HLCs derived from MSCs in ALF animal models

Route	Dose	HLC type	Control group	Inducer	Animal	Effects	Mechanisms	Ref
Orbital vein	1×10^6	HLCs derived from ADMSCs and BMMSCs	ADMSCs and BMMSCs	CCl_4	Mice	Liver injury ↓	Hepatogenic differentiation ↑	[9]
Intrahepatic/intraperitoneal	1.5×10^6	HLCs derived from AF-MSCs	AF-MSCs and HPL cells	CCl_4	Mice	No liver regeneration in HLC group; however, HPL group showed better liver regeneration	HLCs failed to enter the damaged liver	[10]
Tail vein	2×10^6	HLCs derived from UCMSCs	UCMSCs	D-Gal/LPS	Mice	Similar homing ability, rescued nearly all ALF mice	Host liver regeneration	[109]
Tail vein	1.5×10^7	HLCs derived from ADMSCs	PBS	CCl_4	Mice	Similar effects on liver regeneration	(TGF-β1, IL-6, IL-10) ↑	[111]
Splenic	1.5×10^6	HLCs derived from ADMSCs before liver resection	PBS	Partial hepatectomy	Rats	Hepatocyte survival ↑; hepatocyte apoptosis ↓	Extensive lipid accumulation ↓; (balance of amino acids, acylcarnitines, sphingolipids, and glycerophospholipids) ↑	[112]
Splenic	7×10^7	HLCs derived from BMMSCs	BMMSCs	90%-hepatectomy	Rats	HLCs prevent ALF, while undifferentiated MSCs did not affect fatality	(CXCR4, HGF, TGF-α, c-Met, EGFR) ↑	[113]
Intravenous	1×10^6 and 1×10^5	HLCs derived from UCMSCs	UCMSCs	D-GalN/LPS	Mice	HLCs have inferior potency to repair injury in ALF compared with undifferentiated UCMSCs	(HGF, immunosuppression) ↓	[114]
Tail vein	2×10^6	HLCs derived from BMMSCs	BMMSCs	CCl_4	Rats	Similar effects in reducing transaminases and TBIL and increasing liver regeneration	Engraftment ↑	[115]

Table 6 Evaluation of therapeutic effects of CM or exosomes derived from MSCs in ALF animal models

Route	Dose	CM source/Ex source	Control group	Inducer	Animal	Effects	Mechanisms	Ref
Intrahepatic	25-fold	AF-MSCs	CM derived from HPL cells	CCl_4	Mice	CM derived from HPL cells more efficient than CM derived from AF-MSCs in treatment of the liver	(IL-10, IL-1Ra, IL-13, IL-27) ↑	[10]
Tail vein	50 μg	MenSC-Ex (24 h before treatment with D-GalN/LPS)	PBS	D-GalN/LPS	Mice	(Liver function, survival rates) ↑; liver cell apoptosis ↓	(Macrophage proliferation, caspase-3) ↓	[11]
Peritoneal cavities	15-fold	ESC-MSCs and BMMSCs	Nonconditioned medium	TAA		Liver function ↑; no survival benefit after 1 week	(Primary hepatocyte viability, IL-10) ↑	[13]
Tail vein	1.0 ml	BMMSCs	BMMSCs/ DMEM	D-GalN	Rats	(Liver function, IL-10) ↑; (IFN-γ, IL-1β, and IL-6) ↓	STAT3 ↑	[21]
Intravenous	5×10^5	BMMSCs	NaCl	CCl_4/α-GalCer	Mice	Hepatitis ↓	NKTreg/NKT17 cell ratio ↑; NKT cells ↓	[116]
Splenic	5×10^6	ADMSCs	PBS	D-GalN	Rats	Hepatocytic proliferation ↑; hepatocyte apoptosis ↓	Anti-inflammatory factors ↑	[117]
Femoral vein	25-fold	ADMSCs	PBS	D-GalN	Rats	Restoration of liver function ↑	(HGF, VEGF) ↑	[117]
Intravenous	25-fold	BMMSCs	2×10^6 BMMSCs	D-GalN	Rats	BMMSCs did not exhibit an additional benefit in ALF rats, while MSC-CM promoted reversion of liver injury	(Migration, chemokines) ↑	[118]
Tail vein	25-fold	MSCs cocultured with hepatocytes (MSC-H-CM)	MSC-CM, CM derived from hepatocytes (H-CM), combination of MSC-CM and H-CM, nonconditioned medium	D-GalN	Rats	Survival rate ↑	Liver injury biomarkers ↓; recovery of liver tissue ↑	[119]
Tail vein	25-fold	BMMSCs	1×10^6 BMMSCs	TAA	Mice	CM did not significantly improve survival rate	Liver repair at later stages of self-recovery ↑	[75]
Intrasplenic	0.4 μg	ESC-MSC-Ex	PBS	CCl_4	Mice	ALF ↓	(Hepatocyte proliferation, NF-κB, STAT3) ↑	[120]
Tail vein	400 μg	ADMSC-Ex	Knockdown of miR-17 in ADMSC-Ex	LPS/GalN	Mice	(ALT, AST, inflammatory factors) ↓	Activation of macrophages ↓	[121]
Tail vein or intragas-tric	20 mg/ml (8 mg/kg, 16 mg/kg, 32 mg/kg)	UCMSC-Ex	PBS	CCl_4	Mice	Oxidative stress-induced apoptosis ↓; liver regeneration ↑	(GPX1, ERK1/2, Bcl-2) ↑; IKKB/NFkB/casp-9/−3 pathway ↓	[122]

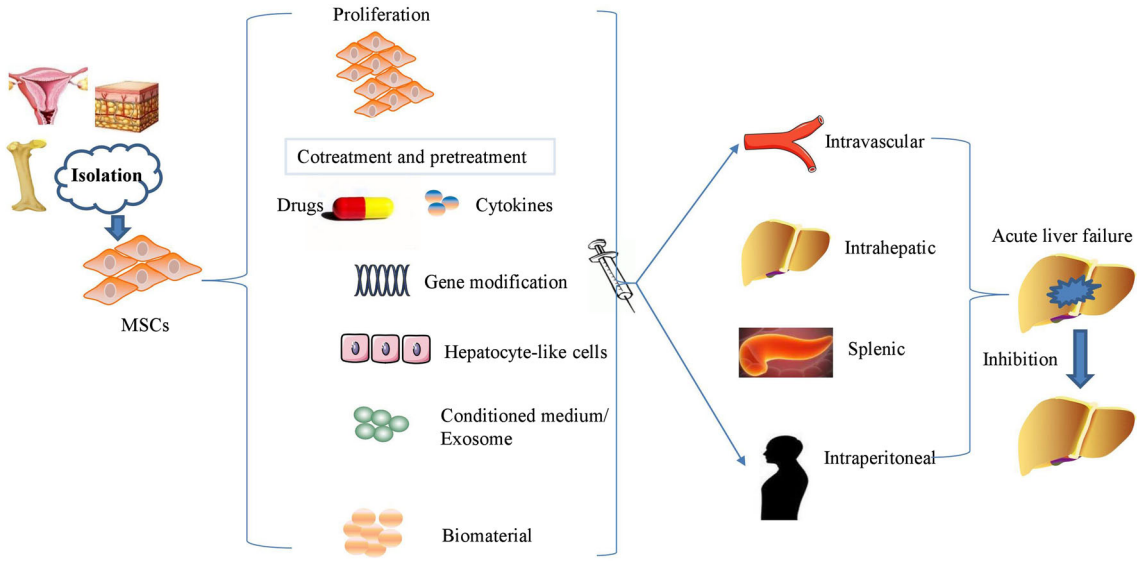

Fig. 2 Transplantation of MSCs and their derivatives via multiple routes can effectively inhibit the development of ALF

Moreover, IL-10 has been suggested to be the most important anti-inflammatory cytokine for the activation of signal transducer and activator of transcription 3 (STAT3) [21]. Injection of MSC-CM and MSCs clearly increased the natural killer T regulatory cell (NKTreg)/IL-17-producing natural killer T (NKT17) cell ratio in liver tissue and decreased the hepatotoxicity of NKT cells in a paracrine, indoleamine 2,3-dioxygenase-dependent manner, consequently attenuating hepatitis in vivo [116]. In addition, both MSC-CM and MSC lysates restored liver function and improved the survival rate of ALF rats via the secretion of HGF and VEGF [117].

Intriguingly, MSCs did not exhibit an additional benefit for ALF rats because of their poor engraftment and immune rejection, while MSC-CM substantially reduced hepatocellular death and bile duct duplication by promoting immune cell migration away from liver tissue and releasing chemokines [118]. Zagoura et al. [10] debated with the opinion that transplantation of CM derived from HPL cells showed a more efficient effect than AFMSC-CM via the secretion of more anti-inflammatory factors, including IL-10, IL-1Ra, IL-13, and IL-27. CM derived from MSCs cocultured with hepatocytes (MSC-H-CM) was the most effective medium for improving cell viability and total protein synthesis, decreasing the levels of lactate dehydrogenase and AST, and inhibiting the apoptosis of D-galactosamine (D-GalN)-treated LO2 cells in vitro than MSC-CM, CM derived from hepatocytes (H-CM), combinations of MSC-CM and H-CM, and nonconditioned medium (NCM). Moreover, MSC-H-CM most efficiently reduced liver injury biomarkers and enhanced the recovery of liver tissue and consequently improved the survival rate of ALF rats [119].

Although MSC and CM significantly improved the gross histopathological appearance of thioacetamide (TAA)-stimulated livers, CM did not remarkably or significantly improve the survival rate since it only enhances liver regeneration at later stages of self-recovery [75]. ESC-MSC is also an important MSC source, which has similar stemness characteristics compared with BMMSCs, but they grow faster than BMMSCs in vitro. An in vitro study showed that ESC-MSC-CM significantly improved the primary hepatocyte viability and upregulated the IL-10 levels in LPS-induced human blood mononuclear cells. However, BMMSC-CM and ESC-MSC-CM did not provide a survival benefit after 1 week of transplantation in ALF animals, although they increased the liver function after 48 h of transplantation [13]. This finding raises concerns as to whether MSC-CM always contributes to the improved outcomes of ALF.

MSC-Ex

Ex are small biological membrane vesicles from CM and contain many active substances (mRNAs and adhesion molecules) for the regulation of cellular and tissue physiology in vitro and in vivo. MSC-Ex express high levels of cytokines, such as angiopoietin-2, Axl, angiogenin, osteoprotegerin, IL-6, IL-8, insulin-like growth factor binding protein-6, and intercellular cell adhesion molecule (ICAM)-1. In vitro, MSC-Ex can be taken up by AML12 cells (a mouse hepatocyte cell line) and migrate to inhibit the apoptosis of D-GalN/LPS-induced AML12 cells [11]. Moreover, MSC-Ex in vitro inhibited APAP- and H_2O_2-induced hepatocyte apoptosis via the upregulation of Bcl-XL and promotion of hepatocyte proliferation but not via alleviation of oxidative stress [120].

After transplantation in vivo, MSC-Ex significantly reversed CCl_4-induced ALF in mice by promoting hepatocyte proliferation and upregulation of NF-κB and STAT3 [120]. MSC-Ex significantly reduced the serum levels of ALT, AST, and inflammatory factor secretion by prohibiting the

activation of macrophages, and miR-17 is an indispensable factor that targets thioredoxin-interacting protein for the suppression of NLRP3-mediated inflammation in ALF [121]. It also engrafted in the liver to serve as an antioxidant and inhibit oxidative stress–induced apoptosis via the delivery of glutathione peroxidase-1 (GPX1), upregulation of ERK1/2 and Bcl-2, and downregulation of the IKKB/NFkB/casp-9/3 pathway [122]. MSC-secreted prostaglandin (PG) E_2 activated Yes-associated protein via upregulating the level of PGE_4 and enhancing the phosphorylation of cAMP, and Yes-associated protein activated the mammalian target of rapamycin via suppressing phosphatase and tensin homolog for enhancing the cell proliferation of hepatocytes and promoting the recovery of ALF [123]. Moreover, pretreatment with MSC-Ex before the induction of ALF inhibited macrophage proliferation and the expression of active caspase-3 in injured livers, consequently improving liver function and enhancing survival rates in ALF mice [11]. Thus, we believe that MSC-Ex represent a highly attractive therapeutic approach compared with MSCs without the risk of iatrogenic tumor formation or pulmonary embolisms in ALF.

Biomaterials for improving MSC transplantation efficacy

Biomaterials with perfect biocompatibility, an applicable microstructure, and a proper degradation rate have gradually attracted attention for improving MSC attachment, proliferation, and secretion of beneficial cytokines via supplementing them with oxygen, nutrition, and growth factors. A nanoparticle that carries MSC-derived regenerative factors and is coated with red blood cell membranes has lower macrophagic internalization and significantly improves the proliferation of liver cells in vitro, and these coated nanoparticles can be well maintained in the injured liver of ALF mice and mitigate the liver injury after transplantation [124]. The IL-1Ra chitosan nanoparticles that have a targeting ability and controlled-release features can also improve the efficacy of MSC transplantation. Cotreatment with IL-1Ra chitosan nanoparticles and MSC transplantation significantly improved liver function and promoted hepatocyte proliferation by improving the levels of HGF and VEGF and suppressing inflammation in ALF swine [125].

The coculture of MSCs and hepatocytes in poly (lactic acid-glycolic acid) (PLGA) scaffolds at 1:5 showed a higher proliferation rate and higher hepatic synthesis function than coculture in ratios of 1:2.5 or 1:10, and this treatment could significantly decrease the levels of ALT, AST, and TBIL in mouse serum stimulated by D-GalN compared with MSC-PLGA or hepatocyte-PLGA scaffold treatments. (MSC + hepatocyte)-PLGA scaffold treatment significantly improved liver function and increased the survival rate of ALF mice via the downregulation of IL-6 and IL-1β compared with MSC-PLGA or hepatocyte-PLGA scaffold treatments. In addition, the (MSC + hepatocyte)-PLGA scaffold-treated ALF mice showed a weaker immunogenic response than the other two groups [126]. Alginate scaffold-MSCs promoted liver recovery by enhancing the secretion of albumin and glycogen, thus improving the survival rate and liver function in rats with hepatectomy-induced ALF more than alginate scaffolds after placing them onto the surface of the liver wound [127]. Furthermore, Xu et al. [128] determined that MSC-seeded regenerated silk fibroin (RSF) scaffolds that were placed onto the liver surface of ALF mice substantially improved the angiogenesis and hepatogenic differentiation of MSCs and downregulated the infiltration of inflammatory cells in vivo more than neat RSF scaffolds, attributed to their increased biocompatibility and enhancement of hepatogenic differentiation. Yagi et al. [129] highlighted the therapeutic effects of liver assist devices (LADs) that contain cocultures of MSCs and hepatocytes via decreasing inflammation and improving the survival benefit in ALF animal models compared with other coculture systems and monocellular control LADs. These effects may be attributed to the coculture system increasing the rate of engraftment and reducing the immune response of the MSCs and hepatocytes.

Therefore, biomaterial scaffolds protect MSCs against harsh microenvironments in vitro and in vivo, in addition to providing physical and directional support for liver regeneration.

Conclusion

MSC transplantation benefits liver injury in ALF models via engraftment into liver tissue, hepatogenic differentiation, immunoregulation, promotion of host hepatocyte proliferation, secretion of anti-inflammatory factors and antioxidants, and the enhancement of liver regeneration in vivo; moreover, the burgeoning application of MSC-CM and MSC-Ex mainly protect ALF animals from progressive injury via immunoregulation and paracrine effects (Fig. 2). We have previously demonstrated the optimized procedures of MSC application in vitro and in vivo in the main text; thus, we highlight several key points as follows. As gene modifications directly alter the gene phenotype of MSCs, treatment with physical or chemical factors on MSCs possesses an absolute advantage. MSCs acquire chromosomal aberrations and spontaneous malignant transformation in vitro and in vivo [130]; thus, we suggest analyzing the chromosomal integrity of MSCs before transplantation in vivo to improve the safety of the procedure. It is worth noting that rare human studies of MSC transplantation were executed to rescue ALF, and it is obligatory for us to carry out multicenter-clinical trials for MSC-based therapy in treating ALF patients. Importantly, we highlight that

autologous or allogenic MSC transplantation should not be considered if the donor is bearing a genetic disease associated with a tumorigenic risk [84]. In summary, further breakthroughs are required to establish safer, more stable, and more effective stem cell–based therapy by MSCs and their derivatives in rescuing liver injury in ALF. We are looking forward to reversing acute injury before it progresses into ALF and decreasing the mortality of ALF patients worldwide via MSC-based therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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