Stem Cell Research & Therapy

REVIEW   **Open Access**

Current understanding of adipose-derived mesenchymal stem cell-based therapies in liver diseases

Chenxia Hu[†], Lingfei Zhao[†] and Lanjuan Li[*]

Abstract

The liver, the largest organ with multiple synthetic and secretory functions in mammals, consists of hepatocytes, cholangiocytes, hepatic stellate cells (HSCs), sinusoidal endothelial cells, Kupffer cells (KCs), and immune cells, among others. Various causative factors, including viral infection, toxins, autoimmune defects, and genetic disorders, can impair liver function and result in chronic liver disease or acute liver failure. Mesenchymal stem cells (MSCs) from various tissues have emerged as a potential candidate for cell transplantation to promote liver regeneration. Adipose-derived MSCs (ADMSCs) with high multi-lineage potential and self-renewal capacity have attracted great attention as a promising means of liver regeneration. The abundance source and minimally invasive procedure required to obtain ADMSCs makes them superior to bone marrow-derived MSCs (BMMSCs). In this review, we comprehensively analyze landmark studies that address the isolation, proliferation, and hepatogenic differentiation of ADMSCs and summarize the therapeutic effects of ADMSCs in animal models of liver diseases. We also discuss key points related to improving the hepatic differentiation of ADMSCs via exposure of the cells to cytokines and growth factors (GFs), extracellular matrix (ECM), and various physical parameters in in vitro culture. The optimization of culturing methods and of the transplantation route will contribute to the further application of ADMSCs in liver regeneration and help improve the survival rate of patients with liver diseases. To this end, ADMSCs provide a potential strategy in the field of liver regeneration for treating acute or chronic liver injury, thus ensuring the availability of ADMSCs for research, trial, and clinical applications in various liver diseases in the future.

Introduction

The liver, the largest organ with multiple synthetic and secretory functions in mammals, consists of hepatocytes, cholangiocytes, hepatic stellate cells (HSCs), sinusoidal endothelial cells, Kupffer cells (KCs), and immune cells, among others [1]. Hepatocytes and cholangiocytes constitute the majority of liver parenchymal cells and play critical roles in maintaining liver function and biliary secretion; thus, the liver participates in the regulation of energy metabolism and detoxification. Under physiological conditions, HSCs, or fat-storing cells, are located

in the parasinusoidal space; they mainly store retinoids and produce extracellular matrix (ECM) that is used in the generation of the basement membrane [2]. Liver sinusoidal endothelial cells are known to secrete several growth factors that promote hepatocyte proliferation, and they are responsible for forming new vasculature [3]. Liver KCs represent approximately 20% of the non-parenchymal cells in the liver and serve as an immune barrier for liver tissue; the activation of Kupffer cells acts as the priming force for hepatocyte proliferation [4]. Natural killer (NK) cells, natural killer T (NKT) cells, eosinophils, and other cells constitute the majority of cells associated with innate immunity in the liver and contribute to liver regeneration [5, 6]. Various causative factors, including viral infection, toxins, autoimmune defects, and genetic disorders, can impair liver function and result in chronic liver disease or acute liver failure. Although liver tissue has a remarkable ability to

* Correspondence: ljli@zju.edu.cn
†Chenxia Hu and Lingfei Zhao contributed equally to this work.
Kidney Disease Center, First Affiliated Hospital, College of Medicine, Zhejiang University; Key Laboratory of Kidney Disease Prevention and Control Technology, Institute of Nephrology, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China

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regenerate after injury, orthotopic liver transplantation (OLT) is still required to rescue patients with end-stage liver disease or liver failure involving large numbers of necrotic and apoptotic hepatocytes at the irreversible stage [7]. However, the application of OLT is limited by donor scarcity, the side effects of immunosuppressants, and ethical issues [8, 9]. A potential alternative to OLT, hepatocyte transplantation (HT), is simpler, less invasive, and safer; however, the application of HT is limited by the finite proliferation capacity and limited liver functions of primary hepatocytes [10]. Fortunately, mesenchymal stem cells (MSCs) from various tissues have emerged as potential candidates for cell transplantation to promote liver regeneration [11]. These multipotent cells are fibroblast-like and can differentiate into adipocytes, osteocytes, chondrocytes, hepatocytes, and other types of cells [12].

Bone marrow-derived MSCs (BMMSCs) have become the most common source of multipotent cells for transplantation in experimental studies and clinical trials since they were first isolated in 1970 by Friedenstein et al. [13]. To standardize MSCs, the International Society for Cell Therapy suggests the following minimal criteria [14]: adherence to plastic in conjunction with a fibroblastoid phenotype; expression of CD105, CD73, and CD90 and lack of expression of CD45, CD34, CD14 (or CD11b), CD79α (or CD19), and HLA-DR surface molecules; and the capacity to differentiate into chondrocyte, adipocyte, and osteocyte lineages. The low rate of immunological rejection of such cells makes it possible to use them in both autotransplantation and allogeneic transplantation applications [15]. MSCs have been reported to participate in repairing tissue or organ injury mainly through their paracrine effects, namely, stimulation of angiogenesis, protection of other cells from apoptosis, and recruitment of host MSCs or other progenitor cells and stimulation of their proliferation and differentiation [16]. MSCs also have anti-oxidative capacity that helps protect tissues against reactive oxygen species (ROS)-induced injury [17]. Moreover, cell fusion of MSCs also contributes to the repair of tissues and organ function [18]. These advantages allow MSCs to be used in the treatment of various diseases and to be clinically applied in the field of regenerative medicine.

The use of the iliac crest for bone marrow extraction is painful, and there is high risk of infection following this procedure [19]. Adipose-derived MSCs (ADMSCs) are collected from adipose tissue by liposuction, washing, collagenase digestion, and centrifugation in a process that is less invasive and easier than the harvesting of bone marrow cells; this permits wide use of ADMSCs [20]. The isolated stromal vascular fraction (SVF) of adipose tissue contains circulating blood cells, fibroblasts, pericytes, endothelial cells, and ADMSCs

[21]. SVF is reported to contain 0.02 to 0.06% ADMSCs, whereas bone marrow mononuclear cells consist of only 0.001 to 0.01% BMMSCs [22]. The isolated undifferentiated ADMSCs express MSC surface markers and liver-specific genes including alpha fetoprotein (AFP), cytokeratin (CK)-18, CK-19, and hepatocyte nuclear factor (HNF)-4; moreover, they also weakly express albumin (ALB), glucose-6-phosphate, and α1-antitrypsin [23]. ADMSCs effectively maintain endothelial and vascular function via the secretion of vascular endothelial growth factor (VEGF) and nitric oxide (NO) [24, 25], and they exert an anti-oxidative effect via the upregulation of superoxide dismutase (SOD) and malondialdehyde (MDA) [26]. ADMSCs also participate in the stimulation of regulatory T cells (Tregs) and in the simultaneous suppression of Th1, Th2, and Th17 cells via the upregulation of immunomodulatory factors including IL-10, TGF-β, indolamine 2, and 3-dioxygenase and the downregulation of inflammatory factors such as IL-4, IL-12, IL-17, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, t-bet, CD80, CD83, and CD86 [27, 28]. It is worth noting that IL-4 is primarily known for its anti-inflammatory effects due to its capacity to suppress Th1 responses and induce protective immunity against intracellular pathogens [29], while IL-4-producing Th2 cells directly mediate tissue destruction and can cause autoimmune disease if transferred to an immune-deficient host [30]. Intriguingly, ADMSCs were shown to survive for up to 4 months after transplantation in vivo [31]. Although ADMSCs share some of the biological properties of BMMSCs, they also have some distinct properties. For example, CD106, which is also known as vascular cell adhesion molecule 1 and is involved in cell migration, is expressed at significantly lower levels in ADMSCs than in BMMSCs [32]. On the other hand, both ADMSCs and BMMSCs express high levels of OCT4, NANOG, SOX2, alkaline phosphatase (ALP), and SSEA4 [33]. BMMSCs from aging donors demonstrated lower cell activity and differentiation capacities, whereas the cell activity of ADMSCs from aging donors is not limited [34, 35]. ADMSCs are superior in immune regulation compared to BMMSCs [36]; ADMSCs were shown to secrete higher levels of interleukin (IL)-6, IL-8, interleukin 1 receptor alpha (IL-1Rα), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein 1, nerve growth factor (NGF), and hepatocyte growth factor (HGF) than BMMSCs for elimination of liver injury [37]. Although ADMSCs secreted more NGF and transforming growth factor (TGF)-β1 than BMMSCs, they inhibited the proliferation and activation of HSCs to a comparable degree while promoting the apoptosis of HSCs for eliminating liver fibrosis [38]. In addition to a paracrine pathway, ADMSCs possess

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hepatogenic differentiation potential similar to that of BMMSCs as shown by their similar levels of expression of CK-18, CK-19, AFP, ALB, cytochrome (CYP), and other liver-enriched transcription factors but can be cultured for a longer period and have higher proliferation capacity [39, 40]. After transplantation in vivo into mice with acute liver failure (ALF), ADMSCs decreased the levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) and improved liver histopathology more effectively than BMMSCs [41].

Given that ADMSCs are superior to BMMSCs in some respects, including ease of manipulation, abundance, and potentially higher stemness, herein we comprehensively analyze landmark studies of the isolation, proliferation, and hepatogenic differentiation of ADMSCs and summarize the therapeutic effects of ADMSCs in animal models with liver diseases. We also discuss key points for improving the hepatic differentiation of ADMSCs via exposure to cytokines and growth factors (GFs), extracellular matrix (ECM), and physical parameters in in vitro culture. The optimization of culturing methods and transplantation route will contribute to the further application of ADMSCs in liver regeneration and help improve the survival rate of patients with liver diseases in the near future.

The source of ADMSCs

Adipose tissue can be collected from subcutaneous tissue [42], viscera [43], omentum [44], inguinal fat pads [45], peritoneal fat [46], and other sources. Although ADMSCs isolated from visceral adipose tissue appeared larger than those isolated from subcutaneous adipose tissue, both sets of ADMSCs showed similar pluripotency and plasticity and expressed MSC markers (CD105 and CD13) as well as other markers (SOX2, OCT4, LIF, and NANOG) [43]. ADMSCs isolated from human liver falciform ligaments showed higher levels of hematopoietic- and mesenchymal-epithelial transition (MET)-related surface markers than ADMSCs obtained from human abdominal subcutaneous adipose tissue, whereas both groups of cells display similar proliferation, multi-lineage capacity, and hepatic induction [47]. Considering that ADMSCs from visceral and subcutaneous tissues are comparable in pluripotency, plasticity, and hepatogenic differentiation, the ease of acquisition currently makes subcutaneous adipose tissue the optimal source of ADMSCs.

Allogeneic ADMSCs are isolated from a cell donor other than the cell recipient, while autologous ADMSCs are isolated from the cell recipient. Autologous ADMSCs serve as the ideal source since their use involves no ethical issues and they display high histocompatibility and low immune rejection [48]. Strong et al. demonstrated that ADMSCs isolated from animals with

chronic inflammatory diseases such as obesity and multiple sclerosis were less effective in immunomodulation [49], while Hu et al. demonstrated that ADMSCs isolated from ALF pigs have stem cell characteristics and cell activities similar to those of ADMSCs from control pigs; however, ADMSCs from ALF pigs showed increased expression of several liver-specific genes [50]. Although BMMSCs from patients with chronic hepatitis B infection proliferated poorly and were limited to hepatogenic differentiation, ADMSCs from these patients were not susceptible to infection by hepatitis B virus [51]. These findings indicate that allogeneic ADMSCs can be used in the treatment of patients with liver diseases.

Although the cellular phenotype and level of apoptosis displayed by ADMSCs obtained from infants, adults, and elderly people are similar, ADMSCs isolated from infants display a higher capacity for proliferation and migration. ADMSCs derived from adults and elderly people were significantly less efficient at suppressing T cell proliferation and showed increased production of IFN-γ and decreased production of IL-10 compared with infant-derived ADSCs, indicating that an age-associated decline in the immunomodulatory capacity of ADMSCs occurs [52]. Sequential passage in vitro exerts a negative impact on the multipotency of ADMSCs [53], and long-term culture results in replicative senescence, genetic instability, and upregulated immune responses in ADMSCs and consequently reduces their therapeutic efficacy [54, 55]. Thus, ADMSCs isolated from infants or early-passage cells may have greater potential to be effective in promoting liver regeneration than ADMSCs obtained from adults and elderly people and late-passage ADMSCs.

Hepatogenic differentiation in vitro and application of HLCs in vivo

Hepatogenic differentiation in vitro

ADMSCs are easily differentiated into hepatocyte-like cells (HLCs) as they change in morphology and cell function after treatment with specific cytokines and when exposed to a liver-damaged internal microenvironment [56]. ADMSC-derived HLCs exhibit several liver-specific functions, including ALB secretion, glycogen synthesis, urea formation, low-density lipoprotein uptake, CYP enzyme activity, and expression of carbamoylphosphate synthetase [11, 57]. HLCs derived from ADMSCs express periportal functions, including carbamoylphosphate synthetase 1 and the entry enzyme of the urea cycle, as well as perivenous functions, including CYP450 subtype 3a11 and CD26 [58]. Furthermore, the gene expression profiles of HLCs reveal a striking similarity between HLCs and liver tissue in their gene clusters, genes, and signaling pathways and MET transition [59]. ADMSCs can be induced to differentiate into hepatocytes by culturing for 2 weeks in hepatogenic medium

containing dexamethasone, insulin, HGF, and epidermal growth factor (EGF); the ADMSCs then complete the hepatogenic differentiation process via activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling pathway [56]. Step-by-step hepatogenic differentiation of MSCs promotes the generation of HLCs, as demonstrated by the appearance of early markers (ALB, alpha-2-macroglobulin, complement protein C3, and selenoprotein P1) and late markers (CYP, apolipoprotein E, acyl-CoA synthetase long-chain family member 1, and angiotensin II receptor, type 1). The loss of stem cell phenotype by these cells was detected by loss of expression of THY1 and inhibitor of DNA binding 3 [60].

Although current studies use various types of differentiation protocols, ADMSC-derived HLCs have immature hepatocyte functions; thus, specialists have attempted to develop new methods to improve the functions of HLCs. Serum from rats that underwent 70% partial hepatectomy (PH) promoted the hepatogenic differentiation of ADMSCs in vitro by upregulating the secretion of IL-6 and HGF [61]. In addition, ADMSCs exhibited more rapid changes in cellular morphology and expressed higher levels of AFP and ALB after incubation with liver extract than after culture in the presence of chemicals including HGF, fibroblast growth factor (FGF), and oncostatin M [62]. Trichostatin A, a specific histone deacetylase inhibitor, significantly enhanced the hepatogenic differentiation of ADMSCs by upregulating the expression of miR-122, ALB, HNF4α, and HNF6 while downregulating the AFP level [63]. Dimethyl sulfoxide, a common cryoprotectant, accelerated the hepatic differentiation of ADMSCs as shown by rapid changes in cell morphology, increased expression of ALB, CK18, HNF4α, and HNF6 and greater glycogen storage in the differentiated ADMSCs [64]. After incubation with activin A and FGF4 for 3 days and subsequent incubation with HGF, FGF1, FGF4, oncostatin M, dexamethasone, insulin–transferrin–selenium, dimethyl sulfoxide, and nicotinamide for 10 days, ADMSCs acquired the functional properties of primary human hepatocytes in vitro [65]. Using a three-step protocol involving incubation with IDE1 and CHIR99021; incubation with IDE1, FGF4, and HGF; and a final step that included exposure of the cells to HGF, EGF, oncostatin M, dexamethasone, and insulin–transferrin–selenium, Xu et al. induced ADMSCs to transform into HLCs with the functions of mature hepatocytes within 9 days [66]. In addition to culture in hepatic medium, gene modification also contributes to promotion of the hepatogenic differentiation of ADMSCs. Overexpression of OCT4 and SOX2 did not alter the expression of MSC markers or morphology in ADMSCs but did enhance the expression of ALB, urea, and glycogen in hepatogenic ADMSCs [67].

MicroRNAs (miRNAs) are small noncoding RNAs that help regulate diverse biological processes such as metabolism, proliferation, the cell cycle, and differentiation. The possible mechanism through which this occurs may be microRNA-mediated expression of GFs and cytokines, as miR-122 and miR-27b have been reported to play a critical role in the hepatogenic differentiation of ADMSCs [68, 69]. ADMSCs can be differentiated into HLCs by stable miR-122 overexpression and let-7f silencing without other stimulation. These genetically modified ADMSCs showed significantly increased expression of hepatocyte markers including ALB, AFP, CK-18, CK-19, and HNF-4a and upregulated urea, ALB, and glycogen production [70].

In recent years, the biochemical and mechanical signals provided by the ECM have been shown to effectively enhance the proliferation and differentiation of ADMSCs. When cultured on spots containing HGF and collagen I, ADMSCs showed significantly upregulated expression of ALB, AFP, and α1-antitrypsin compared to ADMSCs cultured on spots containing only collagen I [71]. Fabricated gelatin scaffolds with high biocompatibility promoted the adhesion and proliferation of ADMSCs without any adverse effects and significantly enhanced the hepatogenic differentiation of ADMSCs compared to culture on two-dimensional tissue culture polystyrene [72]. Furthermore, ADMSCs cultured on a three-dimensional scaffold consisting of gelatin cryogel and laminin displayed increased attachment and improved liver functions similar to those of HepG2 cells [73]. In the presence or absence of GFs, a liver decellularized matrix enhanced the hepatic differentiation of ADMSCs into mature hepatocytes significantly more effectively than other coating matrices including collagen, fibronectin, and Matrigel [74]. The ultimate aim of in vitro hepatogenic differentiation of ADMSCs is the acquisition of functional mature hepatocytes for HT in vivo; the safety of using modified cell culture microenvironments and of using the ADMSCs themselves should also be a matter of concern (Fig. 1).

Application of ADMSC-derived HLCs in vivo

Transplantation of HLCs before ischemia ameliorated hepatic dysfunction and improved liver regeneration after extended resection-induced ALF via attenuation of metabolic overload and normalization of amino acid, acylcarnitine, sphingolipid, and glycerophospholipid levels [46]. HLCs also reduced the levels of expression of ALT, AST, and ammonia and restored liver functions, including ammonia and purine metabolism, in ALF mice [65]. These ADMSC-derived HLCs showed more consistent gene expression and a more normal hepatogenic differentiation profile than HLCs from BMMSCs; moreover, transplantation of ADMSCs, BMMSCs, and HLCs

Fig. 1 Transplantation of HLCs and ADMSCs contributes to liver regeneration in various liver diseases
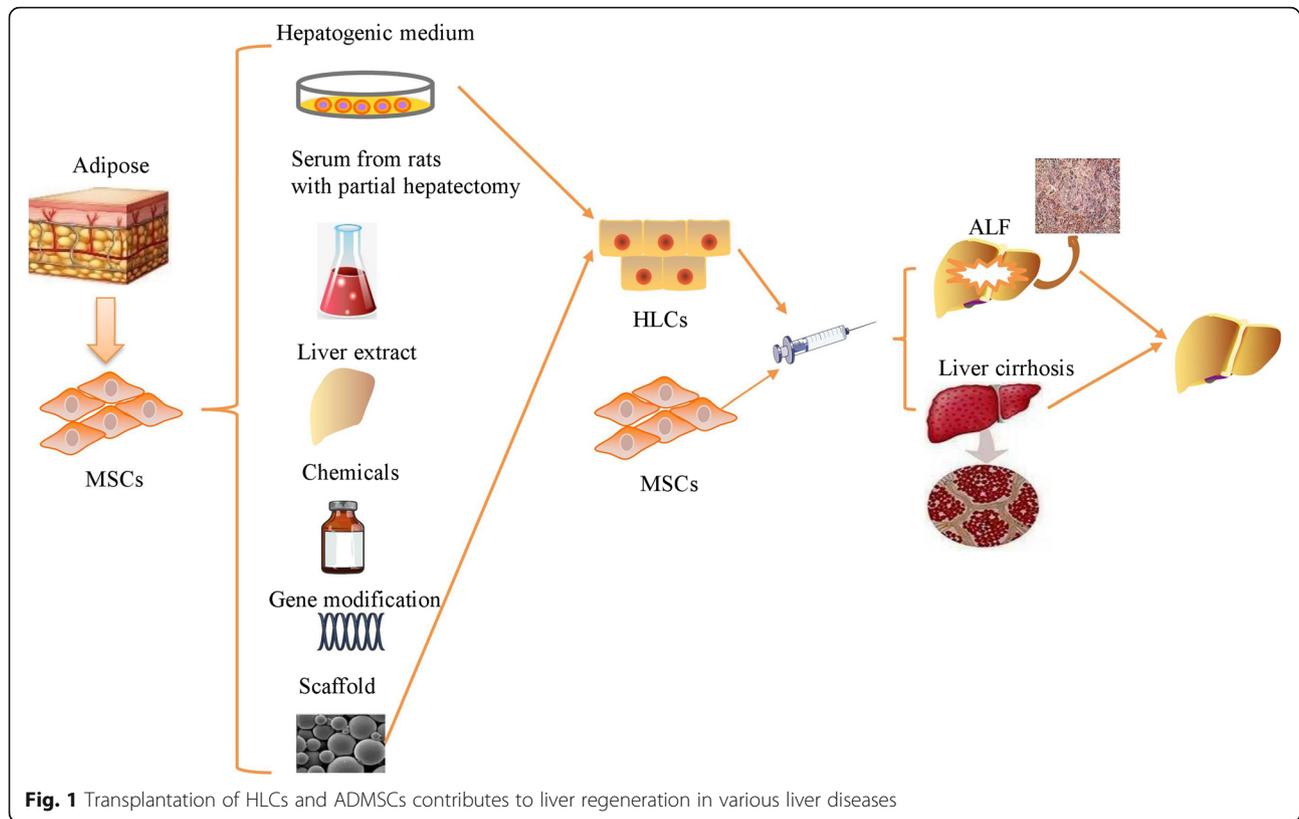
derived from ADMSCs and of HLCs derived from BMMSCs promoted liver regeneration in carbon tetrachloride (CCl_4)-induced ALF mice to comparable degrees [75]. However, there is a debate concerning the use of ADMSCs and HLCs in vivo. As Guo et al. demonstrated, transplantation of ADMSCs and HLCs improved liver function and rescued CCl_4-treated mice with liver injury, but ADMSC transplantation improved liver functions more effectively than transplantation of HLCs [76]. HLCs significantly restored liver function and prolonged the survival of mice with CCl_4-induced ALF by engraftment into the injured liver, but infusion of the liver with primary hepatocytes was not effective [66]. Furthermore, transplantation of HLCs eliminated CCl_4-induced liver fibrosis and preserved liver functions via the secretion of TGF-β1, IL-6, and IL-10 [77]. Engineered hepatic grafts that combined acellular human amniotic membrane with HLCs derived from ADMSCs significantly decreased the degree of CCl_4-induced liver injury by improving the expression of ALB, HNF-4α, and CYP450 2B6 [78]. However, Bruckner et al. demonstrated that these HLCs decreased the amount of collagen, the portal venous pressure, and the splenic weight but had no effect on the improvement of liver dysfunction, fibrillary collagen content, the balance of matrix metalloproteinases (MMPs) and metalloproteinases (TIMPs), or the activation of HSCs [79]. To this end, hepatogenic ADMSCs can be

used in the treatment of various liver diseases, but future studies should further investigate the potential mechanisms through which HLCs function in liver regeneration. The therapeutic effects of HLCs derived from ADMSCs can then be further improved for application in experimental and clinical trials.

ADMSC transplantation for liver regeneration

ADMSCs engraft in vivo and repair injured tissue via differentiation, immunomodulatory effects, and paracrine effects [80] (Fig. 2). Injured liver tissue and hepatocytes secrete various inflammatory factors and chemotactic cytokines that attract ADMSCs to the site of injury. ADMSCs are reported to produce tonofilaments and to then enter the injured sites after activation of the stromal-derived factor-1 (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) axis in the injured liver [81]. Furthermore, engrafted ADMSCs secrete various cytokines, including HGF and FGF, that promote the regeneration of endogenous hepatocytes and thereby help maintain the normal structure of the liver [82, 83]. ADMSC transplantation significantly increased regeneration of the remaining liver following repeat PH, as demonstrated by upregulation of the liver-to-body-weight ratio, HGF, and PCNA levels and downregulation of aminotransferases, total bilirubin (TBIL), and hepatic vacuolar degeneration at 24 h post-hepatectomy; moreover, the liver showed complete
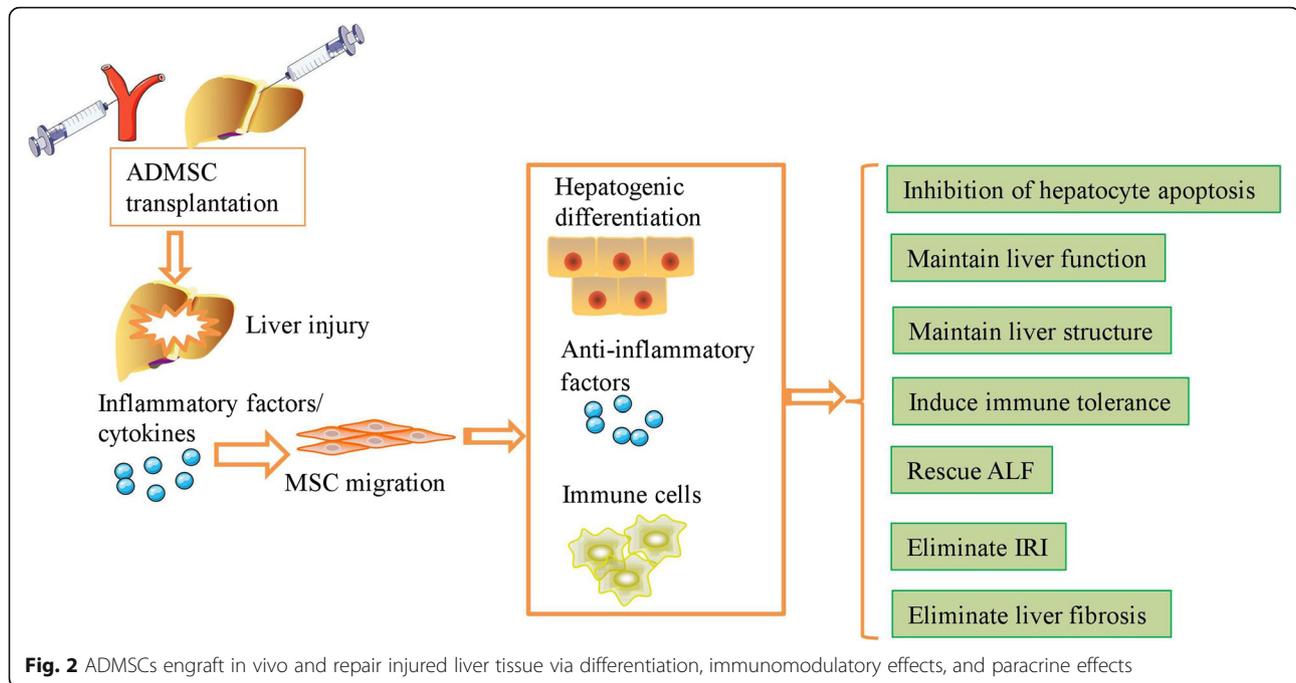
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Fig. 2 ADMSCs engraft in vivo and repair injured liver tissue via differentiation, immunomodulatory effects, and paracrine effects

recovery at 168 h after ADMSC transplantation [84]. MiR-27b-overexpressing ADMSCs enhanced liver regeneration and preserved hepatic function via the downregulation of inflammatory cytokines and the upregulation of HGF, HO-1, and mitochondrial biogenesis in a PGC-1α-dependent manner in PH rats [85].

Intravenously injected ADMSCs engrafted into various tissues, including brain, thymus, heart, liver, and lung, while PH enhanced the integration of ADMSCs into the liver and increased the regeneration of injured liver [86]. Transplantation of ADMSCs via the tail vein reduced biochemical parameters such as ALT, AST, and ammonia in CCl$_4$-induced liver injury more effectively than transplantation via the portal vein or direct liver parenchymal injection [87]. Transplantation of ADMSCs via the peripheral vein or the splenic vein decreased the levels of proinflammatory cytokines, including IL-1, IL-6, IL-8, and IFN-γ, and increased the levels of anti-inflammatory cytokines, including IL-4 and IL-10, HGF, and VEGF in an ALF animal model, while transplantation via the splenic vein significantly decreased the levels of serum liver enzymes and increased the number of engrafted ADMSCs in the liver more effectively than transplantation via the peripheral vein [88]. Wang et al. reported that administration of ADMSCs via the portal vein significantly decreased the hepatic arterial perfusion index but increased portal vein perfusion and microcirculation in rats with liver fibrosis [89]. According to current evidence regarding transplantation route, transplantation via the peripheral vein appears to be the most convenient method, but determination of which route is the most effective requires further study.

Ischemia/reperfusion-induced injury

Ischemia-reperfusion injury (IRI) of the liver is a well-known cause of morbidity and mortality after OLT and HT. ADMSCs decreased the apoptosis of hepatocytes, decreased the levels of ALT, AST, TBIL, IL-2, and IL-10, and maintained the tissue structure in rats with OLT via alleviation of acute rejection [90]. ADMSCs improved the survival rate of rats with liver IRI by downregulating IL-6, IL-21, and CD70 and activating the neurogenic locus Notch homolog protein pathway; the necrotic areas showed improved liver function and improved liver regeneration and maintained normal histology [91, 92]. Intrahepatic transplantation of ADMSCs markedly reduced the apoptosis of hepatocytes and decreased the severity of pathological changes via downregulation of Fas, Fas ligand, caspase-3, caspase-8, and caspase-9 and upregulation of the Bcl-2/Bax ratio in pigs with IR combined with laparoscopic hepatectomy [93]. ADMSCs significantly decreased the serum levels of ALT, AST, TBIL, and lactate dehydrogenase (LDH) via upregulation of SOD, suppression of myeloperoxidase (MPO) and MDA, and suppression of autophagy in swine with IRI [94]. On the other hand, administration of ADMSCs decreased hepatic oxidative stress and the expression of TNF-α, TGF-β, IL-1β, IL-6, endothelin-1, MMP-9, plasminogen activator inhibitor-1, Bax, caspase-3, and intercellular adhesion molecule but increased the levels of endothelial nitric oxide synthase, Bcl-2, IL-10, quinone oxidoreductase 1, and HO-1 in liver with IRI [95]. Sudden and prolonged interruption of the arterial blood flow to the liver accompanied by reperfusion initiated oxygen and

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nutrient deprivation, upregulation of oxidative reactions, and activation of inflammation in the liver, while ADMSCs are effective in eliminating IRI in liver tissues.

Chemically induced acute liver injury

As we know, the liver is the first organ to come into contact with various orally ingested drugs after intestinal absorption; thus, it is susceptible to chemically induced injury, and such injury can result in acute and chronic liver disease [96]. Banas et al. showed that ADMSC transplantation markedly improved liver functions and maintained the levels of ammonia, uric acid, and transaminases in animals with CCl$_4$-induced injury [37]. Animals treated with ADMSCs prior to CCl$_4$-induced ALF also demonstrated lower levels of ALT and IL-6 and higher expression of regeneration markers, accompanied by improved histopathology and survival rate [97]. In addition, spheroid-derived ADMSCs significantly increased liver regeneration in mice with CCl$_4$-induced ALF compared to ADMSCs derived from constant monolayer cultures [98], and regenerated silk fibroin (RSF)-treated ADMSCs rescued CCl$_4$-induced ALF animals via upregulation of angiogenesis and hepatogenic differentiation more effectively than ADMSCs on neat RSF scaffolds [99].

On the other hand, ADMSCs significantly decreased the levels of ALT, AST, and ammonia and returned prothrombin time to normal levels in acetaminophen (APAP)-induced ALF rats via inhibition of liver stress and inflammatory signaling and enhancement of liver regeneration [42]. In addition, ADMSC transplantation significantly attenuated the severity of APAP-induced liver injury and improved the survival rate of APAP-induced ALF mice via suppressing MAPK signal activation, reducing the level of toxic nitrotyrosine and upregulating NF-E2-related factor 2 (Nrf2) expression and anti-oxidant activity [44]. The immunomodulatory effect of ADMSCs may serve as an important mechanism in enhancing liver regeneration and maintaining liver histology without necrosis in the livers of mice with concanavalin A (ConA)-induced hepatitis. Kubo et al. demonstrated that ADMSCs significantly downregulated the levels of liver enzymes, decreased the histopathological changes and increased the survival rate of mice with ConA-induced fulminant hepatitis via suppression of inflammatory cytokines and a reduction in the number of CD11b$^+$, Gr-1$^+$, and F4/80$^+$ cells [100–102].

To improve the therapeutic effects of ADMSCs in vivo, preconditioning with lysophosphatidic acid (LPA) and/or sphingosine-1-phosphate (S1P) has been used. Treatment of cells with these agents synergistically enhanced the anti-stress effects of ADMSCs via Gi protein, the RAS/ERK pathway, the PI3K/AKT pathway, upregulation of IL-10, and promotion of the nuclear translocation of nuclear factor-kappa B (NF-κB). These LPA- and/or S1P-pretreated ADMSCs obviously ameliorated the histological damage, oxidative stress, inflammation, and lipid metabolism dysfunction in galactoside (Gal)/lipopolysaccharide (LPS)-induced ALF mice [103]. Although zeaxanthin dipalmitate (ZD)-pretreated ADMSCs exerted no adverse effects on healthy animals, they significantly improved liver function in a Gal/LPS-induced ALF model via upregulation of microRNA-210 and subsequent suppression of apoptosis, inflammation, and ROS in ADMSCs [104].

Liver fibrosis

Sustained hepatitis virus infection, alcohol consumption, and fat deposition lead to repeated and chronic liver injury, and the resulting accumulation of aberrant myofibroblasts and extracellular matrix results in liver fibrosis with poor prognosis. ADMSC transplantation significantly reduced serum levels of glutamic pyruvate transaminase and TBIL and reduced liver fibrosis as evidenced by Sirius Red staining [105]. Harm et al. concluded that the detailed mechanism through which ADMSCs eliminate liver fibrosis involves hepatic differentiation, reduction of inflammatory activity, and inhibition of HSC activation [106]. Furthermore, ADMSCs significantly reduced the expression of collagen I, collagen III, α-smooth muscle actin (α-SMA), hyaluronic acid, and hydroxyproline and inhibited liver fibrogenesis via inhibition of the activation of HSCs, enhancement of HSC apoptosis, upregulation of HGF, and downregulation of NGF and TGF-β1 [107]. A clinical study enrolled four patients with liver cirrhosis for ADMSC transplantation. The study found that ADMSCs maintained liver function well and that they upregulated the expression of liver regeneration-related factors (HGF and IL-6) in patients with liver cirrhosis [108].

Splenectomy prior to MSC administration suppressed liver fibrosis via upregulation of stromal cell-derived factor-1 and HGF, which facilitate the migration of MSCs into injured sites [109]. After incubation with serum from rats with acute CCl$_4$ injury, ADMSCs demonstrated polygonal morphology and expressed AFP, ALB, and CK8 and other hepatocyte markers. Moreover, ADMSCs preconditioned with serum from rats with acute CCl$_4$ injury significantly improved liver function and reduced liver fibrosis in CCl$_4$-induced liver fibrosis, as demonstrated by higher expression of hepatic and pro-survival markers and improvement in liver structure [110]. Exposure to basic fibroblast growth factor obviously upregulated the proliferation and differentiation of ADMSCs in vitro and enhanced the ability of ADMSCs to suppress the progression of liver fibrosis via elevation of HGF expression, promotion of HSC apoptosis, and enhancement of hepatocyte proliferation [83].

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Table 1 ADMSCs effectively participate in liver regeneration in the treatment of various liver diseases

Dose	Route	Pretreatment	IR method	Animal	Effect	Mechanism	Ref.
2×10^6	Tail vein	N/A	Liver transplantation	Rat	Decrease the apoptosis of hepatocytes; decrease the levels of ALT, AST, and TBIL; maintain the tissue structure	Decrease the expression of IL-2 and IL-10	[90]
1×10^5	Jugular vein	N/A	70% partial hepatectomy	Mice	Liver regeneration	Integration of ADMSCs into the liver	[86]
$1\text{–}2 \times 10^6$	Tail vein	Before ischemia	70% partial hepatectomy	Mice	Improve histopathological changes; decrease serum levels of hepatocyte markers	Enhance hepatocyte proliferation	[91]
4×10^6	Liver lobe	N/A	Bulldog clamp for 90 min and clamp removal	Rats	Decrease necrotic areas and improve liver function	Suppress the proinflammatory cytokines IL-6, IL-21, and CD70; activate the NOTCH Pathway	[92]
2×10^6	Portal vein	N/A	Repeated partial hepatectomy	Rats	Increase body weight ratio; improve liver function; improve PCNA-labeling index	Upregulate expression of HGF	[84]
1×10^6/kg	Liver parenchyma	N/A	Partial hepatectomy	Pigs	Reduce pathological and ultrastructural changes and decrease the number of apoptosis-positive cells	Downregulate the expression of Fas, Fas ligand, caspase-3, caspase-8, and caspase-9; upregulate of the ratio of Bcl-2/Bax	[93]
					Decrease the serum levels of ALT, AST, TBIL, and LDH	Upregulate the expression of SOD; suppress the expression of MPO and MDA; suppress autophagy	[94]
1.2×10^6	Liver lobe	N/A	Occlude the vascular supply of the left lobe of the liver for 60 m followed by reperfusion for 72 h	Rats	Reduce plasma aminotransferases; promote liver regeneration	Suppress cellular activation; reduce proinflammatory cytokine release; alleviate oxidative stress; preserve hepatic microcirculation; decrease apoptosis	[95]
2×10^6	Intravenous	Overexpression of MIR-27b	PH	Rats	Enhance liver regeneration and preserve hepatic function	Downregulate the expression of inflammatory cytokines; upregulate the expression of HGF, HO-1, and mitochondrial biogenesis in a PGC-1α-dependent manner	[85]
$1\text{–}2 \times 10^6$	Tail vein	Before CCl₄	CCl₄	Mice	Decrease levels of ALT and serum IL-6; increase the expression of regeneration markers and PCNA; improve histopathology; improve survival rate	Inhibit inflammation and liver necrosis	[97]
4×10^7 cells/kg	Spleen	Spheroid	CCl₄	Mice	Increase liver regeneration	Inhibit hepatocyte necrosis	[98]
1×10^6	Liver lobe	RSF	CCl₄	Mice	Increase the survival rate of ALF animals	Upregulate angiogenesis and hepatogenic differentiation	[99]
1.0×10^6	Intravenously	N/A	Con A	Mice	Increase the survival rate of ConA-induced fulminant hepatitis mice; decrease liver enzyme levels; improve histopathological changes	Suppress inflammatory cytokines	[100]
1×10^5	Tail vein	N/A	Con A	Mice	Liver histology showed an almost normal appearance, with no necrosis	Repress inflammatory cell accumulation	[101]
1.0×10^6	Tail vein	N/A	Con A	Mice	Decrease liver enzyme levels; improve histopathological changes	Decrease inflammation related to IL-6, IL-10, IFN-γ, and TNF-α	[102]
2×10^6	Tail vein	LPA and/or S1P	Gal/LPS	Mice	Enhance survival rate of Gal/LPS-induced ALF mice; ameliorate histological damage;	Reduce oxidative stress, inflammation and lipid metabolism dysfunction	[103]
2×10^6	Tail vein	ZD	Gal/LPS	Mice	Improve liver function of ALF model; exert no adverse	Activation of the PKC/Raf-1/MAPK/NF-κB	[104]

Table 1 ADMSCs effectively participate in liver regeneration in the treatment of various liver diseases (Continued)

Dose	Route	Pretreatment	IR method	Animal	Effect	Mechanism	Ref.
						pathway; upregulate microRNA-210	
1.0×10^6	Tail vein	N/A	CCl_4	Mice	Reduce serum levels of glutamic pyruvate transaminase and TBIL; reduce hepatocyte vacuolar degeneration; decrease serum transaminase levels; inhibit liver fibrogenesis	Increase MMP-3 and MMP-9 levels	[105]
1.0×10^6	Liver lobe	N/A	Thioacetamide	Rats	Eliminate liver fibrosis	Hepatic differentiation; reduce inflammation and inhibit HSC activation	[106]
5×10^6	Portal vein	N/A	CCl_4	Rats	Reduce the fibrotic area; reduce the expression of collagen I and a-SMA in the liver; reduce hydroxyproline level in the live; reduce collagen III and hyaluronic acid levels; inhibit liver fibrogenesis	Inhibit the proliferation and activation of HSCs; enhance HSC apoptosis; increase HGF level; decrease levels of NGF and TGF-b1	[107]
3×10^5/kg and 6.6×10^5/kg	Hepatic artery	N/A	Cirrhosis	Patients	Improve liver function	Increase serum HGF and IL-6 concentrations	[108]
5×10^6	Caudal vein	Splenectomy	CCl_4	Rats	Improve liver function; reduce levels of α-SMA and TGF-β; suppress liver fibrosis	Upregulate the levels of stromal cell-derived factor 1 and HGF; enhance the migration of ADMSCs into injured sites; promotes HSC apoptosis	[109]
1.5×10^6	Intrahepatic	Serum from acute CCl4 injury rat	CCl_4	Rats	Improve liver functions; reduce liver fibrosis	Increase the homing of ADMSCs	[110]
1.5×10^6	Tail vein	Overexpression of FGF21	Thioacetamide	Mice	Decrease serum hyaluronic acid; reduce serum ALT, AST, and hyaluronic acid levels; reduce expression of fibrosis-related factors such as α-SMA, collagen and TIMP-1;	Inhibition of p-JNK, NF-κB, and p-Smad2/3 signaling and secretion of LA and LTF	[111]
1×10^5	Tail vein	Overexpression of MiR-122	CCl_4	Mice	Decrease serum levels of ALT, AST, and liver hydroxyproline content; reduce mature Col1A1 protein level	Suppress the proliferation of and collagen maturation in HSCs; decrease the expression levels of TGF-β1 and α-SMA in the liver	[112]

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Genetically modified ADMSCs are currently being used in the treatment of liver fibrosis since they are able to accelerate repair of liver injury in vivo. HGF-overexpressing ADMSCs significantly decreased the serum levels of ALT and AST, ameliorated radiation-induced liver fibrosis via downregulation of α-SMA and fibronectin, and promoted hepatocyte regeneration [82]. Transplantation of FGF-21-overexpressing ADMSCs significantly attenuated thioacetamide-induced liver fibrosis via inhibition of p-JNK, NF-κB, and p-Smad2/3 signaling and secretion of α-lactoalbumin and lactotransferrin [111]. Overexpression of miR-122 enhanced the therapeutic efficacy of ADMSCs by suppressing proliferation and collagen maturation in HSCs in the treatment of CCl_4-induced liver fibrosis [112].

Conclusions

In comparison to MSCs from other sources, ADMSCs have similar multi-lineage potential, self-renewal capacity, anti-apoptotic effects, anti-oxidative effects, and anti-inflammatory effects after administration in vivo. We suggest that the application of ADMSCs in liver regeneration be increased since they have unique characteristics such as abundant source material and ease of isolation. Although various studies have focused on improving the hepatic functions of HLCs in vitro, these immature hepatocytes easily progress to the cell death pathway. Thus, we still recommend implanting ADMSCs in vivo since they are not extremely sensitive to the damaged microenvironment. Moreover, autologous ADMSCs are recommended because the use of autologous cells reduces the acute rejection rate. The liposuction process causes less trauma than bone marrow aspiration, and adipose tissue can provide a large number of ADMSCs for proliferation and injection. The ideal route of administration, dosage, and timing of ADMSC administration for the treatment of liver disease are highly variable; thus, studies should focus on the optimization of ADMSC efficacy in vivo. However, the short-term and long-term safety of the clinical application of cell transplantation is also an area of active controversy as cell transplantation may result in infections and potentially in tumorigenesis. To this end, ADMSCs provide a potential strategy in the field of liver regeneration for treating acute or chronic liver injury (Table 1), thus ensuring the availability of ADMSCs for research, trial, and clinical applications in various liver diseases in the future.

Abbreviations
ADMSCs: Adipose-derived MSCs; AFP: Alpha fetoprotein; ALB: Albumin; ALF: Acute liver failure; ALP: Alkaline phosphatase; ALT: Alanine transaminase; APAP: Acetaminophen; AST: Aspartate aminotransferase; BMMSCs: Bone marrow-derived MSCs; CCl4: Carbon tetrachloride; CK: Cytokeratin; ConA: Concanavalin A; CXCR4: C-X-C chemokine receptor type 4; CYP: Cytochrome; ECM: Extracellular matrix; ERK: Extracellular signal-regulated kinase; FGF: Fibroblast growth factor; Gal: Galactoside; G-CSF: Granulocyte colony-stimulating factor; GFs: Growth factors; GM-CSF: Granulocyte macrophage colony-stimulating factor; HGF: Hepatocyte growth factor; HLCs: Hepatocyte-like cells; HNF: Hepatocyte nuclear factor; HSCs: Hepatic stellate cells; HT: Hepatocyte transplantation; IFN: Interferon; IL: Interleukin; IL-1Rα: Interleukin 1 receptor alpha; IRI: Ischemia-reperfusion injury; KCs: Kupffer cells; LDH: Lactate dehydrogenase; LPA: Lysophosphatidic acid; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; MDA: Malondialdehyde; MET: Mesenchymal-epithelial transition; miRNAs: MicroRNAs; MMPs: Matrix metalloproteinases; MPO: Myeloperoxidase; MSCs: Mesenchymal stem cells; NF-κB: Nuclear factor-kappa B; NGF: Nerve growth factor; NK: Natural killer; NKT: Natural killer T; NO: Nitric oxide; Nrf2: NF-E2-related factor 2; OLT: Orthotopic liver transplantation; PH: Partial hepatectomy; ROS: Reactive oxygen species; RSF: Regenerated silk fibroin; S1P: Sphingosine-1-phosphate; SDF-1: Stromal-derived factor-1; SOD: Superoxide dismutase; SVF: Stromal vascular fraction; TGF: Transforming growth factor; TIMPs: Metalloproteinases; TNF: Tumor necrosis factor; Tregs: Regulatory T cells; VEGF: Vascular endothelial growth factor; ZD: Zeaxanthin dipalmitate; α-SMA: α-smooth muscle actin

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