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REVIEW

Mesenchymal Stem Cell–Derived Exosomes in Various Chronic Liver Diseases: Hype or Hope?

Lujian Zhu[1,*], Qin Wang[1,*], Maodong Guo[2], Hao Fang[3], Ting Li[4], Yin Zhu[5], Huimian Jiang[6], Peiguang Xiao[2], Minli Hu[2]

[1]Department of Infectious Diseases, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China; [2]Department of Gastroenterology, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China; [3]Department of Traumatology, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China; [4]Department of Emergency Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [5]Department of Infectious Diseases, Taizhou Enze Medical Center (Group), Enze Hospital, Taizhou, People's Republic of China; [6]Department of Infectious Diseases, the First Affiliated Hospital of Ningbo University, Ningbo, People's Republic of China

*These authors contributed equally to this work

Correspondence: Minli Hu, Department of Gastroenterology, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, People's Republic of China, Email huminlignmc@126.com

Abstract: Chronic liver conditions are associated with high mortality rates and have a large adverse effect on human well-being as well as a significant financial burden. Currently, the only effective treatment available for the effects of liver failure and cirrhosis resulting from the progression of several chronic liver diseases is liver transplantation carried out at the original location. This implies that developing novel and effective treatments is imperative. Regenerative medicine has long been associated with stem cell therapy. Mesenchymal stem cells (MSCs), a type of cell with great differentiation potential, have become the preferred source for stem cell therapy. According to recent studies, MSCs' paracrine products—rather than their capacity for differentiation—play a significant therapeutic effect. MSC exosomes, a type of extracellular vesicle (MSC-EV), came into view as the paracrine substances of MSCs. According to research, MSC exosomes can maintain tissue homeostasis, which is necessary for healthy tissue function. All tissues contain them, and they take part in a variety of biological activities that support cellular activity and tissue regeneration in order to preserve tissue homeostasis. The outcomes support the use of MSCs and the exosomes they produce as a therapeutic option for a range of diseases. This review provides a brief overview of the source of MSC-EVs and outlines their physiological roles and biochemical capabilities. The elucidation of the role of MSC-EVs in the recovery and repair of hepatic tissues, as well as their contribution to maintaining tissue homeostasis, is discussed in relation to different chronic liver diseases. This review aims to provide new insights into the unique roles that MSC-EVs play in the treatment of chronic liver diseases.

Keywords: mesenchymal stem cells, exosomes, liver disease, immunomodulation, tissue homeostasis

Introduction

Numerous pathogenic factors, such as viruses, chemicals, and autoimmune reactions, and the excessive consumption of alcohol can result in both acute and chronic liver damage, which leads to inflammation.[1] The liver cannot replace damaged liver cells, which leads to the development of serious problems like hepatic encephalopathy and hepatorenal syndrome, as well as symptoms including jaundice and poor blood coagulation.[2] This, in turn, can result in cirrhosis, liver failure, and, potentially, liver cancer, with a mortality rate ranging from 50% to 90%.[3] Current therapies for many liver diseases, however, are limited. In the case of advanced liver disease, especially cirrhosis and liver failure, the only possible treatment is a liver transplant performed in the patient's body.[4]

However, the limited availability of liver donors, exorbitant expenses, complications after surgery, and the risk of organ rejection are significant deterrents to transplantation.[5] As a result, researchers from all around the world have been exploring alternate types of therapy that can reverse fibrosis, decrease liver inflammation and necrosis, and promote the regeneration of liver cells.[6,7]

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MSCs are found in various tissues and are not restricted to mesodermal organs such as the bone marrow, adipose tissue, muscle, or bone.[8,9] They can also be extracted from the brain, spleen, liver, kidneys, lungs, thymus, and pancreas.[10–12] Owing to their remarkable ability to regenerate, in recent times, MSCs have undergone clinical testing for a broader spectrum of disease indications,[13] and the utilization of MSCs in clinical trials has seen tremendous growth.[14,15] MSCs are easily extracted from adult tissues and have a strong in vitro expansion capacity.[16,17]

Despite being found in a variety of tissues, MSCs share comparable phenotypic characteristics, and some may even have extra features that are indicative of the source tissues.[17–19] MSCs can transform into a minimum of three different cell types, namely, adipocytes, chondrocytes, and osteocytes.[19,20] Because MSCs are able to distinguish, their use in clinical trials has expanded substantially.[21] MSCs are now well recognized as a type of precursor cells that support the stroma and have the ability to differentiate into stroma-supporting cells. They also produce pro-stromal factors and diverse cell growth factors.[22–25]

MSCs[26] play a role in maintaining the hematopoietic stem cell balance within the matrix and also contribute to the maintenance of microenvironmental homeostasis.[27] Self-renewal and differentiation maintain equilibrium between vital and static hematopoietic stem cells within this microenvironment.[28] MSCs are becoming increasingly important in the study of liver diseases due to their capacity for self-renewal and multi-directional differentiation. These cells can be obtained from different tissues, including bone marrow, umbilical cord, and adipose tissue. In addition, they have immunomodulatory, apoptosis-inhibition, cell-regeneration, and anti-fibrotic properties.[13,19,29–33] The liver, being a crucial organ in regulating immunity and metabolism, contains numerous immune cells, including myeloid and lymphoid cells. Disruption of hepatic immune homeostasis is often linked to various liver diseases.[34,35] Recent clinical investigations have shown that therapies utilizing MSCs can reduce liver damage, improve liver function, and promote the regeneration of liver tissue.[36,37]

However, with the growing use and study of MSCs, concerns have emerged regarding their theoretical basis related to their ability to differentiate. Multiple studies have shown that although MSC therapy leads to the restoration of function, only a small number of cells achieve differentiation into appropriate tissues after MSC transplantation.[38,39] Moreover, the therapeutic effect of transplanted MSCs does not depend on the closeness of the transplantation location to the injury site.[40] Currently, there is a prevailing opinion that the immunomodulatory function of MSCs is predominantly accomplished through the paracrine pathway. This pathway leads to the reduction of damage and promotes the process of healing.[41,42] Extracellular vesicles (EVs) are released by phospholipid bilayer-containing cells and play a crucial role in facilitating cell-to-cell communication by carrying membrane and cytoplasmic proteins, lipids, and RNA.[24,40,43–45] MSC-derived EVs (MSC-EVs) are significantly smaller and more readily obtainable and storable compared to MSCs.[46] Following in vivo intravenous administration, MSC-EVs primarily accumulate in the liver. The occurrence of liver diseases is intimately associated with an imbalance in immunological homeostasis, which is closely related to the function of MSC-EVs.[47] Hence, researchers are investigating the utilization of MSC-EVs as a substitute for MSCs in liver ailments.[48] This review examines the advancements in the utilization of MSC-EVs in treating various chronic liver ailments.

Overview of MSCs

MSCs are pluripotent cells present in nearly all types of postnatal organs and tissues.[49] Moreover, MSCs possess a robust capacity for migration.[11] MSCs possess strong immunomodulatory properties.[50] MSCs are being increasingly utilized in clinical trials that focus on tissue repair and regeneration owing to their immunomodulatory and cell survival-enhancing characteristics.[51] Nevertheless, in contrast to expectations, numerous preclinical investigations have reported that MSCs do not integrate effectively into tissues in significant quantities, and the duration of integration is inadequate.[12]

MSCs possess strong immunomodulatory properties, effectively suppressing T cells, B cells, and natural killer (NK) cells in addition to their ability to differentiate in multiple directions.[52] Presently, there is substantial evidence backing the therapeutic and immunomodulatory roles of MSCs, primarily relying on paracrine effects and the release of secretory components. Therefore, many scholars are showing interest in the paracrine functions of MSCs, focusing specifically on paracrine secretions such as EVs.[53,54] According to their findings, MSCs are capable of producing and releasing numerous growth factors, chemokines, and cytokines, which, in turn, have an impact on the neighboring cells.[20] In 2005, Gnecchi et al proposed that MSCs can release protective substances.[55] They intramyocardially injected the culture supernatants from MSCs with high Akt gene expression, as well as MSCs alone, into an animal model of acute infarction. Both animal groups showed

a decrease in the myocardial infarction area to a similar extent, thereby validating their hypothesis.[56] Furthermore, it has been reported that MSCs enhance microcirculation and produce various anti-apoptotic proteins, including Bcl-2 and Akt, thereby averting cell death.[57,58] Numerous studies have confirmed the likelihood of the hypothesis that the healing benefits of MSCs are attributable to their paracrine effects rather than their differentiation effects.

Recently, studies have shown that under appropriate conditions, MSCs can be differentiated into hepatocyte-like cells (HLCs), providing new ideas for the treatment of liver diseases. Liver-specific miR-122 can be efficiently transfected into MSCs and activate their differentiation into HLCs. Transplantation of these engineered MSCs can treat acute liver failure by replenishing hepatocyte function.[59] Various liver diseases can lead to hepatic failure, and obtaining a sufficient number of functional hepatocytes is the key to liver regeneration.[60] Since the functions of primary hepatocytes are unstable, directing the differentiation of MSCs and other stem cells into HLCs, as well as developing and utilizing more stable and safer extracellular vesicle products, are the directions that researchers are paying attention to. These studies have provided a theoretical basis for the application of MSCs in the treatment of liver diseases.

Overview of MSC-EVs

Research on MSCs' release of extracellular vesicles has advanced significantly to date, and it has been shown that MSCs secrete a variety of extracellular vesicles, including exosomes, microvesicles, and microparticles.[61] Studies indicate that MSC-EVs have a significant impact on the transfer and regulation of intercellular information.[47,62] Scientists initially conducted thorough investigations into the mechanism of MSC-EVs generation. The study determined that the generation of MSC-EVs predominantly takes place through the endoplasmic reticulum and multivesicular body pathways.[63] The endoplasmic reticulum pathway involves the formation of vesicles on the endoplasmic reticulum, which bind to membrane transport proteins (eg, MVB) via fusion and are ultimately released outside the cell.[64,65] The multivesicular body pathway includes the discharge of MSC-EVs by shedding vesicles directly from the cellular membrane in reaction to the equilibrium of the plasma membrane.[66] Gaining knowledge about these pathways provides a basis for further investigation into the biological functions of MSC-EVs.

In addition, a comprehensive examination of the components of MSC-EVs was carried out. A multitude of bioactive constituents, including proteins, nucleic acids, and lipids, have been identified inside MSC-EVs.[48] Proteomics analysis revealed that MSC-EVs contain a diverse range of operational proteins, such as cell adhesion agents, enzymes that degrade the extracellular matrix, and factors that modulate the immune system.[67] Furthermore, MSC-EVs contain miRNAs, mRNAs, and various nucleic acids in abundance, which have the potential to influence gene expression and cellular functions when transferred to recipient cells.[68] MSC-EVs demonstrate various biological capabilities and potential uses in medical practice.[69] Based on empirical evidence, MSC-EVs have been demonstrated to exhibit anti-inflammatory, anti-fibrotic, angiogenesis-promoting, and immune-modulating properties.[70]

Furthermore, extensive research has been conducted on the use of MSC-EVs to manage diverse ailments, including cardiovascular disorders, neurological conditions, and immune-related diseases.[71–73] The potential applications of MSC-EVs in disease treatment and tissue regeneration are being investigated through preclinical and clinical trials, which have shown promising results.

Currently, exosomes are the most characterized extracellular vesicles as they have more biologically and biochemically defined parameters that can be detected in conventional laboratories.[74] Exosomes range from 40–100 nm in size, have a density of 1.10–1.18 g/mL on a sucrose gradient, and are linked to marker proteins such as Alix and Tsg101, as well as tetrameric transmembrane proteins including CD9, CD63, and CD81.[75,76] Exosomes, derived from introns,[76] are the sole extracellular vesicles that are currently recognized. Introns form vesicles with several layers and a large number of luminal vesicles by invaginating the endosomal membrane.[76] When they fuse with the cell membrane, luminal vesicles are released to form exosomes.[77] This distinguishes the process of exosome formation from that of other extracellular vesicle types. Through extensive examination of MSC-EVs, it has been established that these minuscule extracellular vesicles have a significant impact on the communication and regulation between cells.[76] Additional investigation will reveal the precise molecular mechanism of MSC-EVs, improve their composition, and broaden their potential uses in a therapeutic context. This has the potential to provide innovative strategies and techniques for enhancing the management of chronic liver disease and promoting tissue regeneration.[78]

Functions of MSC-EVs

MSC-EVs have received extensive research attention as a novel extracellular messaging medium.[79] The primary role of exosomes is to transmit cellular elements from secretory cells to receptor cells in one direction, thereby controlling the activity of the latter.[80] Similarly, MSC-EVs are responsible for intercellular communication.[81] Exosomes derived from MSCs can control the activity and role of immune cells through the transportation of diverse regulatory proteins, miRNAs, and other biologically active substances.[82] Clinical investigations demonstrate that MSC-EVs can perform an immunomodulatory effect by suppressing T cell activation, regulating macrophage polarization, and modifying B cell activity.[83] Treatment of autoimmune liver diseases[84] relies on this immunomodulatory property.

Furthermore, MSC-EVs can secrete bioactive compounds that consist of anti-inflammatory proteins and miRNAs, thereby controlling inflammatory reactions and preventing the release of inflammatory mediators.[85] Studies show that MSC-EVs can mitigate the inflammatory pathological process, thereby mitigating the progression of inflammatory liver condition.[86] Moreover, MSC-EVs can transport numerous biologically active substances that enhance the growth of cells and the formation of new blood vessels, ultimately aiding in tissue healing and renewal.[87] Several studies have indicated the role of MSC-EVs in the regeneration of cardiovascular, neural, hepatic, skeletal, and various other tissues.[84,88–90] Tissue regeneration function could be a novel approach to treating liver tissue injury. In addition, MSC-EVs, as a natural nanoparticle, have potential applications in drug delivery.[91] Researchers have modified the composition and internal structure of exosomes to enable the precise delivery of drugs to specific cells and tissues.[92] Targeted therapy and personalized medicine may be based on this drug delivery mechanism to treat liver diseases. MSC-EVs generally have a wide range of activities, such as immunomodulation, inflammation reduction, tissue regeneration promotion, and drug delivery facilitation[79,91,93,94] (Figure 1). More studies on MSC-EVs can lead to a better

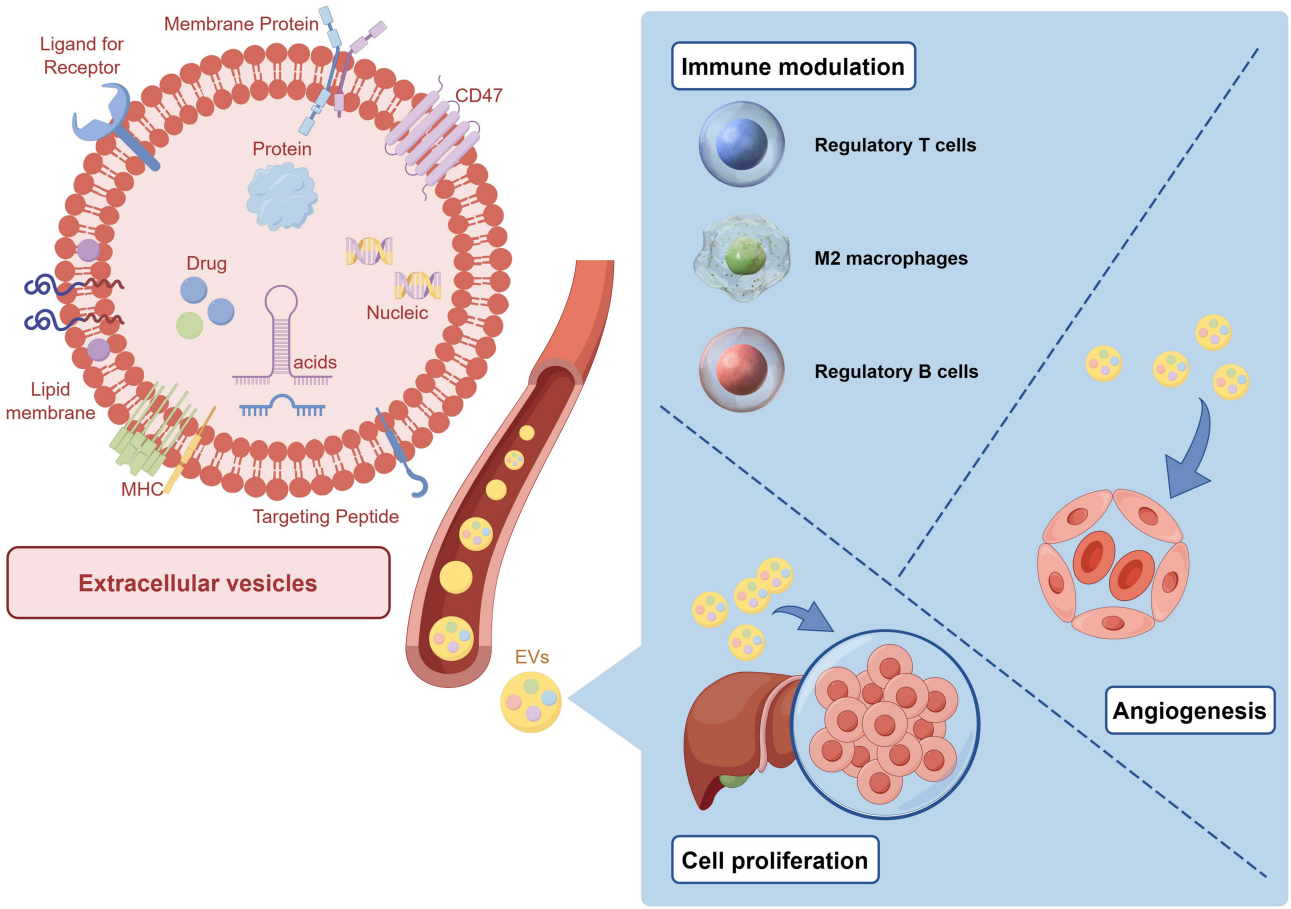

Figure 1 Functions of MSC-EVs. MSC-EVs can transport a variety of bioactive substances, promote cell growth and the formation of new blood vessels, and control the activity and role of immune cells. In addition, MSC-EV, as a natural nanoparticle, has potential applications in drug delivery.
Note: This figure is originally drawn by Figdraw platform (www.figdraw.com).

understanding of their molecular mechanisms, which will facilitate the development of more effective clinical implementation strategies and provide new therapeutic prospects for the treatment of disease and tissue regeneration.

MSC-EVs in the Treatment of Liver Diseases

Lately, a growing body of research has demonstrated the profound promise of MSC-EVs made from MSCs as a medicinal strategy for treating liver conditions.[95] In the human body, the liver is the primary organ responsible for metabolism and immune function.[96] The majority of the blood that enters the liver is from the portal vein instead of the hepatic artery.[97] The liver parenchyma receives blood primarily through the periportal vessels and is then drained from the liver parenchyma by the central hepatic vein through the intricate system of hepatic sinusoids.[98] Hepatic sinusoids serve as anatomical locations that maintain immune homeostasis.[99] Lymphocyte extravasation is promoted by the prolonged contact between lymphocytes and antigen-presenting cells due to the sluggish circulation in the hepatic sinusoids.[100] Besides hepatocytes and cholangiocytes, there are nonparenchymal cells such as hepatic stellate cells (HSCs) and hepatic sinusoidal endothelial cells, as well as numerous immune cells such as T cells, macrophages, and dendritic cells in the liver.[101,102] Liver diseases are typically linked to an imbalance in immune equilibrium, and this immune control includes the varied functionality of macrophages and dendritic cells, the proportion of various T-cell subsets (such as T helper [Th]17 and regulatory T [Treg] cells), the equilibrium between inflammatory and anti-inflammatory cytokines, and other factors related to immunity, which align perfectly with the role of MSC-EVs.[99,103,104] MSC-EVs primarily accumulate in the liver when administered intravenously in vivo.[68] Through immunomodulation, the regulation of MSV-EVs can lead to a decrease in the release of interleukin (IL)-6 and IL-1β by macrophages, and a decrease in the expression of CD154 by $CD4^+$ T cells. This reduction in cytokine release and CD154 expression could potentially alleviate hepatic inflammation and provide relief in various liver diseases.[63,69,105]

Role of MSC-EVs in Liver Diseases

This section provides an overview of preclinical investigations on MSC-EVs as a treatment modality for liver diseases such as liver failure, steatohepatitis linked to metabolism, autoimmune hepatitis (AIH), hepatic fibrosis, and hepatic ischemia reperfusion injury (HIRI). Table 1 summarizes the specific role of MSC-EVs in different liver diseases. The emphasis is on understanding the molecular mechanisms and regulation of MSC-EVs, as discussed in relevant studies.[48,106,107]

MSC-EVs and Acute Liver Injury

The two main pathophysiological features of liver failure that impact the organism as a whole and the liver specifically are immunological dysfunction and inflammation.[138] The rapid occurrence of apoptosis and necrosis in a large number of liver cells due to severe immune damage is followed by the development of ischemia, hypoxia, and endotoxemia. This sequence of events drives and speeds up the advancement of liver failure, commonly referred to as the "triple whammy" theory of liver failure.[138–140] Membrane permeability increases in the injured hepatocytes in the early stages of liver injury. This results in the release of molecules linked to damage-associated intracellular patterns. These molecules then bind to Toll-like receptors, triggering the activation of downstream signaling pathways such as c-jun amino terminal kinase, mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB), and signal transducer and activator of transcription 3.

Consequently, inflammatory cells are induced to infiltrate the affected area, further exacerbating liver failure through the promotion of tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, and other inflammatory factors, as well as cascade reactions.[141] Exosomes derived from various MSCs have been used recently by researchers to treat various liver failure models, proving that they can protect against liver damage.[142] Yan et al[143] administered human umbilical cord MSC-derived exosomes (ucMSC-Ex) at low (8 mg/kg), medium (16 mg/kg), and high (32 mg/kg) doses to mice with carbon tetrachloride (CCl_4)-induced liver failure. Large areas of steatosis and hepatocellular necrosis were found in the liver tissues of mice administered the low dose, whereas the liver tissue lesions in mice in the medium- and high-dose groups were significantly alleviated. EVs promoted recovery from hepatic oxidative injury by delivering GPX1. Besides, several animal experiments have confirmed that the protective role of MSC-Ex in various models of liver failure included inhibition of inflammation, anti-apoptosis effects, and attenuation of oxidative stress.[144]

Table 1 Is a Summary of the Specific Role of MSC-EVs in Different Diseases

Disease	Species Sex	Damage	Cell Source	Diameter (nm)	EV Treatment Group (Method/Dose)	Therapy Time	Therapy Mechanism	Ref.
AIH	C57BL/6 mice male	S100	mBMSC	30–100	i.p./20 μg/mL	Day 21/28/35 after injure	Regulate NLRP3 and caspase-1 by miR-223	[108]
AIH	C57BL/6 mice male	S100	Mouce-BMSC	40–100	i.v./2 μg/g (200 μL)	Day 21/35 after injure	Regulate macrophages by miR-223-3p	[109]
AIH	C57B6 mice male	Con-A induce AIH	Mouse-BMSC	135	i.v./10 μg (0.1 mL)	Once and three times after injure	Anti-apoptosis, increase Ki-67 d and Treg	[110]
AIH	BALB/c mice	Con-A induce AIH	Mouse-BMSC	120	i.v./5 mg/kg (100 μL)	Single after injure	Anti-inflammation	[111]
ALF	C57BL/6 mice male	D-GalN/LPS	MenSC	30–100	i.v./1 μg/μL	Single before injure	Anti-apoptosis	[112]
ALF	C57Bl/6 mice male	TNF-α/D-GalN	hBMSC/mouse-BMSC	116 ± 46	i.p./2 × 1010 particles	Single after injure	Anti-apoptosis	[113]
ALF	C57BL/6 mice male	LPS/D-GalN	hUCMSC	100	i.v./100 μg (250 μL)	Single 1 h after injure	Anti-NLRP3 inflammasome	[114]
ALF	C57BL/6J mice	LPS/D-GalN or TNF-α/D-GalN	Mouse-ADSC	40–100	i.v./400 μg (300 μL)	Single after injure	Anti-TXNIP/NLRP3 inflammasome	[115]
ALF	C57BL/6 mice male	CCl4	hESC	55–65	i.s./0.4 μg (100 μL)	24 h after injure	Activate proliferation and regeneration, anti-apoptosis	[116]
ALF	C57BL/6 mice male	LPS + D-GalN	hUCMSC	30–150	i.v./100 mg	1 h after injure	Anti-NLRP3	[117]
I/RI	Wistar rats male	IRI or CCL4	rat-BMSC	165 ± 3	i.v./50 μg	Single after injure	Antioxidant	[118]
I/RI	C57BL/6 mice male	I/RI	Mouse-BMSC	115 ± 48	i.v./2 × 1010 particles	Single before injure	Anti-apoptosis, anti-inflammation	[119]
I/RI	Sprague-Dawley rats male	I/RI	hUCMSC	178 ± 64	i.v./10 mg/kg	Single after injure	Antioxidant, anti-neutrophil inflammatory response	[120]
I/RI	C57BL/6 mice Male	THS	Mouse-BMSC	90–142	Femoral artery/20 μg	Single after resuscitation	Regulate Kuppf cells by IL-10	[121]
I/RI	C57BL/6	I/RI	hUCMSC	0–200	i.v./2.5 × 1012	Single after injure	Regulate GSK3β/Wnt/β-catenin pathway by miR-1246	[122]
I/RI	C57BL/6 mice male	I/RI	hUCMSC	30–150	i.v./100 μg/100 μL	Single after injure	Regulate CD4+ T cells by Ca2+-calcineurin-NFAT1 signaling pathway	[123]
I/RI	C57BL/6 mice male	I/RI	hAMSC	120–200	i.v./1 × 109 particles (200μL)	Single after injure	Anti-inflammation, increase Ki67	[124]
Liver fibrosis	FVB.129P2-Abcb4tm1Bor mice male	PSC	hBMSC	45–372	i.p./9.1 × 109 particles/mL (100 μL)	Once a week for 3 weeks after injure	Reduce granulocytes and T cells, increase VCAM-1	[125]
Liver fibrosis	Swiss albino mice female	CCL4	hADSC/WJMSC	40–120	i.v./250 μg	Single after injure	Anti-inflammatory, anti-fibrosis	[84]
Liver fibrosis	C57BL/6 mice male	TAA	hADSC	94.2 ± 4.7	i.v./200 μL (1 × 107; 1×108)	Single after injure	Anti-fibrosis	[126]
Liver fibrosis	C57BL/6 mice Male	CCL4	hTMSC	50–290	i.v./150 mg (100 μg/mL)	Once a week for 3 weeks after injure	Inactivate hedgehog signaling by miR-486	[127]
Liver fibrosis	Kunmingbai strain mice	CCL4	hUCMSC	40–100	Liver directly injected/250 μg (330 μL)	Single after injure	Inhibit EMT	[128]
Liver fibrosis	Wistar rats male	TAA	hES-MSC	190.8 ± 18	i.p./350 μg (400 μL)	Single after injure	Anti-fibrosis, anti-inflammation, anti-apoptosis, promote regeneration	[129]
Liver fibrosis	Sprague-Dawley rats male	CCL4	hAMSC	80–110	i.v./15 μg/kg and 20 μg/kg (200 μL)	Single after injure	Anti-fibrosis, reduce Kupfer cells	[130]
Liver fibrosis	Sprague-Dawley rats female	CCL4	hBMSC	30–100	i.v./250 mg (500 μL)	Single after injure	Alleviate liver fibrosis through the Wnt/β-catenin	[131]
Liver fibrosis	Sprague-Dawley albino rats male	CCL4	rat-BMSC	113.7	i.v./80 μg	Single after injure	Anti-fibrosis	[132]

Liver fibrosis	ICR mice Male	CCL4	hESC	120–140	i.v./NA	Twice a week for 4 weeks after injure	Anti-fibrosis by miR-6766-3p	[133]
Liver fibrosis	C57BL/6 mice male	TAA	Mouse-ADSC	117 ± 7	i.v./(1 × 107 particles), or (1 × 108 particles)	Single or three times after injure	Anti-fibrosis	[134]
Liver fibrosis		CCL4	Mouse-ADSC	30–150	i.v./0.4 μg/μL, 100 μL	Twice a week for 8 weeks	Inhibit CXCL1 by miR-150-5p	[135]
MAFLD	Sprague-Dawley rats male	MAFLD	hUCMSC	96	i.v./100 μg (500 μL)	Once a week for 2 months	miR-627-5p inhibit FTO, improve glycolipid metabolism	[136]
MAFLD	C57BL/6 J, female	MAFLD	Mouse-ADSC	95.8 ± 1.2	i.v./100 μg/100 μL	Twice a week starting from the second week of diet	Anti-fibrotic by miR-223-3p/E2F1	[137]

Abbreviations: EV, extracellular vesicle; MSC, mesenchymal stem cell; NA, not available; ALI, acute liver injure; NAFLD, nonalcoholic fatty liver disease; AIH, autoimmune hepatitis; IRI, ischemia-reperfusion injury; PSC, primary biliary cirrhosis; BMSC, bone marrow mesenchymal stem cell; UCMSC, umbilical cord mesenchymal stem cell; ADSC, adipose-derived mesenchymal stem cell; ESC, embryonic stem cell; AMSC, amnion-derived mesenchymal stromal cell; TSC, tonsil-derived mesenchymal stromal cell; MenSC, menstrual blood-derived mesenchymal stem cell; i.p., intraperitoneal; i.s., intrasplenic; i.v., intravenous injection; CCl4:carbon tetrachloride; TAA; thioacetamide; D-GalN/LPS; D-galactosamine (D-GalN) and lipopolysaccharide (LPS); DEN, diethylnitrosamine.

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Human endometrial MSC-EVs have been found to alleviate liver function and reduce hepatocyte apoptosis in a mouse model of ALI, potentially by migrating to the injured liver and suppressing inflammatory responses.[112] Bone-marrow-derived MSC-EVs could also accumulate in the injured liver tissue and attenuate damage after systemic administration in a lethal mouse model of acute liver failure, with improved survival rates compared to controls.[113] The therapeutic effects were partly mediated by a highly enriched Y RNA fragment in MSC-EVs.

Adipose tissue-derived MSC-EVs (AMSC-Exos) could protect against inflammasome-induced inflammation in acute liver failure by shuttling miR-17 into hepatic macrophages and suppressing NLRP3 activation via downregulating TXNIP.[115] This highlights the involvement of exosomal microRNAs as important therapeutic agents. AMSC-Exos may suppress the production of inflammatory cytokines and restore liver function by targeting NLRP3-regulated inflammation. Another study found human umbilical cord MSC-EVs attenuated NLRP3 inflammasome activation and reduced liver injury markers in a mouse model of acute liver failure, likely via dampening inflammatory responses of macrophages.[114] The effects were associated with decreased expression of NLRP3, Caspase-1, IL-1β and IL-6. Furthermore, systemic administration of BMSC-EVs activated regenerative mechanisms and inhibited apoptosis to protect against toxicant-induced acute liver injury in mice.[116] The hepatoprotective effects involved upregulation of proliferation and cell cycle-related genes.

In summary, accumulating evidence has characterized the therapeutic potentials of MSC-EVs in various models of acute liver damage, which are exerted through suppression of inflammasome-mediated inflammation responses, activation of hepatocyte regeneration, and inhibition of cell death pathways. Further research is warranted to understand better the responsible bioactive molecules shuttled by MSC-EVs and optimize treatment strategies.

MSC-EVs and Metabolism-Associated Fatty Liver Disease (MAFLD)

MAFLD is a condition where there is an accumulation of fat in liver tissues even in the absence of significant alcohol consumption or related metabolic disorders.[145] Presently, MAFLD poses a major challenge. The latest data suggest that its occurrence is on the rise worldwide, reaching 24% and resulting in a substantial financial burden.[146] A study found that human umbilical cord MSC-EVs containing miR-627-5p could improve insulin tolerance, alleviate liver injury, modulate glucose/lipid metabolism and reduce lipid deposition in an MAFLD rat model.[136] The effects were associated with miR-627-5p targeting and suppressing Fat Mass and Obesity associated (FTO) gene expression. In vitro, miR-627-5p overexpressing EVs also promoted cell viability, inhibited palmitic acid-induced apoptosis of LO-2 hepatocytes, and regulated a panel of metabolism-related genes. The data support miR-627-5p enriched MSC-EVs as a promising approach to improve MAFLD progression by enhancing metabolic profiles and reducing liver damage.

Another study reported that adipose-derived MSC-EVs delivering anti-fibrotic miR-223-3p could attenuate lipid accumulation and liver fibrosis by targeting E2F1, which plays a key role in hepatic stellate cell activation.[137] The anti-fibrotic effects were validated in a mouse model of diet-induced MAFLD. This highlights the therapeutic potential of functional MSC-EVs achieved by manipulating exosomal microRNA cargo. MSC-EVs can also regulate lipid metabolism. In addition, some studies show that MSC-EVs can enhance the process of fatty acid oxidation and hinder the synthesis of fatty acids, ultimately leading to decreased fat accumulation.[147]

Furthermore, MSC-EVs affect the differentiation and viability of adipocytes and decrease the inflammatory reaction in adipose tissues.[148] The advancement of MAFLD is known to be frequently accompanied by hepatic fibrosis, and MSC-EVs can impede the progression of liver fibrosis.[137] According to studies, MSC-EVs can regulate the activity of matrix metalloproteinases and reduce the formation and accumulation of collagen in the liver, which decreases the severity of fibrosis.[149]

In conclusion, MSC-EVs have emerged as a promising therapeutic agent for MAFLD, with a range of beneficial activities demonstrated in preclinical studies. These activities include resolving metabolic disorders, suppressing inflammation, inhibiting fibrosis progression, and promoting liver regeneration. Further research is required to understand the underlying mechanisms better and facilitate the translation of findings into clinical practice.

MSC-EVs and AIH

AIH is a chronic, progressive liver disease caused by autoimmune disorders. It is characterized by liver inflammation, infiltration of lymphocytes and plasma cells, and elevated ALT, AST, and immunoglobulin G (IgG) levels in the blood.[150] The clinical diagnosis of AIH relies on liver biopsy to evaluate for interfacial hepatitis, rosette nodules, and lymphocytic infiltration.[151] The current initial therapy for AIH involves the use of corticosteroids with or without azathioprine.[152] Although the precise origin of AIH is unknown, scientists believe that a mix of environmental variables, genetic susceptibility, and molecular mimicry may be responsible for lowering autoimmune tolerance.[153,154]

The primary method of treating AIH at the moment is either hormone therapy alone or in conjunction with immunosuppressants. It has significant drawbacks and limitations, such as prolonged treatment duration, adverse responses, insufficient patient adherence, and failure to stop the advancement of liver fibrosis and cirrhosis, even though it may, to some extent, increase patients' chances of survival.[152] Therefore, the treatment of AIH has always been a clinical focus owing to these challenges.

MSC-EVs has emerged as a promising cell-free therapy for autoimmune hepatitis. Studies have shown that MSC-exosomes can attenuate liver injury and inflammation in mouse models of autoimmune hepatitis induced by concanavalin A or liver antigen S100.[110,111] The anti-inflammatory and hepatoprotective effects of MSC-exosomes are comparable to mesenchymal stem cell transplantation.[110] Tracking experiments revealed that MSC-exosomes could target the injured liver after injection. MSC-exosomes can transfer microRNAs, such as miR-223-3p, to hepatocytes and immune cells like macrophages in the liver, regulating cell death, inflammation and immune responses.[109] It also inhibited the activation of NLRP3 inflammasome and caspase-1 activation, thus reducing IL-1β production and pyroptosis.[108] MSC-exosomes modulate the polarization of macrophages from pro-inflammatory M1 phenotype to anti-inflammatory M2 phenotypes and increase the number and function of regulatory T cells in the liver through immunosuppressive molecules like TGF-β.

Furthermore, therapeutic molecules like dexamethasone can be loaded into MSC exosomes to enhance the treatment outcome. The drug-loaded exosomes exhibit better liver distribution and anti-inflammatory effects.[111] MSC-exosomes show promise as a cell-free therapy for AIH through multiple mechanisms of action. Further investigations are warranted to promote the clinical translation of MSC-exosomes.

MSC-EVs and Liver Fibrosis

Liver fibrosis is a degenerative condition that occurs as a result of long-term liver damage. It is a persistent inflammatory response characterized by the replacement of normal liver tissue with fibrous tissue. Many chronic liver diseases, such as hepatitis B and hepatitis C, fatty liver, and alcoholic liver disease, often lead to liver fibrosis. Additionally, the presence of extensive fibrosis may result in cirrhosis and liver insufficiency.[155] HSCs that are activated in the liver are the primary fibroblasts and have a crucial function in the development of liver fibrosis. Normally, HSCs stay dormant and store vitamin A and bilirubin. However, when the liver is damaged, HSCs become active and transform into cell types linked to fibrosis. HSCs that have been activated release collagen, fibronectin, and other substances that stimulate the production of collagen fibers, resulting in the development of liver tissue fibrosis.[156]

Angiogenesis plays a crucial role in the development of liver fibrosis, resulting in the development of new blood vessels with a supply of oxygen and vital nutrients.[157] Activation of vascular endothelial cells by vascular endothelial growth factors (eg, vascular endothelial growth factor-A) and fibronectin-inducing factors (eg, transforming growth factor β) leads to the stimulation of abnormal vasculogenesis and perivascular inflammatory reactions.[158]

However, the primary cause of liver fibrosis[157] is the persistent inflammatory reaction resulting from long-term liver disease. Inflammation-causing agents include cytokines (TNF-α, IL-1, IL-6) and chemokines (alcohol, compounds from oxidative stress). These substances induce hepatocellular damage and apoptosis by stimulating the development of liver fibrosis.[40] Furthermore, the accumulation of collagen and other matrix components in a fibrosed liver modifies the organization and performance of the liver. Activated HSCs release matrix metalloproteinase inhibitors, which inhibit matrix metalloproteinase activity, leading to matrix accumulation and deposition and promoting the progression of fibrosis.[132]

In addition to the mechanisms mentioned above, several other molecular pathways, including the TGF-β/Smad pathway, Wnt/β-catenin pathway, and NF-κB pathway, are also considered significant in the development of liver fibrosis. Numerous preclinical investigations have recently reported the anti-fibrotic properties of MSC-EVs in the liver,[159] lungs,[160] kidneys,[161] and heart.[162] As stated previously, the progression of liver fibrosis is intricately linked to a persistent inflammatory reaction, and MSC-EVs exert anti-inflammatory effects. MSC-EVs can suppress the synthesis of inflammatory cytokines, including TNF-α and IL-1β, leading to a decrease in inflammation.[141] The transformation of epithelial cells to mesenchymal cells can be inhibited by MSC-EVs, leading to the amelioration of CCl_4-induced hepatic fibrosis.[128]

Furthermore, the activation of HSCs can be significantly inhibited by MSC-EVs. The primary feature of hepatic fibrosis is the accumulation of collagen and other matrix components. MSC-EVs can reduce the progression of fibrosis by suppressing collagen production and regulating matrix metalloproteinase activity.[132] MSC-EVs can directly act on hepatic stellate cells to inhibit their activation and proliferation, thereby reducing the expression of collagen and other fibrosis-related genes.[127,128,133] Suppressing the excessive proliferation of hepatic stellate cells and the over-deposition of collagen is an important mechanism for the anti-fibrotic effects of MSC-EVs. MSC-EVs can also reduce the accumulation and activation of inflammatory cells, such as macrophages in the liver,[125,130] since inflammatory responses and immune cell activation are closely related to the process of liver fibrosis. By inhibiting inflammatory signaling pathways such as NF-kB,[125,131,133] MSC-EVs can exert inhibitory effects on fibrosis. In addition, MSC-EVs can inhibit EMT processes and protect hepatocytes from apoptosis.[128,129]

The occurrence of EMT can lead to an increase in fibroblasts, while hepatocyte apoptosis is also a feature of liver fibrosis. MSC-EVs reduced EMT by upregulating markers such as E-cadherin. Some studies have indicated that the therapeutic effects of MSC-EVs are superior to MSC themselves[131,132] because EVs can avoid some of the problems of cell therapy, such as the risk of embolism. Surface-engineered EVs can improve targeting,[134] while sustained-release formulations can prolong the action time of EVs in target organs.[129] Furthermore, miRNAs enriched in MSC-EVs, such as miR-223 and miR-486,[109,127,133] are also involved in their anti-fibrotic mechanisms. These miRNAs can downregulate hepatic stellate cell activation-related genes to exert effects. miR-150-5p can inhibit hepatic stellate cell activation by targeting CXCL1.[135]

Therefore, MSCs and their secreted EVs exert synergistic effects through multiple pathways, inhibiting activation of hepatic stellate cells and inflammatory cells, reducing EMT, protecting hepatocytes and other mechanisms to treat liver fibrosis.

(Figure 2). This provides a theoretical basis for MSC-EVs to become a new extracellular therapy for liver fibrosis, but further research and clinical validation are still needed.

MSC-EVs and HIRI

HIRI is a tissue injury and inflammatory response caused by hepatic ischemia (insufficient blood supply) and reperfusion (blood resupply). Liver surgery, liver transplantation, and shock often result in HIRI. Severe HIRI can result in liver failure and organ damage.[163] The intricate process of HIRI entails interactions between several cellular types and chemical communication channels. Ischemia impairs cellular activity and disturbs energy metabolism in the liver by depriving it of oxygen and nutrients.[164] Ischemia also leads to a decrease in intracellular ATP levels, resulting in elevated intracellular calcium ion levels, compromised mitochondrial function, and the development of oxidative stress.[165]

When blood is being resupplied to the liver during reperfusion, several negative effects are associated with this process. Reperfusion worsens oxidative stress, which generates significant amounts of reactive oxygen radicals and inflammatory agents. These changes cause apoptosis, necrosis, and inflammatory responses, all of which contribute to tissue damage.[164] Oxygen radicals and inflammatory mediators cause lipid peroxidation of cell membranes, protein oxidation, and DNA damage, which worsen cellular damage and inflammatory responses.[165]

Studies show that $CD4^+$ T lymphocytes have a crucial function in initiating hepatic inflammatory reactions in response to HIRI. Following reperfusion, a notable increase is observed in the proliferation, infiltration, and aggregation of $CD4^+$ T cells that have specificity for antigens in ischemia-affected tissues. CD154, a cell-surface protein weighing 32–39 kDa and belonging to the TNF superfamily, is abundantly expressed by activated $CD4^+$ T cells. This protein can

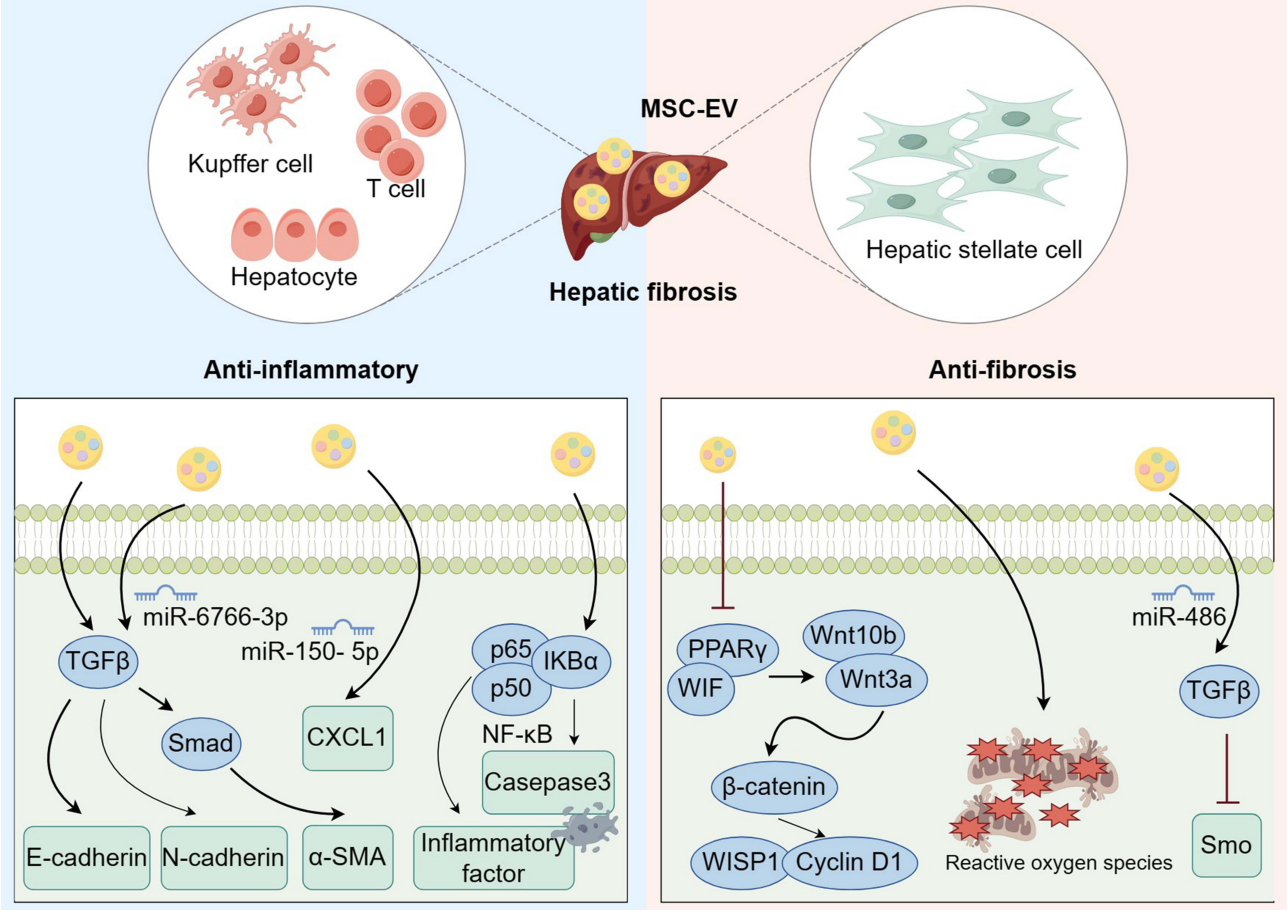

Figure 2 Mechanisms of mesenchymal stem cells in treating liver fibrosis. MSC-EVs can inhibit liver fibrosis by reducing inflammatory response, suppressing hepatic stellate cell activation and proliferation, and promoting hepatocyte regeneration.
Note: This figure is originally drawn by Figdraw platform (www.figdraw.com).

enhance the immune response, promote platelet production, and worsen hepatocellular injury by interacting with CD40/CD154. Low levels of CD154 are typically expressed during normal circumstances. $CD4^+$ T cell activation leads to the continuous synthesis and expression of a significant level of CD154 on the cell surface. CD154 binds to CD40 on B cells, natural killer (NK) cells, dendritic cells, macrophages, basophils, and eosinophils, triggering the activation of downstream transcription factors that stimulate cytokine production.

Furthermore, CD154 is a protein that can undergo rapid degradation over a short period.[105] Hence, early therapeutic strategies are essential to regulate the manifestation of CD154 on hepatic $CD4^+$ T cells to alleviate HIRI and decrease the complications and mortality in OLT. Thus, the hepatoprotective effects of MSC-EVs are attributed to the suppression of CD154 expression on $CD4^+$ T cells in the liver. Additional mechanistic investigations revealed that chaperone proteins that include the TCP1 subunit 2 in MSC-EVs were moved to $CD4^+$ T cells. These cells are responsible for controlling the calcium influx/NFAT1 signaling pathway, affecting the synthesis and expression of CD154.[123]

Moreover, MSC-EVs can reduce serum aminotransferase levels, alleviate tissue necrosis, increase the number of Ki-67-positive hepatocytes, and inhibit the transcription of inflammation-related genes, demonstrating regenerative repair potential.[118,124] Compared to unfractionated conditioned medium, the fractionated EVs secreted by MSCs have greater cytoprotective and restorative capacities.[118] In mouse IRI models, MSC-EVs can reduce apoptotic and caspase-3 positive cells, decrease inflammatory factor mRNA levels, increase hepatocyte viability, and inhibit oxidative stress and NF-κB activation, thereby suppressing inflammatory responses and cell apoptosis.[119,120] This is related to the antioxidant enzyme MnSOD carried and delivered by MSC-EVs.[120] Exogenous MSC-EVs can transport IL-10 into the liver, be

taken up by Kupffer cells, induce PTPN22 expression, polarize Kupffer cells towards an anti-inflammatory phenotype, and alleviate inflammation and liver injury.[121] Studies have demonstrated that miR-1246 originating from MSC-EVs interacts with GSK 3β in liver cells, significantly suppressing Wntl, Wnt 3a, and β-catenin expression. This interaction activates the Wnt/β-catenin signaling pathway and reduces the production of HIRI-induced TNF-α, IL-6, and IL-1β.

Consequently, this reduction in inflammation alleviates HIRI.[122] The exploration of employing MSC-EVs to enhance HIRI is presently in its preliminary stages, thus raising several unresolved inquiries. Further research is required to conduct comprehensive investigations into the mechanisms and lasting impacts of MSC-EVs in the treatment of HIRI.

Limitations of MSC-EVs and Improvements in Clinical Applications

Pluripotent stem cells can be employed to acquire MSC-EVs, which exhibit potential for clinical application. However, within a clinical setting, MSC-EVs are linked to specific disadvantages that require attention. Moreover, the methods for manufacturing MSC-EVs have not yet been well standardized. The exploitation of these resources in a clinical setting is impeded by the obstacles presented by their diversity and limited length of use. Different techniques of preparation produce various batches of MSC-EVs, each possessing unique features and functions. Therefore, it is crucial to create standardized preparation processes in order to ensure consistency and reliability when utilized for clinical purposes.

Significant challenges also lie in the concentration and purification of MSC-EVs. The commonly used method of isolating extracellular vesicles (EVs), ultracentrifugation, is ineffective for characterizing individual EVs. To address this issue, a number of innovations in EV isolation techniques are currently being employed. These include techniques such as fluorescent marking and subsequent analysis using high-resolution flow cytometry,[166] specialized flow cytometry,[167] and the use of laser tweezers and Raman spectroscopy[168] for quantitative and qualitative assessment. The purity and functionality of MSC-EVs may be affected by the presence of other cell types or impurities during the current techniques used for isolation and enrichment. Hence, it is imperative to improve the efficacies of the separation and enrichment techniques to enhance the purity of MSC-EVs.

Additionally, the long-term viability of MSC-EVs is also a matter of concern. Possible alterations in the functionalities of MSC-EVs may occur as a consequence of several conditions, including temperature, pH, and oxygen levels, in both in vitro and in vivo settings. These changes have the potential to lead to a reduction or complete cessation of their functions. Recent studies focus on delivering EVs locally using tissue-engineered substances, which could be a potential approach to enhancing their practical use. Polymer networks in hydrogels have a three-dimensional arrangement that enables them to absorb substantial quantities of water or biofluids.[169] Hydrogels exhibit biocompatibility and remarkable mechanical characteristics.[170]

Furthermore, hydrogels can maintain EV release, boost their stability, and enhance their efficacy.[48] Thus, the identification of a suitable modifier may enhance the durability of MSC-EVs. Nevertheless, our understanding of the processes by which MSC-EVs exert their effects and their biological activities is limited. Notwithstanding this limitation, MSC-EVs are recognized for their anti-inflammatory, anti-fibrotic, and regeneration-promoting properties. Therefore, further investigation is necessary to further understand the roles of MSC-EVs in a therapeutic context.

Summary and Future Prospects

The utilization of MSCs is critical in the management of disorders that alter tissue function, as emerging data indicates that the compounds they produce have a therapeutic impact. MSC-EVs, being the primary constituent of MSC paracrine substances, can protect against different types of chronic liver ailments. The liver offers a range of cell types that can serve as recipient cells, including hepatic macrophages for exosomes.[171] Recently, small sample clinical studies have been conducted to explore the safety and initial effectiveness of exosomes in the treatment of cirrhosis (ChiCTR2300075676). Nevertheless, the evaluation of MSC-EVs in clinical studies remains limited, and a significant disparity exists before their utilization in a therapeutic environment. In clinical trials involving MSC-EVs, it is crucial to address the challenges of optimizing the culture conditions of MSC, establishing standardized protocols for extracting and identifying EVs, and determining the optimal disease states that could benefit from treatment.[172] Coupling gene editing tools or various biomaterials to tissue-specific extracellular vesicles may enable precise modulation of target genes to improve therapeutic efficacy.[173] For example, loading CRISPR-Cas9 ribonucleoproteins into extracellular

vesicles derived from activated hepatic stellate cells enabled targeted delivery to liver tissue.[174] This extracellular vesicle-mediated gene editing system specifically accumulated in the liver in vivo and exhibited significant therapeutic potential in models of acute liver injury, chronic liver fibrosis and hepatocellular carcinoma.

In conclusion, MSC-EVs have great potential for treating liver disease. These problems will undoubtedly be answered as research on MSC and its EVs progresses, opening up exciting new therapeutic opportunities for the treatment of chronic liver diseases. In the future, we anticipate that MSC-EVs will be a useful therapeutic approach for treating liver disorders.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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