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REVIEW

Current Status and Prospect of Delivery Vehicle Based on Mesenchymal Stem Cell-Derived Exosomes in Liver Diseases

Xinfeng Lu[1,2,*], Haijun Guo[2,3,*], Xuyong Wei[2,3], Di Lu[2,3], Wenzhi Shu[2,4], Yisu Song[2,4], Nasha Qiu[1,2], Xiao Xu[1–4]

[1]The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, 310000, People's Republic of China; [2]Key Laboratory of Integrated Oncology and Intelligent Medicine of Zhejiang Province, Hangzhou, 310006, People's Republic of China; [3]Department of Hepatobiliary and Pancreatic Surgery, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, 310006, People's Republic of China; [4]Zhejiang University School of Medicine, Hangzhou, 310058, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xiao Xu, Department of Hepatobiliary and Pancreatic Surgery, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, 310006, People's Republic of China, Email zjxu@zju.edu.cn; Nasha Qiu, The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Key Laboratory of Integrated Oncology and Intelligent Medicine of Zhejiang Province, Hangzhou, 310006, People's Republic of China, Email qiunasha@163.com

Abstract: With the improvement of the average life expectancy and increasing incidence of obesity, the burden of liver disease is increasing. Liver disease is a serious threat to human health. Currently, liver transplantation is the only effective treatment for end-stage liver disease. However, liver transplantation still faces unavoidable difficulties. Mesenchymal stem cells (MSCs) can be used as an alternative therapy for liver disease, especially liver cirrhosis, liver failure, and liver transplantation complications. However, MSCs may have potential tumorigenic effects. Exosomes derived from MSCs (MSC-Exos), as the important intercellular communication mode of MSCs, contain various proteins, nucleic acids, and DNA. MSC-Exos can be used as a delivery system to treat liver diseases through immune regulation, apoptosis inhibition, regeneration promotion, drug delivery, and other ways. Good histocompatibility and material exchangeability make MSC-Exos a new treatment for liver diseases. This review summarizes the latest research on MSC-Exos as delivery vehicles in different liver diseases, including liver injury, liver failure, liver fibrosis, hepatocellular carcinoma (HCC), and ischemia and reperfusion injury. In addition, we discuss the advantages, disadvantages, and clinical application prospects of MSC-Exos-based delivery vectors in the treatment of liver diseases.

Keywords: exosomes, mesenchymal stem cells, liver disease, nanocarriers

Introduction

The WHO identified that the world's overweight and obesity rates have nearly tripled since 1975. By estimation, more than 1.9 billion (39%) adults (≥18 years) are overweight and 650 million (13%) individuals are obese.[1] With the improvement of the average life expectancy and increasing incidence of obesity, the burden of various liver diseases, including non-alcoholic fatty cell liver disease (NAFLD), liver cirrhosis, and liver failure, is increasing.[2] These high rates of morbidity pose a great threat to human health.[3] Chronic liver disease and cirrhosis contribute to 2 million deaths per year globally, with a high burden of disability and treatment costs.[4,5] The prevalence of alcoholic liver disease and NAFLD in the general adult population is approximately 7.4% and 20–33%, respectively. Hepatitis B virus (HBV) infection affects at least 2 billion people worldwide, and without radical treatment, all forms of chronic hepatitis eventually progress to end-stage disease. Hepatocellular carcinoma (HCC) was reported to be the leading cause of cancer death worldwide.[6] Liver cancer is the fourth leading cause of cancer-related death worldwide and the second most lethal cancer with a 5-year survival rate of 18%.[7,8] In 2030, the WHO estimates that more than 1 million patients will die

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from liver cancer.[8] The liver disease accounts for about 2 million deaths worldwide every year,[9] which poses a great threat to human health. Liver transplantation is the only effective treatment for many advanced liver diseases when other medical therapies have failed.[10–12] However, we have to admit that the shortage of liver donors, high cost, postoperative complications, and other issues restrict the popularization of liver transplantation. Therefore, we hope to find effective alternative measures to cope with the occurrence and development of liver diseases.

MSC-Exos can inhibit the occurrence and development of liver injury, liver fibrosis, liver cancer, and other liver diseases through signal transduction, immune regulation, tissue regeneration promotion, drug delivery, and other pathways. However, at present, the mechanism of MSC-Exos in the treatment of liver disease has not yet been fully clarified. Preclinical studies are insufficient; therefore, the clinical application still faces several challenges. In this review, we summarize the roles and functions of MSC-Exo-based delivery systems in liver diseases, thereby enabling us to better understand the latest findings in the field and tackle accompanying clinical challenges.

Mesenchymal Stem Cells

MSCs, a kind of stromal cells with self-renewal and multilineage differentiation abilities,[13] were discovered in the 1960s and 1970s.[14,15] MSCs can be isolated from various adult tissues, such as the bone marrow, umbilical cord, adipose, peripheral blood, liver, and tooth root.[16] (Figure 1) Human-derived MSCs express relatively constant markers, including CD90, CD73, and CD105. However, the surface markers and characteristics of MSCs from different sources are slightly different.[17] MSCs can be induced to differentiate into cells of the mesodermal lineage, such as adipocytes, skeletal cells, and muscle cells.[18] Therefore, MSCs can promote tissue repair, proliferation, and regeneration. The regeneration of liver, kidney, heart, and

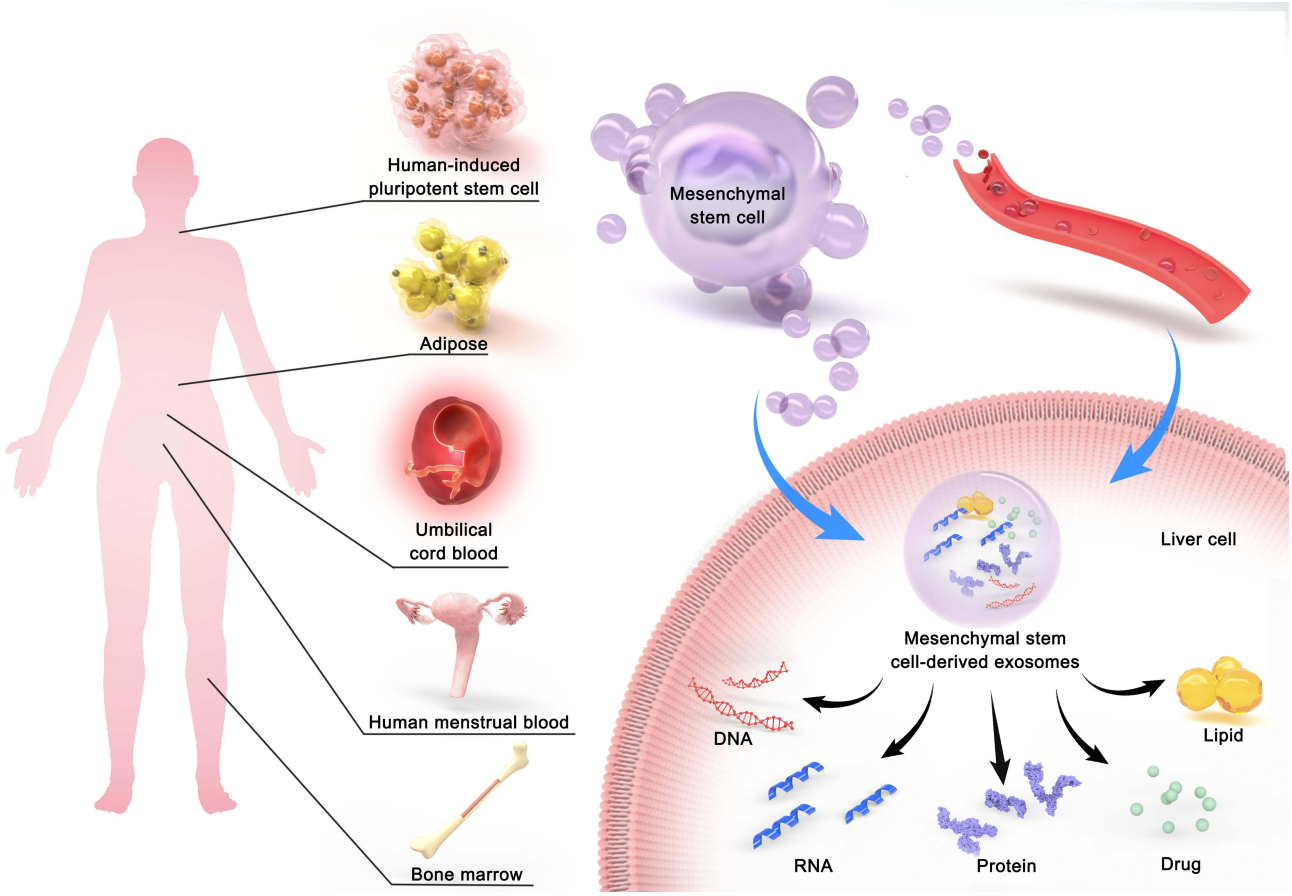

Figure 1 MSC-Exos have proven potential as delivery vehicles and have the opportunity to treat liver diseases. MSCs can be isolated from various adult tissues, such as the human-induced pluripotent stem cell, bone marrow, umbilical cord blood, adipose, human menstrual blood. MSC-Exos play a therapeutic role in liver by secreting DNA, RNA, protein, lipid and drug through paracrine and blood transport.

pancreas tissues can be promoted under the action of MSCs.[16,19–22] MSCs have been demonstrated to possess nutritional, anti-inflammatory, immunomodulatory, anti-apoptotic, and antibacterial properties.[23] These characteristics provide MSCs with great advantages in the treatment of diseases. MSCs are effective in treating bone, brain, nerve, cardiovascular, and autoimmune diseases and promoting wound and soft tissue regeneration.[23] In recent studies, researchers have found that MSCs have great potential in the treatment of liver diseases, including liver fibrosis, liver failure, liver cirrhosis, metabolic-associated fatty liver disease, and liver regeneration.[24–28]

Several studies have shown that mesenchymal stem cells are the most promising alternative therapy for the treatment of liver diseases, especially liver cirrhosis, liver failure, and complications of liver transplantation.[29] However, case reports have suggested that MSC treatment may cause unexpected differentiation and unknown proliferative lesions,[30] which may have potential tumorigenicity.

Exosomes

Extracellular vesicles (EVs) are cell-derived membrane vesicles that are secreted from almost all types of cells and play an important role in intercellular communication and regulation.[31,32] EVs mainly include apoptotic bodies, microvesicles, and exosomes.[33] Apoptotic cells can produce apoptotic bodies, and during this process, apoptotic cells can actively package their biomolecules into vesicles, so that drugs such as nucleic acids can be loaded into apoptotic bodies.[34] However, there is still a considerable blank space in the study of the mechanism and function of apoptotic bodies.[35] Microvesicles are derived from the direct budding of the plasma membrane in living cells and carry active components that can affect target cells and alter their behavior.[36] Exosomes originate from the fusion of clathrin-coated vesicles, forming multivesicular endosomes by fusing with early endosomes, and eventually fusing with the cell membrane and shedding exosomes.[37] EVs, typically between 50 nm and 500 nm in size, are important mechanisms of intercellular communication and are involved in a variety of physiological and pathological processes.[38] EVs play a good role in the regulation of immunity, tumor progression, specific modulators of cell behaviors, and targeted delivery of drugs.[38,39] Noncoding RNAs (ncRNAs) account for a small but important proportion of EV cargo, and MSC-EVs have been reported to contain ncRNAs related to various molecular mechanisms in liver diseases.[37] Exosomes, as an important component of EVs, were first discovered in the 1980s.[40,41] Almost all mammalian cells can secrete and absorb exosomes.[42,43]

Exosomes are small vesicles that are shed from the surface of the plasma membrane through outward budding with diameters ranging from 40 nm to 160 nm.[44] According to the position statement from the International Society for Extracellular, at least two different techniques are generally used to characterize individual EVs. Exosomes, as one of the EVs, also follow this rule. Nanoparticle tracking analysis (NTA), dynamic light scattering, or resistive pulse sensing could be used to measure the concentration and size distribution of exosomes. Exosomes were visualized by transmission electron microscopy using the transmission electron microscope, atomic force microscopy, and Western blot analyses. The International Society for Extracellular also suggests that investigators report the amounts of 3 or more proteins in at least a semiquantitative manner in any exosome preparation.[45]

Since the first discovery of exosomes in the 1980s, research on exosomes has been advancing. Exosomes were confirmed to be formed from lipid bilayers derived from the plasma membrane. They have the same topology as cells and are rich in sugar conjugates, proteins, lipids, nucleic acids, and metabolites.[46] The ExoCarta database (http://www.exocarta.org) lists thousands of proteins, RNA, and lipids that can all be biological cargoes delivered by exosome-based delivery vectors.[47] Noncoding RNAs in exosomes have been shown to play a therapeutic role in a variety of liver diseases by inhibiting inflammatory response, alleviating oxidative stress in the liver, and inhibiting the activation and proliferation of hepatic stellate cells.[48]

Due to their good histocompatibility and material exchangeability, exosomes have become an important way of intercellular communication and regulating various physiological and pathological activities of the body. Exosomes have been proven to have many biological characteristics, including stability, histocompatibility, and good material exchange ability.[49] Exosomes play an important role in many aspects, including disease development, immunity, cancer, and tissue regeneration through the intercellular vesicle transport pathway.[46] Additionally, exosomes have the advantages of targeted delivery, low immunogenicity, and high repairability.[50] Exosomes are naturally secreted by cells and have low immunogenicity, which can prevent immune rejection.[51] The cell-free structure of exosomes helps prevent potential tumorigenic effects. Exosomes had been shown to have targeting properties, and exosomes from different cell sources

affected biodistribution.[52] The targeting properties of exosomes could be changed by exosome modification, so exosomes have the potential to become biological carriers for targeted drug delivery. In a mouse model of pulmonary metastases, exosomes released from macrophages are delivered through the airway and colocalize almost completely with cancer metastasis. Exosomes were shown to target cancer cells and effectively deliver paclitaxel (PTX, a chemotherapeutic agent).[53] Modified exosomes could target the lesion region of the ischemic brain and effectively inhibit inflammation and apoptosis in this lesion region.[54] Therefore, MSC-Exos have proven potential as drug delivery vehicles and may have the opportunity to treat liver diseases.

Mesenchymal Stem Cell-Derived Exosomes

EVs derived from MSCs are critical mediators of intercellular communication.[55] EVs deliver materials from MSCs to effector cells,[33] allowing MSCs to function.[56] MSCs do not engraft and replace damaged tissues directly but exert therapeutic effects through secreted paracrine effectors by these cells. Therefore, the therapeutic effect of MSCs can be largely attributed to paracrine effectors, of which exosomes are considered to be critical.[57] The biological functions of MSC-Exos are similar to those of their parental cells.[58] Similar to general exosomes, MSC-Exos also contain a variety of biological substances, such as sugar conjugates, proteins, lipids, nucleic acids, and metabolites. Most exosomes expressed an evolutionarily conserved set of proteins, including the tetraspanin protein family (CD81, CD63, and CD9), heat shock proteins (HSP60, HSP70, and HSP90), ALIX, and tumor susceptibility gene 101 (TSG101).[59] However, exosomes also express cell type-specific proteins, which correlate with their cellular origin. MSC-Exos expressed not only CD81 and CD9 but also mesenchymal stem cell surface markers such as CD44, CD73, and CD90.[59] Among the different types of MSC-derived exosomes, half of the proteins were similar among all proteins.[50]

MSC-derived exosomes are derived from a wide range of sources (Figure 1), including bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos), adipose tissue-derived mesenchymal stem cell-derived exosomes (AMSC-Exos), human umbilical cord mesenchymal stem cell-derived exosomes (hUCMSC-Exos), exosomes derived from human menstrual blood-derived stem cells, and exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells (hiPSC-MSC-Exos).

MSC-Exos, like MSCs, can help maintain tissue homeostasis and help tissues achieve optimal function.[56] MSC-Exos can regulate cell migration, proliferation, and differentiation. Furthermore, MSC-Exos can remodel matrix synthesis and deliver signals and molecules to other cells.[55,60–62] In studies on myocardial repair after acute myocardial infarction and autoimmune diseases, MSC-derived exosomes showed similar effects as MSCs, including anti-apoptosis, angiogenesis promotion, and immunomodulation.[50,59,63] In some studies, MSC-derived exosomes (MSC-Exos) have shown better results than MSCs.[59,64,65] MSC-Exos can prevent the challenges of microvascular obstruction, allogeneic rejection, and abnormal chromosomal differentiation of MSCs.[59] MSC-Exos have been shown to enhance wound healing and tissue regeneration.[49,66,67] MSC-Exos can exert beneficial effects in neurological, bone, renal, and heart diseases, as well as cancer.[49,68–71] Additionally, MSC-Exos possess immunosuppressive properties and can effectively alleviate autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, type-1 diabetes, uveitis, rheumatoid arthritis, and inflammatory bowel disease.[59] Furthermore, MSC-Exos can be used as an alternative MSC-based therapy and play a role in the treatment of liver diseases.

As delivery vectors, exosomes can be isolated and prepared in various ways (Table 1). Exosomes can be isolated by differential ultracentrifugation and density gradient centrifugation, immunoaffinity chromatography, size exclusion chromatography, polymer precipitation, and microfluidic technologies. Differential ultracentrifugation and density gradient centrifugation are considered to be the "gold standard" for the isolation of exosomes.[72] Different separation methods have their advantages and disadvantages, and the best separation method can be selected according to the actual needs and conditions. These methods can be used in combination to partially alleviate their limitations and improve extraction yield and purity to meet the needs of research and disease treatment.[57] Table 1 provides a summary of the exosome isolation methods and a comparison of their advantages and disadvantages. Moreover, at present, biological cargoes can be loaded into MSC-Exos in various ways, including electroporation, transfection, and overexpression. Transfection is the most commonly used method to load RNA. Electroporation generally introduces hydrophilic cargoes into MSC-Exos, and overexpression is usually used to introduce proteins into MSC-Exos.[73] The methods of drug encapsulation by exosomes and exosome culture vary.[74] Exosomes treated in different ways have different effects on

Table 1 Separation and Purification of MSC-Exos

Separation and Purification Method	Advantage	Disadvantage	Ref.
Differential ultracentrifugation	High purity, simple method, common method for exosome extraction	The separation time is long and the efficiency is low	[80,81]
The two-ultracentrifugation cycle protocol that incorporated a 30% sucrose cushion	Less pollution	Impact EVs size, structure and cargo	[82]
Density-gradient ultracentrifugation	Minimize lipoprotein contamination when isolating exosomes from blood plasma	The separation efficiency needs to be evaluated	[83]
Combined tangential flow filtration (TFF) with 3D culture	Improve the yield of exosomes to a cumulative extent of 140-fold.	Expensive and easily polluting.	[73,84]
Size-exclusion chromatography (SEC)	Quick, cheap, easy and no expensive equipment is needed. Vesicles can be easily separated from proteins and HDL.	The separation of vesicles with small size (less than 70 nm in diameter) was less effective	[85]
Purifying exosomes by polyethylene glycol-based method (ExtraPEG)	Enriches exosomes rapidly and inexpensively	There is a lack of good extraction effect for some subsets of exosomes	[86]
Integrating acoustics and microfluidics	The purity and yield are high, and the structural integrity of exosomes is less affected	Depends on the extraction instrument	[87]
Commercial exosomes extraction kits	The operation is relatively simple, and the technical requirements for equipment and operators are low	The isolation and purification effect needs to be verified	[88]

Abbreviations:MSC-Exos, mesenchymal stem cell-derived exosomes; EVs, Extracellular vesicles; HDL, high-density lipoprotein cholesterol.

the liver disease when used as drug delivery vehicles. Electroporation is widely used for exosome drug loading, which has the best drug encapsulation efficiency.[51] When norcantharidin (NCTD) is loaded into purified BMSC-Exos by electroporation, BMSC-Exos-NCTD provides a continuous and slow release of the drug.[75] Li et al[76] found that the 3D culture of hUCMSCs (3D-hUCMSCs) promoted cell yield and stemness maintenance. 3D culture of exosomes (3D-Exos) has a better anti-liver fibrosis effect than 2D-Exos.[77] Compared to 2D-tumour-cell-derived microparticles (2D MPs), 3D-tumour-cell-derived microparticles (3D MPs) can achieve effective internalization into target cells, ultimately improving their ability to deliver drugs.[78] The stability of proteins and microRNAs in MSC-Exos was significantly increased. Therefore, the retention of MSC-Exos in vivo was increased.[79] The above reports have guiding significance for the future application of MSC-Exos in liver diseases, and we should pay attention to the effect of the way of MSC-Exos culture on the therapeutic effect. The above methods and technologies have laid a foundation for the large-scale preparation of exosomes and delivery of biological cargoes and drugs, making MSC-Exos-based delivery vectors feasible for the treatment of liver diseases.

Application of Mesenchymal Stem Cell-Derived Exosome-Based Delivery Vectors in Liver Diseases

To apply MSC-Exos-based delivery vectors in the treatment of liver diseases, the following section will evaluate the role of MSC-Exos as delivery vectors in the treatment of liver diseases, including liver injury, liver failure, liver fibrosis, HCC, and ischemia and reperfusion (I/R) injury (Table 2) (Figure 1).

Liver Injury and Liver Failure

MSC-Exos can play an effective protective role in various organ damages, including spinal cord injury, traumatic brain injury, acute lung injury, and cardiac injury.[109–113] Similarly, in recent years, researchers have found that MSC-Exos exhibit great promise for liver injury therapy. MSC-Exos have been shown to induce hepatoprotective effects against drug-induced liver

Table 2 The Role of MSC-Exo-Based Delivery Vectors in the Treatment of Liver Diseases, as Discussed in the Text

Liver Disease	MSC-Exos Types	Loaded Small Molecule	Animal Model	Disease Model	Mechanism of Action	Effect	Ref.
Liver injury	hUCMSC-Exos	miR-455-3p	Eight-week-old male mice (C57BL/6)	CCl_4-induced acute liver injury	Block the activation of the IL-6 signaling pathway by targeting the PIK3r1 gene	Suppress the over activation of monocytes/macrophages and improve liver damage and systemic homeostasis	[89]
Liver injury	BMSC-Exos	miR-223	Wild-type (WT) male C57BL/6 mice with ages of 4–6 weeks	Liver injury caused by hepatic S100-induced AIH	Attenuation of NLRP3 and caspase-1	Attenuation of liver injury	[90]
ALF	AMSC-Exos	miR-17	Mice (C57BL/6J, aged 5–6 weeks)	LPS/GalN-induced ALF	Suppress NLRP3 inflammasome activation by TXNIP inhibition	Play a protective role in ALF	[91]
Liver fibrosis	AMSC-Exos	miR-181-5p	Eight-week-old male C57BL/6 mice	TGF-β1-induced and CCl_4 -induced liver fibrosis	Inhibit the STAT3/Bcl-2/Beclin 1 pathway	Increase autophagy, reduce liver fibrosis	[92]
Liver fibrosis	MSC-Exos	circDIDO1			Target miR-141-3p/PTEN/AKT pathway	Suppress HSCs activation	[93]
Liver fibrosis	AMSC-Exos	miR-122	C57BL/6 mice (6 weeks)	CCl_4-induced liver fibrosis	Reduce IGF1R expression, enhance the G0/G1 arrest of HSCs, reduce P4HA1 expression level, prevent the up-regulation of TGF-β1 and α-SMA	Inhibit proliferation, block collagen maturation in HSCs, suppress HSCs activation	[64]
Liver fibrosis	MSC-Exos	miR-148a	The male C57BL/6 J mice (6–8 weeks)	CCL_4-induced liver fibrosis	Deliver miR-148a to target KLF6/STAT3 pathway in macrophages	Ameliorate the inflammatory response by the remodeling of macrophage phenotypes in vivo	[94]
Liver fibrosis	hUCMSC-Exos	BECN1	Mice (BALB/c, female, 4–5 weeks)	CCl_4-induced liver fibrosis	Induce ferroptosis via the downregulation xCT/GPX4 pathway	Target hepatic stellate cells activation in vitro and in vivo	[95]
Liver fibrosis	MSC-Exos	siRNA or ASO	Female Balb/c mice (8-weeks-old)	CCl4-induced liver fibrosis	Enhance STAT3 targeting efficiency, suppress STAT3 levels and ECM deposition in liver fibrosis	Significantly improve liver function	[96]

HCC	AMSC-Exos	MiR-199a-3p	Male BALB/c nude mice (6 weeks old)		Target mTOR pathway	Increase the sensitivity of HCC cells to chemotherapeutic agents	[97]
HCC	AMSC-Exos	miR-122	Male Balb/c nude mice (6 weeks old)		Negative regulation of the expression of miR-122 target genes, enhance cell apoptosis and cell cycle arrest	Increase the chemosensitivity of HCC cells	[98]
HCC	BMSC-Exos	miR-127-3p	Thirty-two male BALB/c nude mice (6 weeks old, 20–22 g)		Regulate a C5orf66-AS1/miR-127-3p/DUSP1/ERK axis	Block malignant behaviors of HCC-sourced CSCs	[99]
HCC	hUCMSC-Exos	miR-451a			Suppress the paclitaxel resistance, cell cycle transition, proliferation, migration and invasion, and restrict the epithelial-mesenchymal transition of HCC cells	Promote apoptosis of HCC cells	[100]
HCC	BMSC-Exos	miR-338-3p			Down-regulating EST1	Inhibit HCC cell proliferation, invasion and migration, and induce cell apoptosis	[101]
HCC	BMSC-Exos	NCTD	4-week-old male BALB/c nude mice		Promote cellular uptake, induce cell cycle arrest, reduce tumor cell proliferation, increase apoptosis	Exert obvious antitumor effects	[75]
I/R injury	hUCMSC-Exos	miR-1246	C57BL/6 mice		Regulate GSK3β-mediated Wnt/β-catenin pathway	Improve hepatic I/R injury	[102]
I/R injury	hUCMSC-Exos	miR-1246	Male C57BL/6 mice		Modulate the balance between Tregs and Th17 cells via miR-1246-mediated IL-6-gp130-STAT3 axis.	Improve hepatic I/R injury	[103]
I/R injury	hUCMSC-Exos	miR-20a	Male Sprague Dawley rats of about 300 g in body weight		Inhibit Beclin 1- and FAS-mediated autophagy and apoptosis	Alleviate hepatic I/R injury	[104]

(*Continued*)

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Table 2 (Continued).

Liver Disease	MSC-Exos Types	Loaded Small Molecule	Animal Model	Disease Model	Mechanism of Action	Effect	Ref.
I/R injury	BMSC-Exos	miR-124-3p	Specific pathogen free (SPF) SD rats		Downregulate Steap3 expression to inhibit ferroptosis	Attenuating graft I/R injury	[105]
I/R injury	BMSC-Exos	miR-29a-3p	Clean-grade male Sprague-Dawley (SD) rats		Suppress ferroptosis by targeting Ireb2	Alleviation of steatotic liver I/R injury	[106]
NAFLD	hUCMSC-Exos	miR-627-5p	Male rats (6 weeks old, weighting 240–260 g)	NAFLD rat model was established by high-fat high-fructose (HFHF) feeding for 8 weeks.	Improved glucose and lipid metabolism of L-O2 cells by targeting FTO	Improved liver damage, lipid deposition and glucose and lipid metabolism in vivo, ameliorated the progression of NAFLD	[107]
Liver regeneration	hUCMSC-Exos	miR-124	Sprague-Dawley rats (male, 8 weeks old)	70% partial hepatectomy	Promote liver regeneration and inhibit liver injury via negatively regulating Foxg1	Promote liver regeneration after partial hepatectomy	[163]

Abbreviations:hUCMSC-Exos, human umbilical cord mesenchymal stem cell-derived exosomes; AIH, autoimmune hepatitis; ALF, acute liver failure; TXNIP, thioredoxin-interacting protein; STAT3, signal transducer and activator of transcription 3; CircDIDO1, a circRNA derived from 2 to 6 exons of DIDO1 gene; α-SMA, Alpha-smooth muscle actin; HSCs, hepatic stellate cells; KLF6, Kruppel-like factor 6; GPX4, glutathione peroxidase 4; ASO, antisense oligonucleotide; HCC, hepatocellular carcinoma; ECM, extracellular matrix; mTOR, a serine/threonine kinase; C5orf66AS1, one of long noncoding RNAs; DUSP1, dual-specificity phosphatase 1; CSCs, cancer stem cells; EST1, E26 transformation specific-1; I/R injury, ischemia and reperfusion injury; IL-6, interleukin-6; gp130, The interaction between miR-1246 and IL-6 signal transducer; Ireb2, Iron response element-binding protein 2; NAFLD, non-alcoholic fatty cell liver disease; FTO, a fat mass and obesity-associated gene.

injury. MSC-Exos inhibited the acetaminophen (APAP)- and hydrogen peroxide (H_2O_2)-induced hepatocyte apoptosis mainly through activation of antiapoptotic, proliferative, and regenerative responses by upregulation of Bcl-xL protein expression.[114] MSC-Exos significantly attenuated CCl_4-induced lipid peroxidation and reduced other iron ptosis markers, including decreased expression of SLC7A11 and increased expression of Ptgs2 and LOXs, thus alleviating CCl_4-induced liver injury.[115] Phosphoinositide 3-kinase (PI3K) plays a key role in the activation of the IL-6-related signaling pathway. hUCMSC-Exos rich in miR-455-3p can regulate the PIK3r1 gene, which encodes the PI3K subunit P85α and inhibits IL-6-related signaling pathways, thus inhibiting macrophage activation and alleviating acute liver injury.[89] Furthermore, in an experimental model of autoimmune hepatitis (AIH), BMSC-Exos are effective in liver injury, which could be related to miR-223 regulation of NLRP3 and caspase-1.[90]

Acute liver failure (ALF) is a rare but life-threatening critical illness that usually results from viral infections and drug-induced liver injury.[116] NLRP3 inflammasome has been identified as a potential mediator of hepatocyte damage, immune cell activation, and hepatitis amplification.[117] AMSC-Exos can reduce the activation of NLRP3 inflammasome in macrophages through miR-17-mediated thioredoxin-interacting protein (TXNIP) inhibition. Therefore, AMSC-Exos play a protective role in lipopolysaccharide and d-galactosamine (LPS/D-GalN)-induced ALF.[91] hUCMSC-Exos have been shown to significantly improve LPS/D-GalN-induced hepatitis by the downregulation of NLRP3.[118] BMSC-Exos can promote autophagy and effectively reduce hepatocyte apoptosis in LPS/D-GaIN-induced ALF.[119] In a study, MSC-Exos migrated to sites of injury and AML12 cells (a mouse hepatocyte cell line) after fulminant hepatic failure (FHF). Therefore, LPS/D-GalN-induced apoptosis of AML12 cells was inhibited. MSC-Exos significantly inhibited apoptosis in hepatocytes, improved liver function, and increased survival rates by reducing the number of mononuclear cells and the expression of caspase-3.[120] Glutathione peroxidase1 (GPX1) is a critical antioxidant in the human body.[121]

In conclusion, MSC-Exos can be used as a delivery vector to overexpress target miRNAs and deliver these miRNAs to target tissues, thus playing a role in effectively reducing liver injury and liver failure. This may become a new approach to the treatment of liver injury and liver failure.

Liver Fibrosis

Liver fibrosis is caused by chronic liver damage, inflammation, and excess accumulation of extracellular matrix (ECM) components.[122–124] Several studies have found that hepatic stellate cell (HSC) activation is a key driver of hepatic fibrosis.[125–127] In a CCl_4-induced liver fibrosis model, AMSCs translocated miR-181-5p to damaged hepatocytes by selectively transferring exosomes to mouse HSCs. In vitro analysis confirmed that miR-181-5p-rich AMSCs were secreted extracellularly and subsequently taken up by stem stellate cells, thus allowing miR-181-5p to be transferred. MSC-Exos could be used to deliver miRNAs to HSCs. miR-181-5p-modified AMSC-Exos effectively inhibited liver fibrosis by increasing autophagy of HSCs by inhibiting the STAT3/Bcl-2/Beclin 1 pathway and decreasing TNFα, IL-6, and IL-17 levels.[92] CircDIDO1 (a circRNA derived from 2 to 6 exons of DIDO1 gene) mediated by MSC-Exos was confirmed to inhibit HSC activation by sponging miR-143-3p, which was an activator of the activation of the PTEN/AKT pathway.[93] MSC-Exos induced the transformation of proinflammatory macrophages to the anti-inflammatory phenotype and subsequently reduced liver fibrosis. MiR-148a, as the therapeutic effector of MSC-Exos, regulated the STAT3 signaling pathway by directly targeting KLF6.[94] Wang et al[77] showed that miR-6766-3p in the exosomes derived from human embryonic stem cells (hESC-Exos) inactivates recombinant mothers against decapentaplegic (SAMD) signaling by restraining TGFβ type II receptor (TGFβRII) expression, consequently attenuating LX2 cell and HSC activation and suppressing liver fibrosis. By delivering miR-122 content into HSCs, exosomes led to altered expression of miR-122-target gene in HSCs, thereby enhancing the therapeutic effect of AMSCs on liver fibrosis.[64] The signal transducer and activator of transcription 3 (STAT3) has been proven to be an important transcription factor related to the pathogenesis of liver fibrosis.[96] Compared to scrambled siRNA control, siRNA-STAT3, or ASO-STAT3, MSC-Exos carrying siRNA or antisense oligonucleotide (ASO) treatments enhanced STAT3 targeting efficiency and suppressed STAT3 levels and extracellular matrix (ECM) deposition in established liver fibrosis in mice. Liver function was significantly restored.[96] In addition to playing an effective role as a delivery vector for the treatment of liver fibrosis, MSC-Exos could inhibit liver fibrosis. Rong et al found that the expression of several proteins (PPARγ, Wnt3a, Wnt10b, and β-catenin) in the Wnt signaling pathway can be downregulated by BMSC-Exos, which in turn inhibited downstream gene (WISP1, Cyclin D1)

expression. BMSC-Exos could inhibit the activation of HSCs through inhibition of the Wnt/β-catenin signaling pathway, inhibit the expression of α-SMA, alleviate liver inflammation, improve liver function, promote hepatocyte regeneration, reduce liver fibrosis, and improve liver function.[128] Ferroptosis has been reported to play an important role in liver fibrosis.[129] MSC-Exos can enhance HSCs ferroptosis through the exosome/BECN1/xCT/GPX4 pathway, thereby ameliorating liver injury and alleviating liver fibrosis.[95] Altogether, the delivery vector based on MSC-Exos has a good effect in the treatment of liver fibrosis and is expected to become a new method for the treatment of liver fibrosis in the future.

Hepatocellular Carcinoma

Liver cancer is the most common fatal malignancy and the second leading cause of cancer-related death worldwide.[130,131] In recent years, the incidence of hepatocellular carcinoma (HCC) is increasing.[3,132] Tumor occurrence is closely related to the physiological state of the tumor microenvironment (TME), which is involved in tumor biology, tumorigenesis, development, and treatment response.[133,134] Surprisingly, TME can be regulated by exosomes.[135–137] Recently, researchers have found that MSC-Exos can exert an inhibitory effect on HCC through various pathways. Exosomes may be able to enhance or expand their therapeutic ability in cancer through chemical or biological modification.[138] AMSC-Exos can mediate the delivery of miR-199a-3p between AMSCs and HCC cells, thus the sensitivity of HCC cells to chemotherapeutic drugs can be effectively improved by miR-199a-3p-modified AMSC-Exos by targeting the mTOR (a serine/threonine kinase) pathway.[97] AMSC-Exos mediated miR-122 communication between AMSCs and HCC cells and further altered miR-122-target gene expression in HCC cells. By enhancing cell apoptosis and cell cycle arrest, the sensitivity of HCC cells could be enhanced by miR-122-modified AMSC-Exos.[98] By downregulating E26 transformation specific-1 (EST1), the miR-338-3p-modified BMSC-Exos could delay the development of HCC, which inhibited the proliferation, invasion, and migration of HCC cells, and induced cell apoptosis.[101] MiR-451a-modified hUCMSC-Exos inhibited the epithelial-mesenchymal transition of HCC cells by repressing ADAM10 (a target gene of miR-451a). By this means, hUCMSC-Exos inhibited paclitaxel resistance, cell cycle transition, proliferation, invasion, and migration of HCC cells, thereby promoting apoptosis of HCC cells.[100] HCC can be inhibited by miR-125a and miR-125b, which repressed proliferation, stem cell properties, and migration of HCC cells through the CD90 pathway.[139] Delivery of miR-125a/b by MSC-Exos may be a new therapeutic approach for HCC. In conclusion, MSC-Exos rich in different miRNAs can inhibit HCC cells through various effects, resulting in a therapeutic effect on HCC. MSC-Exo-based HCC treatment has a certain potential to become an alternative therapy for HCC. Compared with the anticancer drug, norcantharidin (NCTD) treatment alone, BMSC-Exos-NCTD delivery system showed a more significant antitumor effect, which was reflected in promoting cellular uptake, inducing cell cycle arrest, reducing tumor cell proliferation, and increasing apoptosis. Moreover, BMSC-Exo-NCTD increased cellular proliferation and inhibited hepatocyte oxidation without showing body toxicity.[75] Additionally, adipose stem cell exosomes (ASC-Exos) have been shown to inhibit the hepatoma cell line growth and promote the normal liver cell line growth.[51] Therefore, MSC-Exos have been hypothesized to have the ability to inhibit the growth of liver cancer cells and promote the growth of normal liver cells, thereby exerting a therapeutic effect on HCC.

Liver cancer stem cells (CSCs) are a unique subset of HCC cells with stem cell characteristics, which have the ability of self-renewal and differentiation.[140,141] However, this role can be inhibited by MSC-Exos. Exosomes released by CSCs induced Nanog expression and regorafenib resistance in differentiated cells[142] and induced tumor development and progression in vivo.[137] Furthermore, exosomes can affect CSCs. Gu et al[99] evidenced that the malignant behaviour of liver CSCs was blocked by exosomes through the C5orf66AS1/miR-127-3p/DUSP1/ERK axis. However, several researchers have previously found that MSCs promote HCC.[143,144] MSCs interact with tumor cells in a myriad of ways that can support or suppress tumor growth. Klopp et al[144] indicated that the effect of MSCs on tumors can be affected by several factors, including the heterogeneity of MSCs, the effects of propagating cells in vitro, the time of MSCs that enter the TME in vivo, and the variability of MSCs from patient to patient. This suggests that researchers should pay attention to the influence of the above factors on the results when studying the effect of MSC-Exos on HCC, because they may produce results that MSC-Exos have a promoting effect on HCC.

In summary, MSC-Exos inhibit HCC by delivering different types of miRNA, drug delivery, and blocking the stemness of liver CSCs, thereby achieving the therapeutic effect of HCC. However, attention should be paid to the potential role of MSC-Exos in promoting HCC to better apply them in clinical treatment.

Ischemia and Reperfusion Injury

Exosomes have been shown to play a protective role in organ ischemia and reperfusion (I/R) injury of organs, such as the brain and heart.[145–147] Exosomes protect cardiomyocytes from acute myocardial I/R injury by transmitting survival signals to the ischemic myocardium and inhibiting cardiomyocyte apoptosis in vivo.[148] Studies have shown that MSC-Exos are used as delivery carriers in the ischemia and reperfusion (I/R) injury of various organs, such as the brain, spinal cord, heart, kidney, and liver.[149–152] hUCMSC-Exos regulated the glycogen synthase kinase 3β (GSK3β)-mediated Wnt/β-catenin pathway by delivering miR-1246 and finally alleviated hepatic I/R injury.[102] Furthermore, hUCMSC-Exos could alleviate hepatic I/R injury by delivering miR-1246, targeting the IL-6/gp130/STAT3 axis to regulate the balance between Tregs and Th17 cells.[103] hUCMSC-Exos-enriched miR-20a could alleviate hepatic I/R injury by alleviating the abnormal expression of genes related to apoptosis and autophagy.[104] Ferroptosis was associated with the I/R injury of liver transplantation (LT) with a severe steatotic donor liver. Wu et al[105] showed that heme oxygenase oxygen-1 (HO-1)-modified BMSC-Exos (HM-Exos) could inhibit hepatocyte ferroptosis and reduce graft hepatic I/R injury by delivering miR-124-3p to downregulate the Steap3 level. Additionally, HM-Exos could inhibit hepatocyte ferroptosis by delivering miR-29a-3p targeting Ireb2, ultimately reducing hepatic I/R injury.[106] These studies provide a new way to solve the problem of future donor liver shortage. Furthermore, many studies have found that exosomes can reduce the inflammatory response, inhibit cell apoptosis, promote cell proliferation, and liver regeneration, thereby alleviating hepatic I/R injury.[153–156] In summary, MSC-Exos-based delivery vectors can alleviate hepatic I/R injury by delivering multiple miRNAs, which may effectively improve the success rate of LT and the prognosis of patients.

Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is the main cause of chronic liver disease, and the liver-related mortality of patients with non-alcoholic steatohepatitis has increased in recent years.[108] NAFLD affects more than one-third of the population.[157] Exosomes from AMSCs attenuated white adipose tissue inflammation, systemic insulin resistance, dyslipidemia, and hepatic steatosis in a study on obese mice.[158] Exosomes enriched in miR-223 inhibited NAFLD-associated liver fibrosis by transfer into hepatocytes to suppress the hepatic expression of fibrotic genes.[159] hUCMSC-Exos rich in miR-627-5p improved glucose and lipid metabolism and alleviated liver injury by inhibiting FTO (fat mass and obesity-associated gene) expression, thereby meliorating the progression of NAFLD.[107] Moreover, hepatocyte-derived exosomal miR-192-5p was shown to inhibit proinflammatory macrophage activation and disease progression in NAFLD.[160] In conclusion, MSC-Exos can deliver multiple miRNAs, thereby slowing or halting the progression of NAFLD and improving the quality of life of patients.

Liver Regeneration

Exosomes have great potential for liver regeneration, tissue repair, and blood vessel formation. Exosomes, as nanocarriers, deliver active factors or small molecules to promote tissue repair. Preclinical studies of exosomes in tissue engineering and regenerative medicine have been performed in the fields of bone/cartilage repair, skin repair, and nerve, liver, kidney, and vascular tissue regeneration.[161] Hepatic gene expression of cytokines and growth factors related to cell proliferation, angiogenesis, and anti-inflammatory response was upregulated by an MSC-conditioned culture medium (MSC-CM).[162] In the early phase after surgical resection, MSC-derived factors promoted hepatocyte proliferation and regenerative responses. After patients have undergone extensive liver resection or liver transplantation, MSC-derived factors therapy could represent a feasible new strategy to promote liver regeneration.[162] After partial hepatectomy in rats, miR-124 derived from hUCMSC-Exos can downregulate Foxg1 and promote liver regeneration.[163] Xue et al found that ADMSC-Exos could promote vascular endothelial growth factor (VEGF) expression and angiogenesis by activating the protein kinase A (PKA) signaling pathway.[164] The above studies provide new ideas for the application of MSC-Exos-based delivery vector in liver regeneration, which may benefit patients with extensive liver resection.

Advantages and Disadvantages of MSC-Exos

As a relatively novel treatment, exosomes have certain advantages over nano-drug. As mentioned above, exosomes have been shown to promote the growth of normal cell lines and inhibit the growth of hepatocellular carcinoma cell lines which were inhibited through several anti-inflammatory molecules. Compared with nano-drugs, exosomes are naturally secreted by cells, which have the advantages of low immunogenicity and immune rejection prevention.[51] Unlike other lipid nanoparticles,

exosomes have surfaces that are rich in membrane proteins, which can mediate adhesion and target functions between exosomes and plasma membrane of recipient cells, thereby regulating exosome uptake.[38,161] These advantages may allow MSC-Exo-based delivery vectors to play a good therapeutic effect in the treatment of liver diseases. The use of MSCs as cell therapy carries some risks, such as potential tumorigenicity and immunological rejection.[165] As a cell-free therapy, MSC-Exos can effectively reduce this risk. Although derived from MSCs, MSC-Exos sometimes have better results than MSCs.[128] In addition, many in vivo studies have shown that MSC-derived exosomes can enter the liver.[38] This suggests that MSC-Exo-based delivery vector is a promising alternative to MSC therapy, especially in liver disease.

In the treatment of liver diseases, MSC-Exo-based delivery vectors have a wider application space. Concurrently, we must admit that MSC-Exos should be used cautiously in the treatment of HCC because the role of MSC-Exos in tumor development has not been fully elucidated.[137] The choice of exosome drug loading method and surface targeting peptide needs to be fully considered.[138]

MSC-Exos in Clinical Trials

Currently, 139 clinical trials of exosomes are available at www.ClinicalTrials.gov and nine of them are on MSC-Exos. However, currently, clinical trials on the treatment of liver diseases based on MSC-Exos are lacking. This situation is in part due to the fact that translating MSC-Exos therapy from preclinical studies to the clinic requires key parameters.[65]

Conclusions and Prospects

The above findings add substantially to our understanding of the therapeutic effect of MSC-Exo-based delivery vectors in liver disease. In the past years, MSC-Exo-based therapy has raised considerable concern. With in-depth research on cell-free therapy, exosomes have a broad application value in liver diseases, including drug delivery, liver cancer, and liver transplantation. MSC-Exos have shown therapeutic potential in various liver diseases and are expected to become a new treatment method for liver diseases. Besides, MSC-Exos can be used as a carrier for drug delivery to assist the more accurate delivery of clinically used drugs to target tissues, which not only improves the efficacy of drugs but also reduces systemic toxic side effects. As a kind of biological carrier, MSC-Exos provide a new idea for the current drug delivery scheme and expand the drug delivery system in the treatment of liver diseases. Therefore, MSC-Exos have the potential to become biological agents for the treatment of liver diseases. By delivering biological cargoes or drugs, MSC-Exos have the potential to be an alternative treatment option for various liver diseases, whether benign or malignant liver disease and early or advanced liver disease. MSC-Exo-based delivery vectors have been widely shown to reduce normal cell apoptosis, promote liver regeneration, increase autophagy of hepatic stellate cells, and inhibit the growth of hepatocellular carcinoma cells. However, MSC-Exos do not exhibit systemic toxicity. Through these mechanisms, MSC-Exos have shown good therapeutic effects in drug-induced liver injury, liver I/R injury, liver resection, HCC, and other liver diseases by delivering biological cargoes or drugs. At present, MSC-Exo-based liver disease therapies are still in the stage of in vitro research and animal models. The current challenges of large-scale production, quality control, long-term storage, cost, and safety of MSC-Exos have not been solved. Therefore, we need to conduct more in-depth research and analysis.[50] The clinical application of MSC-Exos-based delivery vectors, as an emerging treatment for liver diseases, should be fully and effectively evaluated. More preclinical studies should be conducted to accumulate more data and prepare for relevant clinical studies. In the treatment of liver disease, investigators need to fully consider the safety and potential side effects of MSC-Exo-based therapy. Further studies on how to efficiently extract or prepare MSC-Exos on a large scale, reduce the cost, improve the loading efficiency of biological cargoes or drugs, and accurately exert therapeutic effects on effector cells or tissues are needed. In summary, MSC-Exos own high clinical translation and application value. In the future, the treatment of MSC-Exos as a biological carrier may not only assist the existing treatment options for liver diseases but also become a new treatment plan for liver diseases. MSC-Exo-based therapy has the potential to relieve the symptoms of patients with liver disease and improve their quality of life and prognosis.

Abbreviations

2D MPs, 2D-tumour-cell-derived microparticles; 2D-Exos, 2D culture of exosomes; 3D-Exos, 3D culture of exosomes; 3D-hUCMSCs, 3D culture of hUCMSCs; ADAM10, a target gene of miR-451a; AFM, atomic force microscopy; AIH,

autoimmune hepatitis; ALF, Acute liver failure; AMSC-Exos, adipose tissue-derived mesenchymal stem cell-derived exosomes; AMSCs, adipose tissue-derived mesenchymal stem cells; APAP, acetaminophen; ASO, antisense oligonucleotide; Bcl-xL, One of the antiapoptotic genes; BECN1, a crucial regulator of ferroptosis; BMSC-Exos, bone marrow mesenchymal stem cell-derived exosomes; C5orf66AS1, one of long noncoding RNAs (lncRNAs); CircDIDO1, a circRNA derived from 2 to 6 exons of DIDO1 gene; CSCs, cancer stem cells; DUSP1, dual-specificity phosphatase 1; ECM, extracellular matrix; EST1, E26 transformation specific-1; EVs, Extracellular vesicles; FHF, fulminant hepatic failure; FTO, a fat mass and obesity-associated gene; gp130, The interaction between miR-1246 and interleukin 6 (IL-6) signal transducer; GPX1, Glutathione peroxidase1; GPX4, Glutathione peroxidase 4; GSK3β, glycogen synthase kinase 3β; HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; hESC-Exos, exosomes derived from human embryonic stem cell; hiPSC-MSC-Exos, exosomes produced by human-induced pluripotent stem cell-derived mesenchymal stromal cells; HO-1, heme oxygenase oxygen-1; HSC, hepatic stellate cell; hUCMSC-Exos, human umbilical cord mesenchymal stem cell-derived exosomes; I/R injury, ischemia and reperfusion injury; IL-17, interleukin-17; IL-6, interleukin-6; Ireb2, Iron response element-binding protein 2; KLF6, Kruppel-like factor 6; LT, liver transplantation; LX2 cell, Human hepatic stellate cells; MSC-Exos, Exosomes derived from mesenchymal stem cells; MSCs, Mesenchymal stem cells; mTOR, a serine/threonine kinase; NAFLD, non-alcoholic fatty cell liver disease; NCTD, norcantharidin; NLRP3, nucleotide-binding and oligomerization domain-like receptor 3; NTA, Nanoparticle Tracking Analysis; PI3K, Phosphoinositide 3-kinase; PI3K, Phosphoinositide 3-kinase; PKA, protein kinase A; PTX, paclitaxel; ROS, oxygen species; SAMD, Recombinant Mothers Against Decapentaplegic; STAT3, Signal transducer and activator of transcription 3; TEM, transmission electron microscope; TGFβRII, TGFβ type II receptor; TME, tumor microenvironment; TNFα, Tumor necrosis factor α; TXNIP, thioredoxin-interacting protein; VEGF, vascular endothelial growth factor; α-SMA, Alpha-smooth muscle actin.

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Disclosure

The authors declare that they have no competing interests.

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