

REVIEW

Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases

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The administration of mesenchymal stem cells (MSCs) as a therapy for liver disease holds great promise. MSCs can differentiate into hepatocytes, reduce liver inflammation, promote hepatic regeneration and secrete protective cytokines. However, the risks of iatrogenic tumor formation, cellular rejection and infusional toxicity in MSC transplantation remain unresolved. Accumulating evidence now suggests that a novel cell-free therapy, MSC-secreted exosomes, might constitute a compelling alternative because of their advantages over the corresponding MSCs. They are smaller and less complex than their parent cells and, thus, easier to produce and store, they are devoid of viable cells, and they present no risk of tumor formation. Moreover, they are less immunogenic than their parent cells because of their lower content in membrane-bound proteins. This paper reviews the biogenesis of MSC exosomes and their physiological functions, and highlights the specific biochemical potential of MSC-derived exosomes in restoring tissue homeostasis. In addition, we summarize the recent advances in the role of exosomes in MSC therapy for various liver diseases, including liver fibrosis, acute liver injury and hepatocellular carcinoma. This paper also discusses the potential challenges and strategies in the use of exosome-based therapies for liver disease in the future.

Experimental & Molecular Medicine (2017) 49, e346; doi:10.1038/emm.2017.63; published online 16 June 2017

INTRODUCTION

Mesenchymal stem cell (MSC)-based therapy has emerged as a promising strategy for treating liver diseases via tissue repair and immune regulation.^{1–3} However, the use of MSCs has some drawbacks, such as the need of a consistent supply of cells with stable phenotype, high costs and time delays for the generation and handling of these cells. In addition, issues related to ectopic tissue formation, infusional toxicity caused by cells lodged in the pulmonary microvasculature, and cellular rejection or unwanted engraftment, have been reported.⁴ Studies have shown that MSCs can achieve a therapeutic effect *in vivo* via paracrine action^{5,6} and direct differentiation. Subsequent studies indicated that MSC-secreted extracellular vesicles (EVs), including microvesicles (MVs; 0.1–1 μm in diameter) and exosomes (40–100 nm in diameter),^{7,8} may contribute to the therapeutic potency of MSCs by mediating cell–cell micro-communication and transporting paracrine factors during angiogenesis, tissue regeneration and immune regulation.^{9–12}

The administration of MSC-derived exosomes has yielded beneficial effects in a variety of animal models of liver disease, including drug-induced acute liver injury, liver fibrosis and

hepatocellular carcinoma (HCC).^{13–16} Exosomes have advantages over the corresponding MSCs: they are smaller and less complex than cells, so they are easier to produce and store, and have the potential to avoid some of the regulatory issues that face MSCs.¹⁷ Therefore, MSC-derived exosomes may represent an ideal therapeutic tool for liver diseases in the near future.

FUNCTIONS OF EXOSOMES

Exosomes are nano-sized EVs that possess remarkable physiological properties and originate via the inward budding of the membrane of late endosomes called multivesicular bodies (MVBs). Upon the fusion of MVBs with the plasma membrane, exosomes are released into the extracellular milieu and can be either taken up by target cells residing in the microenvironment or carried to distant sites via biological fluids.¹⁸ Exosomes have a narrow diameter range of 40–100 nm and a density of 1.13–1.19 g ml⁻¹ in sucrose solution. They can be sedimented by centrifugation at 100 000 g.¹⁹ Exosome membranes are enriched in cholesterol, sphingomyelin, ceramide and lipid raft proteins.²⁰ Exosomes are reported to contain both proteins and RNAs.²¹ Most exosomes have an evolutionary conserved set of proteins including tetraspanins

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Received 31 August 2016; revised 3 January 2017; accepted 12 January 2017

(CD81, CD63 and CD9), heat-shock proteins (HSP60, HSP70 and HSP90), ALIX and tumor susceptibility gene 101 (TSG101); however, they also have unique tissue/cell type-specific proteins that reflect their cellular source.²²

General physiological functions of exosomes

Exosomes were once thought to be 'trash bags' for cells to discard unwanted proteins. However, over the past decade, they have emerged as an important intercellular communication vehicle for modulating or mediating a variety of cellular processes. Exosome release is currently considered an integral mechanism of cell-to-cell communication that may affect both neighboring cells and distant parts of the body.

In the immune system, exosomes have been demonstrated to have a key role in mediating both adaptive and innate immune responses, thereby participating in antigen presentation by spreading antigens in association with MHC-II and MHC-I molecules. For example, B-lymphocyte- and dendritic cell (DC)-derived exosomes stimulate T cells and initiate host immune responses.^{23,24} When pulsed with tumor peptides, DC-derived exosomes prime cytotoxic T cells and suppress tumor growth in mice.²⁵

Interestingly, virally or bacterially infected cells use exosomes as vehicles to disseminate disease.^{26,27} The exosomes that are secreted from scrapie-infected epithelial and neuroglial cells, which contain infectious prion protein (PrP) scrapie (PrPsc), may contribute to intercellular membrane exchange and the spread of prions throughout the organism.²⁸ Exosomes derived from cells infected by human immunodeficiency virus type 1,²⁹ Epstein-Barr virus,³⁰ Herpes simplex virus-1³¹ and hepatitis C virus³² circulate in the bloodstream, attack and enter their target cells. Viruses that are delivered by exosomes are released and replicate within them, giving rise to a systematic distribution of viral agents that influence the host immune cells.³³ Similar to virally infected cells, bacterially infected cells such as *Mycobacterium tuberculosis*-, *Mycobacterium bovis*- or *Mycobacterium avium*-infected macrophages release exosomes that contain pathogen-derived antigens,³⁴ which have an important role in immune surveillance during intracellular bacterial infections.

Cancer cells also utilize exosomes in tumor progression and metastasis. Highly metastatic melanoma cell-derived exosomes can promote epithelial-mesenchymal transition in primary melanocytes.³⁵ Glioblastoma multiforme cell-derived hypoxic exosomes can secrete and transfer potent growth factors into vascular cells to promote tumor vascularization during tumor development.³⁶ Tumors such as melanoma and colorectal carcinoma also secrete exosomes to induce immunosuppressive myeloid cells and possibly enable tumors to evade the immune system.³⁷ The nucleic acid content of exosomes also appears to be important in tumor metastasis; for instance, the PTEN-targeting microRNAs, which are transferred by astrocyte-derived exosomes, enhance brain metastasis outgrowth.³⁸

Recently, exosomes have also been implicated in degenerative diseases. In Parkinson's and Alzheimer's patients, neuron-

secreted exosomes containing α -synuclein³⁹ and amyloid- β peptide (A β),⁴⁰ respectively, have been reported. These exosomes can contribute to the nucleation or physical dissemination of the α -synuclein and A β aggregates that characterize the two diseases. Another study showed that exosomes from interleukin-1 β (IL-1 β)-stimulated human synovial fibroblasts could induce osteoarthritis-like changes in both *in vitro* and *ex vivo* models: this might represent a novel mechanism involving osteoarthritis-affected joints via pathogenic signal communication.⁴¹

Moreover, exosomes are emerging as mediators in tissue regeneration. During injury, cardiomyocyte progenitor cells secrete exosomes to stimulate the migration of endothelial cells and promote cardiac regenerative activity.⁴² Exosomes have also been shown to be involved in the regeneration of peripheral nerves and in the repair of neuronal injuries.⁴³ Furthermore, their ability to cross the blood-brain barrier has prompted extensive investigations to use them as delivery vehicles to treat neurological disorders.⁴⁴

Functions of MSC-derived exosomes

The physiological function of MSC-derived exosomes has not been defined. They may act as an intercellular communication vehicle for modulating or mediating cellular processes, similar to the exosomes derived from cell types. MSC-derived exosomes may interact with multiple cell types within adjacent and remote areas to elicit appropriate cellular responses; they affect the stromal support functions of MSCs through the maintenance of a dynamic and homeostatic tissue microenvironment.⁴⁵

Similar to exosomes in general, MSC-derived exosomes carry complex cargo, including nucleic acids, proteins and lipids. Through mass spectrometry, antibody array and microarray analysis, >850 unique gene products and >150 miRNAs have been identified in the cargo of MSC-derived exosomes.^{46,47} These exosomal proteins and miRNAs are functionally complex, and are implicated in many diverse biochemical and cellular processes, such as communication, immune regulation, bioenergetics, tissue repair and regeneration and metabolism. Thus, MSC-derived exosomes display the potential to elicit diverse cellular responses and interact with various cell types.

MSC-derived exosomes have a key role in mediating the capacity of MSCs to function as stromal support cells to maintain homeostasis within the tissue and respond to external stimuli. This role is particularly important when the homeostasis of the tissue microenvironment is disrupted by disease or injury; this, in turn, compromises normal tissue function. MSC-derived exosomes are highly enriched in biologically active molecules, such as proteins and RNAs, and are therefore well equipped for this role.⁴⁸ Many of the proteins found in exosomes are enzymes, with activities that are catalytic rather than stoichiometric and are dictated by their microenvironment (for example, substrate concentration or pH). Therefore, the enzyme-centric feature of exosomes may alleviate the risk of over- or under-dosing if they are used as therapeutical agents.⁴⁹ A significant clustering of glycolytic enzymes has been

found in MSC exosomes; if they are given as therapy, these enzymes from MSC exosomes can ameliorate the glycolytic deficit and potentially increase glycolytic flux and ATP production in the reperfused myocardium.⁵⁰

Moreover, exosomes are the ideal vehicles to protect and deliver molecules to the appropriate targets. By encapsulating molecules within their membranes, exosomes can protect enzymes or RNAs against degradation and facilitate their intracellular uptake via the cellular endocytosis of exosomes.^{51,52} The uptake of exosomes has been reported to be modified by microenvironmental acidity,⁵³ and tissue injury is often characterized by tissue acidosis.⁵⁴ Exosomes would be preferentially taken up by cells in injured tissues. Furthermore, exosomes are nanometer-sized particles that can be easily transferred through blood and other biological fluids. Thus, MSC-derived exosomes can mediate cell communication in both adjacent and remote areas via paracrine and endocrine signaling. The capacity of MSC-derived exosomes to restore and maintain the homeostasis of the tissue microenvironment would depend on the biochemical potential of their protein and RNA cargo.

THERAPEUTIC PROPERTIES OF MSC-DERIVED EXOSOMES ON LIVER DISEASE

The unique ability of MSCs to self-renew and their multipotentiality have made them a promising cell-based strategy for treating liver diseases. Recent studies have shown that MSC-derived exosomes are as potent as parent stem cells in the regeneration of various organ injury models, primarily through the transfer of their cargo to the recipient cells, which are consequently modified in their function and/or phenotype.^{55,56} As shown in Table 1, multiple recent studies have presented preclinical data addressing the reparative and regenerative properties of MSC-derived exosomes in liver diseases.^{13–16,57}

MSC-derived exosomes for liver fibrosis

The application of MSCs in animal models of liver fibrosis/cirrhosis and, eventually, in patients ameliorates the progress of the disease.⁵⁸ Similar results were obtained when MSC-conditioned media (MSC-CM) were applied instead,^{58,59} which suggests that MSCs might achieve a therapeutic effect *in vivo* via their secreted EVs, such as exosomes.

Several studies have focused on the therapeutic effects of MSC-derived exosomes in a mouse model of liver fibrosis. Using a carbon tetrachloride (CCl₄)-induced liver injury model in Kunming mice, Li *et al.* found that the exosomes derived from human umbilical cord MSCs ameliorate liver fibrosis by inhibiting both the epithelial–mesenchymal transition of hepatocytes and collagen production. Exosomes were found to significantly restore the serum aspartate aminotransferase activity and inactivate the TGF- β 1/Smad signaling pathway by decreasing collagen type I/III and TGF- β 1 and the phosphorylation of Smad2.¹⁴ Another study showed that chorionic plate-derived MSCs could release exosomes containing miR-125b, mediate miR-125b transfer between MSCs and target cells, such as Hedgehog (Hh)-responsive hepatic stellate cells

(HSCs), and thus alleviate hepatic fibrosis in CCl₄-treated Sprague–Dawley rats by impeding the activation of Hh signaling via the inhibition of Smo expression.¹⁵ Our unpublished data showed that the exosomes produced by adipose tissue-derived MSCs (AD-MSC-122) expressing miR-122 were more effective than were those expressing scramble miRNA or naive exosomes in reducing the proliferation and activation of the human HSC cell line LX2 or primary HSCs from C57BL/6 mice. AD-MSC-122-derived exosomes could transfer miR-122 into HSCs cells and then regulate the expression of miR-122-target genes, such as P4HA1 and IGF1R, which have been shown to be involved in the proliferation and collagen maturation of HSCs.^{60,61} These data indicate that miR-122-modification may improve the therapeutic efficacy of AD-MSCs via exosome-mediated miR-122 delivery, thereby representing a new strategy for treating liver fibrosis.

MSC-derived exosomes for acute liver injury

The therapeutic effects of MSC MVs have been reported in several experimental models of acute kidney, cardiac, neural and lung injury. In acute kidney injury models induced by cisplatin,⁶² glycerol,⁶³ ischemia-reperfusion (I/R),⁶⁴ nephrectomy⁶⁵ and drug toxicity (gentamicin),⁶⁶ MSC exosomes or MVs can improve renal function and reduce the extent of kidney damage. A single administration of MSC exosomes immediately following cisplatin- or I/R-induced acute kidney injury alleviates inflammation, mitigates renal cell apoptosis, and enhances the proliferation of renal epithelial cells.^{10,62} In myocardial I/R injury, the administration of purified MSC exosomes before reperfusion significantly reduces the infarct size and improves the left ventricular function.^{67,68} Further studies have shown that MSC-derived exosomes exert protective effects against myocardial I/R injury possibly through several mechanisms, such as anti-apoptosis, cardiac regeneration, anti-cardiac remodeling, anti-inflammatory effects, neovascularization and anti-vascular remodeling.^{69,70}

However, to date, only a few groups have studied the therapeutic effects of MSC exosomes in acute liver injury.¹³ Tan *et al.* found that HuES9.E1 MSC-derived exosomes elicit hepatoprotective effects both in *in vitro* models of acetaminophen or H₂O₂-induced hepatocyte injury and in a C57BL/6 mouse model of CCl₄-induced acute liver injury, primarily through an increase in hepatocyte proliferation, as demonstrated by elevated proliferating cell nuclear antigen and high cell viability. The increased survival rate is associated with the upregulation of genes involved in the priming phase liver regeneration, which subsequently lead to high expression of proliferation proteins (proliferating cell nuclear antigen and Cyclin D1), the anti-apoptotic gene *Bcl-xL* and the signal transducer and activator of transcription 3 (STAT3). However, the therapeutic effect of MSC-derived exosomes does not occur via the modulation of oxidative stress during hepatic injury.¹³ At the CSH-AASLD & CSH-EASL Joint Scientific Symposiums meeting on Hepatology (Beijing, 2016), we presented our study indicating that transplantation of exosomes released

Table 1 Application of MSC-derived exosomes in liver diseases

MSCs	miRNA/target genes	Animal strain	Disease model	Functions	Ref
hucMSCs	Unknown	Kunming mouse	CCl ₄ -induced liver fibrosis	Inhibit hepatocytes EMT and collagen production through inactivating TGF-β1/Smad pathway	14
CP-MSCs	miR-125b/Smo	Sprague–Dawley rat	CCl ₄ -induced liver fibrosis	Impede Hh signaling activation in HSCs by inhibiting Smo expression	15
AD-MSC-122	miR-122/P4HA1, IGF1R	C57BL/6 mouse	CCl ₄ -induced liver fibrosis	Reduce the proliferation and activation of HSCs more effectively than naïve AD-MSCs	UPD
HuES9.E1 MSCs	Unknown	C57BL/6 mouse	CCl ₄ , APAP or H ₂ O ₂ -induced liver injury	Increase hepatocyte proliferation by upregulating proliferation proteins and anti-apoptosis gene	13
AD-MSCs	Unknown	C57BL/6 mouse	Con A-induced acute liver failure	Reduce serum ALT and AST levels and proinflammatory cytokines production	UPD
AD-MSCs	Unknown	Fischer-344 rat	Rat N1S1 cell-bearing orthotopic HCC model	Suppress HCC by promoting NKT cell anti-tumor responses	16
AD-MSC-122	miR-122/CCNG1, ADAM10, etc.	BALB/c nu/nu mouse	HepG2 cell xenograft nude mice	Sensitize HCC to 5-FU or sorafenib therapy	57

Abbreviations: AD-MSCs, adipose tissue-derived MSCs; AD-MSC-122, miR-122-expressed AD-MSCs; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CP-MSCs, chorionic plate-derived MSCs; HuES9.E1 MSCs, hESC-derived HuES9.E1 MSCs; hucMSCs, human umbilical cord MSCs; UPD, our unpublished data; 5-Fu, 5-fluorouracil.

from AD-MSC can significantly reduce the elevated serum levels of alanine aminotransferase and aspartate aminotransferase, liver inflammation and necrosis in concanavalin A (Con A)-induced hepatitis in C57BL/6 mice as well as the serum levels of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-6, IL-18 and IL-1β, and the inflammasome activation in mouse liver.

MSCs offer an opportunity to treat acute liver injury induced by lipopolysaccharide (LPS),⁷¹ thioacetamide,⁷² I/R,⁷³ and radiation,⁷⁴ and MSC-CM has been shown to exert a protective effect on these models.^{75,76} Thus, we have reason to believe that the transplantation of MSC-derived exosomes may be a novel therapeutic approach for treating various types of acute liver injury.

MSC-derived exosomes for HCC

MSCs have recently gained much attention for their application to tumor therapy because MSCs can mobilize from the bone marrow or other tissues to the tumor microenvironment.^{77,78} Several studies have shown that MSCs can either promote or inhibit tumor progression in different tumor models.^{79–82} However, the mechanisms by which MSCs control tumor cells remain unclear.

MSC-secreted paracrine factors, which are delivered by EVs, have been shown to mediate their effects on tumor progression.⁸³ For example, exosomes that are released from multiple myeloma patient bone marrow (BM)-derived MSCs promote multiple myeloma tumor growth in SCID-beige mice.⁸⁴ MVs derived from human Wharton's jelly MSCs promote renal cancer cell growth and aggressiveness in BALB/c nu/nu mice.⁸⁵ BM-MSC-derived exosomes promote gastric or colon tumor growth in BALB/c nu/nu mice by enhancing the expression of vascular endothelial growth factor in tumor cells,⁸⁶ and they facilitate nasopharyngeal carcinoma progression and migration in non-obese diabetic/severe combined immunodeficient

(NOD/SCID) mice by activating the FGF19-FGFR4-dependent ERK signaling cascade and epithelial–mesenchymal transition.⁸⁷ However, exosomes also exhibit anti-tumor effects. Bruno *et al.*⁸⁸ found that exosomes from human BM-MSCs inhibit the growth and survival of three different human tumor cell lines, and similar results were observed in NOD/SCID mouse models. In another study, mouse BM-MSC-derived exosomes were found to suppress tumor progression and angiogenesis in the mouse breast cancer cell line 4T1 by downregulating the expression of vascular endothelial growth factor *in vitro* and *in vivo* via shuttling miR-16, which is a known effector of vascular endothelial growth factor that is enriched in MSC-derived exosomes.⁸⁹ A different study showed that exosomes derived from menstrual stem cells suppress the secretion of pro-angiogenic factors in prostate tumor cell line PC3 in a reactive oxygen species-dependent manner and inhibit angiogenesis of prostate tumor in PC3-bearing NOD SCID gamma mice.⁹⁰ The different effects of MSC-derived exosomes on tumor growth may be due to the heterogeneity of MSCs. MSCs from different donors or tissue sources may release exosomes containing cell-specific molecules to promote or prevent tumor development. In addition, the different tumor types and *in vivo* tumor models examined and the variation in the way and time exosomes are administered may also influence the effect of exosomes on tumor progression.⁹¹

To date, only a few studies have shown the effect of MSC exosomes on HCC. MVs derived from BM-MSCs inhibit cell cycle progression and induce apoptosis in HepG2 cells. The intra-tumor administration of MVs in established tumors generated by a subcutaneous injection of HepG2 cells in SCID mice significantly inhibits tumor growth.⁸⁸ A recent study showed that AD-MSC-derived exosomes in rat N1S1 cells, which is an orthotopic HCC model, can promote NKT cell anti-tumor responses in rats, thereby facilitating HCC suppression, low-grade tumor differentiation and an increase of

the early apparent diffusion coefficient, which is a measure of the magnitude of water diffusion within the tissue and can be used as an early biomarker of treatment response.¹⁶

In addition to their role in modulating tumor development, MSCs-derived exosomes influence tumor chemosensitivity. Exosomes from human umbilical cord MSCs significantly induce the resistance of gastric cancer cells to 5-fluorouracil in a BALB/c nu/nu mice subcutaneous xenograft tumor model by antagonizing 5-fluorouracil-induced apoptosis by enhancing the expression of multidrug resistance-associated proteins.⁹² In another study, exosomes from anti-miR-9-transfected BM-MSCs delivered anti-miR-9 into temozolomide-resistant glioblastoma multiforme cells and reversed their chemoresistance by affecting the expression of the multidrug transporter P-glycoprotein.⁹³

In one of our previous studies, we found that naïve AD-MSC-derived exosomes have no effect on tumor growth and chemosensitivity in HepG2 cell xenografts in BALB/c nu/nu mice. Because miR-122 has an essential role in increasing the chemosensitivity of HCC cells by targeting CCNG1, ADAM10 and IGF1R, we modified AD-MSCs with miR-122. Exosomes from miR-122-modified AD-MSC (122-Exo) could mediate miR-122 transfer between AD-MSCs and HCC cells, thereby enhancing cell sensitivity to chemotherapeutic agents by regulating miR-122-target gene expression in HCC cells. Moreover, an intra-tumor injection of 122-Exo significantly sensitizes HCC to sorafenib therapy *in vivo*.⁵⁷ The different anti-cancer effects of AD-MSC-derived exosomes on HCC between our study and the study performed by Ko *et al.* in a rat model may be due to differences in the tumor model of HCC investigated. In a previous study, Ko *et al.*¹⁶ used N1S1 rat HCC cells to obtain an orthotopic HCC model in Fischer-344 (F344) rats, which have a normal immune system. They found that AD-MSC-derived exosomes promote NKT cell anti-tumor responses in rats, thereby facilitating HCC suppression. This finding suggests that the anti-cancer effect of AD-MSC-derived exosomes is mainly by promoting anti-cancer immunity. In our study, we used HepG2 human HCC cells to construct a subcutaneous xenograft model in nude mice in which the immune-mediated anti-cancer effect of AD-MSC-derived exosomes could not be observed.

The use of MSC-derived exosomes in cancer therapy must be conducted with caution because their role in tumor growth remains unclear. A better understanding of the mechanisms involved in the regulation of MSC-derived exosomes is important to determine their true role in cancer progression and guide researchers in the development of important therapeutic agents through their specific modification.

MSC-derived exosomes for drug delivery

Advances in biomedical research have generated an increasing number of potential targets for treating liver disease or delaying disease progression. Unfortunately, many of these targets are undruggable because they are intracellular, present in many cell types, poorly soluble or rapidly inactivated. Although pharmaceutical drug vehicles (for example, synthetic lipid

and nanoparticles), have successfully circumvented many of these problems, exosomes, as a mimic of 'nature's delivery systems,' are highly attractive as a potential alternative to deliver drugs to target cells via membrane fusion or endocytosis. Given the problems associated with many of the current nanoparticulate delivery systems, exosomes can avoid phagocytosis or degradation by macrophages and also circulate for extended time periods within the body because of nature's own cellular product. Unlike typical nanoparticulate systems such as liposomes or polymeric nanoparticles, exosomes can potentially avoid the endosomal pathway and lysosomal degradation, and deliver cargoes directly into the cytoplasm. By avoiding the endosomal pathway, the transfection efficiency for molecules such as siRNA can be enhanced.⁹⁴ Moreover, exosomes have other features of an ideal drug delivery vehicle. First, the presence of proteins and RNAs in exosomes indicates that they can be loaded with such biological materials.⁹⁵ Second, exosomes can penetrate the blood-brain barrier,⁹⁶ which has proven to be highly impenetrable to many drugs. Third, exosomes are naturally stable and have inherent targeting properties depending on their composition.^{97,98} Finally, exosomes are amenable to membrane modifications that enhance cell type-specific targeting.^{99,100}

Among the cell types that are known to produce exosomes, MSC are the ideal candidate for the mass production of exosomes for drug delivery. At present, MSC-derived exosomes have been used as drug delivery vehicles in tumor therapy and regenerative medicine in some studies.^{101,102} Upon *in vitro* exposure to high concentrations of paclitaxel (PTX), BM-MSCs can package and deliver this active drug through their exosomes. The PTX-loaded exosomes acquire strong anti-tumor effects on human pancreatic adenocarcinoma (CFPAC-1), *in vitro*.¹⁰³ Exosomes from BM-MSCs overexpressing miR-146b or miR-143 can significantly reduce the growth of glioma xenografts in a Fischer rat model or the migration of osteosarcoma cells *in vitro*, through delivery of these anti-tumor miRNAs into the target tissue or cells, respectively.^{104,105} BM-MSCs loaded with anti-miR-9 can transfer this anti-miR into co-cultured-glioblastoma cells via secreted exosomes, thereby conferring temozolomide chemosensitivity.⁹³

In addition to their use in tumor therapy or chemotherapy, MSC exosome-shuttled therapeutic materials have been used in regenerative medicine. CXCR4-enriched exosomes released from CXCR4-overexpressing rat BM-MSCs can protect cardiomyocytes from ischemic injury both *in vitro* and in a rat model of myocardial infarction. Upregulation of the Akt signaling pathway contributes to these beneficial effects, suggesting that CXCR4-enriched exosomes may serve as an additional therapeutic strategy to promote cell survival and angiogenesis in ischemic hearts following myocardial infarction.¹⁰⁶

To date, there are few studies on the use of MSC-derived exosomes for drug delivery in liver diseases. Because specifically modified MSCs and their conditioned media show better therapeutic efficacy on liver diseases^{1,107}, and because exosomes that contain therapeutic molecules can be mass produced from

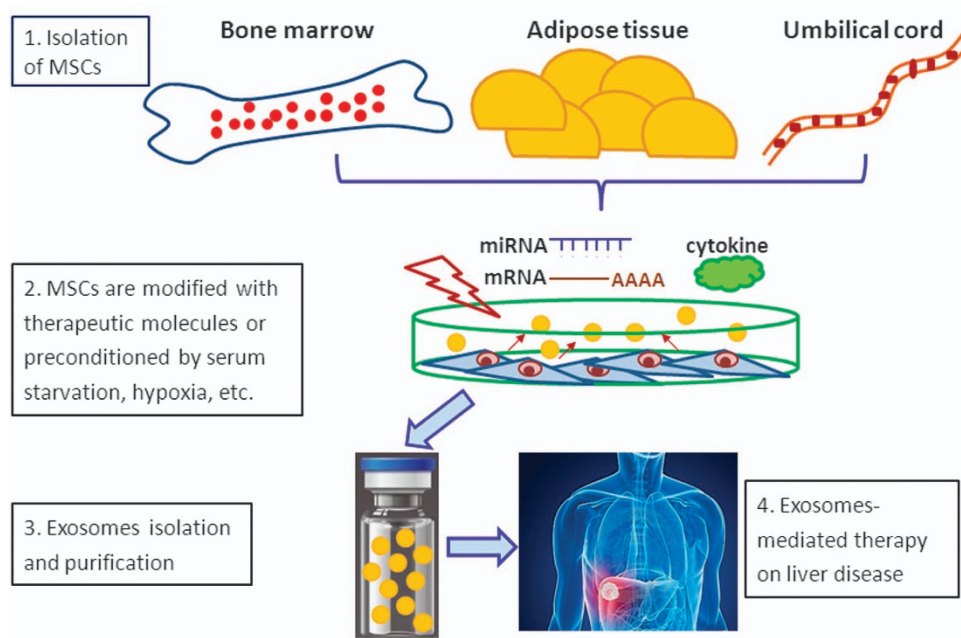


Figure 1 MSC-derived exosomes represent an attractive therapeutic approach for treating liver diseases. Step 1: MSCs are isolated from bone marrow, adipose tissue and umbilical cord. Step 2: MSCs are modified with therapeutic molecules (i.e., mRNA, miRNAs and cytokines) or preconditioned by serum starvation, hypoxia and pharmacological and physical stimulation. Step 3: Exosomes loading therapeutic molecules are isolated from MSC-conditioned media, purified, characterized and quantified. Step 4: Administration of exosomes via veins (i.e., portal vein) to treat liver diseases, including acute liver injury, liver fibrosis and HCC. MSCs, mesenchymal stem cells; HCC, hepatocellular carcinoma.

MSCs, exosome-mediated drug delivery potentially engenders a novel approach for treating liver diseases such as hepatitis, liver fibrosis and HCC.

CONCLUSIONS AND PROSPECTS

As stromal support cells, MSCs target housekeeping processes through their secreted exosomes to restore liver homeostasis and enable hepatocytes to recover, repair and regenerate. This observation provides the rationale for the therapeutic efficacy of MSCs and their secreted exosomes in a variety of liver diseases. Furthermore, by maintaining the therapeutic advantages of MSCs without the risk of iatrogenic tumor formation or of pulmonary embolisms due to intravenous administration, MSC-derived exosomes represent a highly attractive therapeutic approach for treating inflammatory liver diseases and HCC. Such exosomes also can be used as an adjuvant to support and complement other therapeutic modalities (Figure 1).

However, questions about the characterization, potency and quantification of MSC exosomes must be addressed before their clinical use. On the basis of preclinical studies, the amount of MSC exosomes needed to generate an equivalent effect as MSCs in tissue injury is roughly higher.⁷ Although MSCs are relatively easy to expand *in vitro*, their growth in culture is finite. Both new batches of MSCs, through immortalization and expansion by bioreactors, and more effective techniques for large-scale exosome production need to be developed. Moreover, there is still no gold standard to characterize MSC exosomes. Because MSCs are heterogeneous,

the exosomes isolated from them are also heterogeneous, and this heterogeneity may cause different effects on their target cells.^{84,108} In addition, the methods used to precondition MSC in stimulating exosome release, such as serum starvation, hypoxia and inflammation, change the surface and intracellular content of exosomes. Thus, to significantly advance exosome-mediated therapy in clinical trials, effective methods that maintain the homogeneity of MSC-derived exosomes, involve robust and standardized characterization of MSC-derived exosomes, and include standardized safety data from preclinical animal models need to be developed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Fund (81000730 and 81201782), and the Key R&D projects of the Zhejiang province (2015C03045).

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