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Review Article

Role of Exosomal Modulation of Macrophages in Liver Fibrosis

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Abstract

Exosomes are 60–120 nm diameter double-membrane lipid organelles discharged by cells. Various studies have shown that exosomes exert multiple functions in both physical and diseased processes, such as intercellular information exchange, immune response, and disease progression. Repeated chronic injury to the liver often leads to inflammation and liver fibrosis (LF), a disorder that, if unchecked, may progress to cirrhosis, liver failure, portal hypertension, and even hepatocellular carcinoma. As an essential component of host innate immunity against pathogen invasion, macrophages play an important role in modulating inflammation homeostasis by finely tuning the polarization process of macrophages into either M1 or M2 subtypes in response to different microenvironments. As a critical contributor to the inflammation process, macrophages also play a complex and instrumental function in the progression of LF. This review focuses on recent advancements in the role of macrophage-associated exosomes implicated in LF, including macrophage-released exosomes and macrophage-targeted exosomes. In addition, the progress made in exosome-based antifibrotic therapy by *in vivo* and *in vitro* studies is also highlighted.

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Keywords: Exosome; Macrophage; Liver fibrosis; Hepatocyte; Mesenchymal stem cell; Hepatic stellate cell.

Abbreviations: CAMSAP1, calmodulin-regulated spectral-associated protein 1; CLD, clodronate; CTSB, cathepsin B; ECM, extracellular matrix; EDA, ectodysplasin-A; EV, extracellular vesicle; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; KC, Kupffer cell; KLF, Krüppel-like factor; LF, liver fibrosis; LPS, lipopolysaccharide; LSC, liver stem cell; miRNA, microRNA; MSC, mesenchymal stem cell; NIN, nintedanib; ODN, oligonucleotide; RHM, recruited hepatic macrophage; SMAP-5, smooth muscle cell-associated protein-5; TAZ, transcriptional activator with PDZ-binding; TGF-β, transforming growth factor-beta.

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Introduction

Exosomes are extracellular vesicle (EVs) originating from endosomes, with an average size of approximately 100 nm in diameter. While they contribute to cellular homeostasis by eliminating unnecessary components from cells, they can also affect the function and behavior of recipient cells.[1] As an intercellular material and information transport system, exosomes play an essential role in numerous biological and diseased processes, including metabolism, immune responses,[2] infections,[3,4] cardiovascular diseases,[5,6] and cancers.[7,8] Various components such as nucleic acids, proteins, lipids, and metabolites wrapped in exosomes reflect changes in cells or tissues, making exosomes valuable in disease diagnosis and progression prediction through liquid biopsies.[9] In addition, they have also shown promising potential in drug delivery,[10] immunotherapy,[11] and vaccine development.[12] Exosomes have a key role in diagnosing and treating liver diseases.[13]

Liver fibrosis (LF) is a typical consequence of permanent liver damage, and advanced LF, particularly end-stage cirrhosis, is one of the primary reasons for liver-related mortality worldwide.[14] In the progression of LF, repetitive or chronic injury to hepatocytes leads to persistent and excessive inflammation, which may contribute to self-limiting tissue repair processes. However, dysregulated imbalanced inflammatory response aggravates liver injury, leading to the progression of LF.[15,16] It has been generally agreed that increased production and storage of intractable collagen and extracellular matrix (ECM) components induce liver fibrogenesis following chronic persistent liver injuries.[17] Many chronic liver diseases, such as hepatitis B virus, hepatitis C virus infection, alcoholic liver disease, and nonalcoholic steatohepatitis, contribute to LF.[18] Activation of myofibroblasts, which originate from fibroblasts such as hepatic stellate cells (HSCs), portal fibroblasts, and fibroblasts, is one of the typical characteristics of LF. Prolonged injury causes fibroblast proliferation and acquisition of the myofibroblast phenotype due to the extracellular tension induced by various ECMs (e.g., fibronectin and collagen types I and III).[19] Activated myofibroblasts secrete ECM and form stress-related connections that promote further ECM remodeling.[20] Furthermore, uncontrolled ECM deposition and substantial alterations in the topographical distribution of ECM components lead to increased expression of tissue inhibitor of matrix metalloproteinases (referred to

TIMPs) to stimulate liver fibrogenesis.[21,22] However, LF can be reversed if the underlying etiology is removed and the injury heals.

Hepatic macrophages, including resident macrophages (Kupffer cells, KCs) located on the surface of the endothelial sinus lumen or in the per sinus space, as well as macrophages derived from monocytes that infiltrate into the liver, are the primary regulators in this inflammation cascade.[23,24] Crosstalk among macrophages, HSCs, and hepatocytes in the liver has long been the focus of LF. Activated hepatic macrophages and mast cells contribute to the inflammatory process by producing pro-inflammatory mediators and attracting monocytes, neutrophils, lymphocytes, and eosinophils to the liver. While short-term inflammation benefits tissue regeneration and repair after liver injury or triggers the host immune response to defend pathogen infection, excessive and persistent inflammation activates HSCs to become myofibroblasts, which produce large amounts of ECM, leading to the formation of LF. As deliverers of substances and host genetic information, exosomes are undoubtedly involved in the complex regulation network of LF. This review emphasizes the roles of macrophage-targeted and macrophage-released exosomes in LF.

Origin of exosomes and their functions

Exosomes, also called microvesicles, ectosomes, migratory bodies, apoptotic bodies, or tumor bodies, are named based on the shape and size of EVs.[25–28] Exosomes can be small (60–80 nm) or large (90–120 nm).[29,30] Small exosomes contain proteins associated with endosomes and phagocytic vesicles derived from the endosomal compartment. Large exosomes consist of plasma membrane proteins, cell-linked proteins, and late endosomal proteins, suggesting that they may be derived from the plasma membrane and represent atypical exosomes.[31] The formation of exosomes starts with the penetration of the cell membrane, which leads to the formation of endosomes, followed by the transition into multivesicular bodies. These multivesicular bodies can fuse with lysosomes immediately, be transported to the Golgi for recycling, or fuse with cell membranes to form tiny vesicles released outside the cells, creating exosomes.

Exosomes consist of a shell and cargo. Because exosomes are secreted by cells, their shell is analogous to the membrane of the cells from which they are derived and equipped with additional signal recognition, stability, and other function-related molecules.[32] The cargo carried by exosomes consists of RNAs, lipids, and proteins to define their function.[33] Exosomal RNAs, such as mRNAs, microRNAs (miRNAs), lncRNAs, and circRNAs, play essential roles in regulating target cells. Currently, miRNAs are the most intensively investigated class of exosomal RNAs, primarily because of their significance in the emergence and development of various diseases.[34] Studies on the mechanisms of exosome-mediated intercellular communication have been conducted, with the surface signaling hypothesis being the most recognized.[35] This approach necessitates connections between target receptors on recipient cells and exosomal membrane proteins, such as tetrameric proteins.[36] Because they activate functional molecules that activate several signaling pathways, these interactions are essential for cellular communication.[37]

Exosomes exert various functions in peripheral circulation.[38] (1) They function as "messengers" between cells, facilitating signal transduction and playing an essential role in initiating host immunity against invading pathogens. (2) Exosomes can be used as a targeted delivery system for pharmaceuticals. These tiny vesicles can deliver medications to specific organs, providing a potential solution for treating a wide range of diseases.[39,40] (3) Exosomes have a role in immune system control. They can alter the immune response, convey immunomodulatory chemicals and antigens, and promote immune cell communication.[35] (4) Exosomes in the peripheral circulation have been looked at as possible biomarkers for several illnesses, including cancer, cardiovascular disease, and neurological disorders. The state of the cells from which they come may be reflected in their contents.[37]

The role of exosomes in the onset and aggravation of LF has drawn much attention recently. It has been suggested that exosomes produced by cells such as mesenchymal stem cells (MSCs), HSCs, and other cells contribute to the development of LF. For instance, MSC-derived exosomes enriched with miR-181-5P or miR-486-5P have been shown to suppress the activation of HSCs to prevent the onset of LF.[41,42] Exosomes from HSCs, on the other hand, are abundant in cellular communication network factor 2, which promotes the progression of LF.[43]

Macrophages in the liver and their role in LF

Activation of macrophages plays an essential role in tissue remodeling and homeostasis, inflammatory responses, and immune defense.[44] Macrophages are present in nearly all tissues and organs in our body, and a significant amount of heterogeneity exists among macrophages due to different environments where they are present or disease states.[45] Alarmingly, macrophages in the liver account for approximately 90% of all macrophages in the human body, and these macrophages consist primarily of KCs perpetually residing in the hepatic sinusoids and peritoneal- and bone marrow-derived monocyte macrophages.[46] Macrophages involve diverse pathophysiological processes, such as inflammation, tumor formation, tissue repair, and metabolism.[47] Under normal conditions, most liver macrophages are KCs, and their role is maintaining the homeostasis of liver. However, when liver lesions occur, KCs are the first to respond. They differentiate into various phenotypes to produce inflammatory or anti-inflammatory factors and recruit extrahepatic macrophages into the liver, initiating inflammation or tissue repair.

Triggering of macrophages in response to stimulation by infectious agents, inflammatory responses, cytokines, or specific physicochemical factors is referred to as macrophage polarization.[38,48] Briefly, macrophages can be polarized into two distinct phenotypes responding to stimuli: classically activated M1 and alternatively activated M2.[49,50] M1 macrophages predominantly trigger the production of inducible nitric oxide synthase and exert pro-inflammatory action and antitumor effects by releasing various pro-inflammatory cytokines, such as IL1 and IL6.[51,52] Therefore, M1 macrophages are essential in immune response and surveillance since they are involved in antigen presentation and secretion of proinflammatory cytokines. On the other hand, M2 macrophages function in anti-inflammatory response and cancer development through the secretion of IL-10, transforming growth factor (TGF) β, and VEGF,[51,52] playing a significant role in the immune surveillance.[53]

Previous studies have elegantly demonstrated that multiple signaling pathways intricately regulate the macrophage polarization process. TLR4/NF-κB signaling pathway was reportedly involved in the M1 polarization, producing pro-inflammatory factors.[54] In contrast, JAK/STAT6 is one of the crucial pathways involved in the induction of M2 polarization. In addition, STAT3 is also essential for M2 polarization, because inhibiting either the IL-6/STAT3 or the JAK3/STAT3

signaling pathway leads to the repolarization of macrophages from the M2 to M1 phenotype.[55]

Various studies have shown that miRNAs could affect macrophage polarization. miR-30c, miR-99a, and miR-155 inhibit M1 polarization, while miR-let7 and miR-32 stimulate M2 polarization.[56] miRNAs participate in the macrophage polarization process by interacting with host signaling pathways.[57] To take an example, miR-221-3p promotes M1 polarization by inhibiting the JAK3/STAT3 signaling pathway,[58] and miR-1246 facilitates M2 polarization by inhibiting NF-κB and targeting TERF2IP to activate STAT3.[59] Similarly, miR-34a-containing exosomes derived from adipocytes inhibit the expression of Krüppel-like factor (KLF) 4 to suppress M2 polarization.[60] In contrast, miR-124-3p-contaning exosomes derived from MSCs promoted M2 macrophage polarization.[61] It has also been reported that Notch signaling controls macrophage polarization via miRNAs. Li *et al*.[62] observed an increased expression of miR-125a/miR-99b leading to the activation of Notch signaling and M1 polarization. A recent study demonstrated that Notch-induced M1 polarization is mediated by miR-148a-3p.[63] Additionally, EVs derived from adipose stem cells blocked the Notch signaling pathway and M1 polarization to exhibit their anti-inflammatory effects, correlated with the reduced mir148a-3p expression.[64] Not surprisingly, miRNAs wrapped in macrophage-derived exosomes may mediate the biological function of these exosomes. It has been reported that M2 macrophage-derived exosomes stimulated the progression of pancreatic ductal adenocarcinoma via the inhibitory effect of mir-501-3p on TGFBR3 and the suppressed TLR4/NF-κB/NLRP3 signaling through the downregulation of TXNIP expression by miR-148a.[65]

Macrophage-derived exosomes and their roles in LF

Exosomes have been reported to be able to mediate cell-to-cell communication in many cells, including macrophages. On one hand, exosomes affect the polarization and phenotype of macrophages, which determine their roles in pathological and physiological processes. For instance, tumor-derived exosomes have been reported to modulate macrophage polarization, thereby affecting cancer progression.[66] Exosomes derived from MSCs induce macrophages toward M2 differentiation, exerting anti-inflammatory and immunomodulatory properties.[67] On the other hand, exosomes also provide an innovative mechanism for regulating the functions of recipient cells. M2 macrophage-derived exosomal miRNAs are believed to contribute to pulmonary fibrosis.[68] And tumor-associated macrophages promote tumor proliferation and immunosuppression through exosomal lncRNAs.[69] Macrophage-HSC interactions play an essential role in the progression of LF, and exosomes are heavily involved in this process. Macrophages not only induce activation but also directly regulate the behaviors of HSCs in the advance of liver fibrosis;[61,70] and in the reversal of LF, macrophages promote HSCs apoptosis.[71] The activated HSCs could, in turn, accelerate macrophage infiltration and drive M2 polarization.[72] Moreover, liver macrophages secret cytokines to create an inflammatory environment, which impairs hepatocyte survival and further aggravates fibrosis.[73,74]

Macrophage-derived exosomes in the inhibition of LF

The role of hepatic macrophages in the progression of liver inflammation and fibrosis has been extensively explored. In recent decades, many studies have focused on inhibiting LF by targeting activated hepatic macrophages and their interaction with hepatocytes and HSCs via exosomes. Activation of macrophages is typically thought to play an essential role in the development of LF; however, many studies conducted in recent years have demonstrated that certain exosomes released by macrophages play a crucial role in preventing the development of LF. One study showed that IL-6-treated macrophages produce more miR-223-rich exosomes to inhibit the expression of profibrotic transcriptional activator with PDZ-binding (referred to herein as TAZ) motif in hepatocytes,[75] leading to the inhibition of LF progression. Another study shows that miR-690 in KCs-derived exosomes affects different types of cells in the liver, including hepatocytes, recruited hepatic macrophages (referred to herein as RHM), and HSCs. Specifically, miR-690 directly suppresses signaling linked to the progression of LF in HSCs, reduces inflammation in RHM, and restores the normal function of hepatocytes.[76] Activation of HSCs contributes to LF, and blocking HSC activation has been shown to alleviate fibrosis progression. Exosomes produced by M2 macrophages blocked the activation of HSCs significantly. More specifically, exosomal miR-411-5p from M2 macrophages blocks HSCs activation. Mechanistically, miR-411-5p in macrophage-derived exosomes has been shown to suppress the expression of calmodulin-regulated spectral-associated protein 1 (referred to herein as CAMSAP1) in HSCs, preventing them from being activated.[77]

In recognition of the potential role of macrophage-derived exosomes in inhibiting the progression of LF, an increasing number of studies are investigating them as drug targets for treating LFs. Owing to their ability to overcome physiological barriers and serve as effective vehicles for drugs and gene therapy, exosomes have emerged as novel treatment options for diseases such as LF, raising the possibility of "cell therapy without cells." For example, macrophages can bind to relaxin, an antifibrotic peptide hormone, and upon binding, they transform from a profibrogenic to a proresolution phenotype. Exosomes carrying miR-30a-5p are released by macrophages with the proresolution phenotype, and these exosomes regulate the quiescence of activated HSCs.[78] Based on this discovery, scientists created an innovative lipid nanoparticle that contains miR-30a-5p and relaxin gene mimics to selectively target activated HSCs in fibrotic livers, which has been proven to be effective *in vivo* antifibrotic treatment in mice.[78] Another research demonstrated that exosomes from M2 macrophages stimulated the activation of HSCs. However, treatment with vitamin D receptor, a protein linked to macrophage polarization, changed these exosomes' protein composition and reversed this promoting impact. Based on this discovery, researchers created a new LF treatment that combines a vitamin D receptor agonist with a macrophage-targeting exosome secretion inhibitor. The efficacy of this treatment has now been confirmed.[79] Phillygenin, the active ingredient in a traditional Chinese drug called forsythia, has been found to inhibit HSCs activation and exerts significant anti-inflammatory effects. It has been reported that phillygenin inhibited M1 macrophage polarization by downregulating the JAK1/JAK2-STAT1 and Notch1 signaling pathways and reducing HSCs activation by inhibiting miR-125b-5p in macrophage exosomes that target Stard13.[80]

Macrophage-derived exosomes in the promotion of LF

Macrophages exhibit distinct properties and perform various functions within an organism's intricate microenvironment. For example, macrophages are involved in the progression of LF by producing matrix metalloproteinases, such as MMP9, which actively degrade the basement membrane. This degradation facilitates the infiltration of immune cells and recruits fibroblasts into the site of injury. *In vivo*, a close association was observed between macrophages and collagen-producing myofibroblasts.[81] In addition, macrophages are crucial

Table 1. Roles of macrophage-derived exosomes in LF

Origin	Target	Exosome component	Pathway/mediator	Effect	References
Macrophages	Hepatocytes	miR-223	Inhibit TAZ	Inhibit LF	75
KCs	Hepatocytes; RHM; HSCs	miR-690	Restores normal function	Inhibit LF	76
M2 macrophages	HSCs	miR-411-5p	Inhibit CAMSAP1	Inhibit LF	77
Macrophages	HSCs	miR-30a-5p	Bind to relaxin	Inhibit LF	78
M1 macrophages	HSCs	miR-125b-5p	Boost Notch1 pathways	Promote LF	80
LPS-treated macrophages	HSCs	miR-500	Inhibit MFN2	Promote LF	83
LPS-treated macrophages	HSCs	miR-155-5p	Inhibit SOCS1	Promote LF	84
LPS-treated macrophages	HSCs	miR-103-3p	Inhibit KLF4	Promote LF	85
M2 macrophages	HSCs	SMAP-5	Regulate autophagic flux	Promote LF	79

CAMSAP1, calmodulin-regulated spectral-associated protein 1; HSC, hepatic stellate cell; KC, Kupffer cell; KLF4, Krüppel-like factor 4; LF, liver fibrosis; LPS, lipopolysaccharide; MFN2, mitofusin-2; RHM, recruited hepatic macrophage; SOCS1, suppressor of cytokine signaling 1; TAZ, transcriptional activator with PDZ-binding.

in regulating myofibroblast activity by secreting cytokines and growth factors. Different subtypes of macrophages are recruited during hepatic fibrogenesis, ultimately leading to pathological scars or epithelialization.[82]

Increasing evidence suggests that macrophage-derived exosomes contribute to the progression of LF and may also compromise the effectiveness of medical drugs. Studies have shown that exosomes from macrophages treated with lipopolysaccharides (LPSs) have higher expression levels of miR-500, accelerating the activation of HSCs and fibrogenesis by inhibiting MFN2.[83] The authors further implied the significant potential of miR-500 in serum exosomes as a diagnostic for LF. Another study showed that miR-155-5p was increased in the released exosomes from macrophages, such as THP-1, treated with LPS.[84] After coculturing LPS-stimulated THP-1 with HSCs cells, the proliferative and migratory abilities, oxidative stress levels, and expression of fibrosis markers (e.g., collagen type I) of HSC cells were significantly enhanced.[84] The authors concluded that miR-155-5p in the exosomes suppressed the expression of SOCS1 in the HSCs cells and further promoted the expression of p-Smad2/3, Smad2/3, and RhoA protein expression, facilitating the formation of LF. In another independent study, LPS was found to alter the miRNA profile of THP-1-derived exosomes. Specifically, miR-103-3p in exosomes has been found to promote the growth and activation of HSCs by targeting KLF4. Interaction between THP-1 macrophages and HSCs may also play a crucial role in the progression of LF.[85] Further study demonstrated that combining total astragaloside and glycyrrhetinic acid could effectively inhibit HSCs activation triggered by LPS-stimulated macrophage-derived exosomes.[86] This inhibitory effect may be achieved by inhibiting the phosphorylation of Smad2 and Smad3 in the TGF-1/SMAD signaling pathway in HSCs. Although M2 macrophages blunt the immune response, M2 macrophage-derived exosomes promote LF by activating HSCs. For example, highly potent smooth muscle cell-associated protein-5 (referred to herein as SMAP-5), which is assumed to be a crucial effector protein that activates HSCs by regulating autophagic flux, is found in exosomes released by M2 macrophages.[79]

In conclusion, increasing numbers of studies have shown that exosomes released from macrophages regulate the development of LF (Table 1).[75–80,83–85] However, there is still a great deal of ground to cover here for the following reasons: On one hand, macrophages have a role in LF primarily through the release of regulatory cytokines and chemokines, the modification of HSCs activity, and the recruitment of extrahepatic macrophages. Exosomes make up a small part of the treatment methods discovered in this investigation. On the other hand, it is challenging to identify and elucidate the mechanisms by which macrophages regulate LF via exosomes, a term more commonly used in molecular biology and bioinformatics. The same cell subtype may have two opposing effects on LF since macrophages are a very diverse cell population. For instance, SMAP-5 in exosomes produced by M2 macrophages promotes LF, whereas miR-411-5p prevents the development of hepatic fibrosis.

Exosomes targeting macrophages and their roles in LF

After we summarized the role of macrophage-derived exosomes in the progression of LF in the previous section, we moved on to look at those exosomes that target macrophages and their function in

Exosomes targeting macrophages to inhibit LF

MSC-derived exosomes: MSCs, a type of embryonic stem cell, have gained attention because of their potential for the treatment of LF.[87] Studies have shown that MSCs-derived exosomes can enhance liver health by attenuating LF progression, inflammation, and collagen deposition. These exosomes have been found to inhibit TGF-1/SMAD signaling to inhibit LF and stimulate hepatocyte proliferation, leading to a protective effect on hepatocytes.[85,88] Similar anti-LF and hepatoprotective and regenerative effects were also observed with exosomes from various sources of MSCs, including bmMSCs,[89,90] hucMSCs,[91,92] hIMSCs,[93] induced pluripotent MSCs,[94] heMSCs,[95] blood-derived MSCs,[96] and adipose-derived MSCs of adipose origin.[97] Numerous studies have linked the anti-LF impact of macrophages to the exosomes derived from MSCs. It has been reported that exosomes isolated from MSCs inhibit LF by releasing miR-148a, which controls intrahepatic macrophage activity via KLF3/STAT3 signaling.[98] This finding identifies a potential treatment option for LF. MSCs and their exosomes mitigate both short- and long-term liver injury by promoting macrophages toward M2 polarization, leading to the activation of Treg/Breg cells and immune suppression. while the development and maturation of T cells, B cells, DCs, and NK cells were inhibited.[99]

MSCs have been demonstrated to be a promising treat-

ment option for many hard-to-cure diseases, and there is accumulating evidence that exosomes mediate their therapeutic effects. MSC-derived exosomes can deliver anti-inflammatory cytokines and other bioactive proteins to a damaged liver without additional auxiliary cells. In a mouse LF model induced by CCl_4, for example, infusion of exosomes derived from human umbilical cord MSCs effectively inhibited collagen production, alleviating LF.[100] Furthermore, exosomes injected into mice were found to migrate to the liver and block the TGF-1/Smad pathway, decreasing levels of type I/III collagen and TGF-1 in these mice.[100]

Hepatocyte-derived exosomes: Although the vast majority of research indicates that hepatocytes are destroyed during the progression of LF, their role as fibrosis inhibitors is still under investigation. The effects of ezetimibe, a prescription drug commonly used to treat hypercholesterolemia, on liver cells and its therapeutic potential in LF were investigated. Ezetimibe was found to stimulate hepatocyte-derived exosome secretion, and these exosomes modulate hepatocyte-macrophage interaction by directly targeting macrophages. In addition, these exosomes inhibited the inflammatory response of macrophages by inhibiting the NLRP3 inflammasome-IL1β pathway in an autophagy-dependent manner and activating AMPK and TFEB nuclear translocation.[101] In another study, scientists examined changes in exosomes produced by liver stem cells in a CCl_4-induced fibrosis model, and they identified the increased expression of miR-142a-5p, which prevents macrophages toward M1 polarization and facilitated the production of M2 markers through targeting cathepsin B to suppress LF in mice.[102]

Artificial exosome system: Artificial exosomes have been explored to treat LF in recent decades. Contemporary scholarly investigations are mainly concentrated on utilizing unaltered or modified exosomes as an innovative approach for drug administration, explicitly targeting the liver to deliver antifibrotic medications with precision. The present technique is undergoing extensive investigation due to its inherent benefits in terms of heightened specificity and enhanced delivery efficiency compared to conventional medication delivery methods. In the following discourse, we have chosen several artificial exosome systems that specifically target macrophages for examination.

It has been shown that macrophage activation and plasticity are correlated with Notch signaling, which is mediated by the transcription factor RBP-J, and that myeloid-specific modulation of RBP-J expression reduces LF in rats. To target the transcription factor RBP-J, scientists created hairpin decoy oligonucleotides (ODNs) and electroporated ODNs into exosomes produced from the HEK293T cells. It has been shown that exosome-delivered RBP-J-baited ODNs successfully suppressed Notch signaling in macrophages and alleviated LF in CCl_4 or bile duct ligation mouse model. Further research into this technique as a brand-new exosome-delivered innovative treatment is under intensive investigation. And these artificial exosomes were primarily taken up by hepatic macrophages.[103] Similarly, a different research team created the LIVE hybrid exosome-liposome drug delivery system, successfully delivering clodronate (referred to herein as CLD) and nintedanib (referred to herein as NIN) to the liver tissues. Combining the inhibitory effects of NIN on liver fibroblasts and CLD on KCs, this artificial exosomal delivery system showed an enhanced antifibrotic effect in the CCl_4-induced mouse model.[104] Furthermore, researchers have used optically reversible protein-protein interactions to load effector proteins into exosomes. For example, Exo-srIB inhibits NF-κB signaling in KCs to reduce LF through exosomes.[105] They successfully delivered exosomes loaded with the super suppressor IB (Exo-srIB) to the liver using a novel optogenetically engineered exosome technology called "exosomes loaded with proteins via optically reversible protein-protein interactions". Exo-srIB markedly reduced the levels of fibrosis-associated gene expression in HSCs following three-day injections.

Exosomes targeting macrophages to promote LF

Hepatocytes-derived exosomes: Damaged hepatocytes contribute to the progression of hepatic fibrosis by releasing signals to stimulate macrophage infiltration and HSC activation. It has been generally agreed that exosomes play an essential role in this process. For example, lipid activation of DR5 stimulated hepatocytes to produce exosomes, which in turn triggered a macrophage-mediated inflammatory phenotype.[106] Release of exosomes was inhibited by blocking DR5 signaling or rho-related coiled-coil protein kinase 1.[106] In addition, hepatocyte-derived exosomes contained TNF-associated apoptosis-inducing ligands, which also stimulated mouse bone marrow-derived macrophages to produce inflammatory cytokines, such as IL1 and IL6, to maintain the inflammation microenvironment in the liver to promote LF.[106] Furthermore, excessive buildup of fatty acids stimulated hepatocytes to produce excessive exosomes, facilitating liver fibrogenesis by activating surrounding HSCs and macrophages.[107] By inhibiting the Rictor/Akt/FoxO1 signaling pathway, miR-192-5p-rich exosomes released from hepatocytes were found to activate pro-inflammatory macrophages.[108] miR-192-5p was also found to increase vascular endothelial inflammation and vascular permeability and activate the NF-κB signaling pathway and NLRP3 to stimulate LF.[109] miR-155 has been implicated in the high-fat, high-cholesterol, high-sugar diet-induced steatosis and LF, and it is found in exosomes released by hepatocytes.[110] MiR-155 knockout mice had considerably less liver damage, decreased steatosis, and attenuated fibrosis when given a high-fat, high-cholesterol, high-sugar diet or CCl_4 treatment in a fibrosis paradigm. However, KCs extracted from miR-155 KO mice displayed an M2 phenotype even when exposed to M1-stimulated environment.[111] Furthermore, elevated levels of miR21 and Arg1 in hepatocyte-derived exosomes led to increased hepatic fibrosis and enhanced M2 polarization in mice.[112]

HSC-derived exosomes: The role of HSCs in the development of LF is believed to be mediated by macrophages. However, the relationship between HSCs activation and macrophage polarization has yet to be fully described. One study analyzed differential mRNAs expression using exosomal RNA sequencing from activated Lx-2 cells and a DHFR loss-of-function Lx-2 cell model. They found DHFR-containing exosomes are essential in the M1 polarization of macrophages.[113] Another study demonstrated that exosomes from activated HSCs stimulated mouse and human macrophages to produce IL-6 and TNFα, and promoted macrophage migration.[114] In addition, exosomes secreted by activated HSCs increased glycolysis in quiescent HSCs and KCs,[115] indicating they were activated.[116] It was also shown that ectodysplasin-A mRNA plays a significant role in the exosomes produced by HSCs to control macrophage activity, which stimulates the production of IL-6 and TNF-α and improves macrophage migration.[116] Generally speaking, in the progression of LF, macrophages are the core hub of the regulation network (Table 2).[98,100–106,109,110,112,117]

Conclusions and perspective

Present investigations have revealed the significant role of

Table 2. Roles of exosomes targeting macrophages in LF

Origin	Target	Exosome component	Pathway/mediator	Effect	References
MSCs	Macrophages	miR-148a	KLF3/STAT3 signaling	Inhibit LF	98
hucMSCs	Macrophages	Unknown	Inhibit TGF-1/Smad pathway	Inhibit LF	100
Ezetimibe-treated hepatocytes	Macrophages	Unknown	Inhibit NLRP3 Inflammatory vesicles	Inhibit LF	101
LSCs	Macrophages	miR-142a-5p	Inhibit CTSB	Inhibit LF	102
Artificial exosomes	Macrophages	RBP-J-ODNs	Inhibit Notch signaling	Inhibit LF	103
Artificial exosomes	Macrophages	CLD and NIN	Regulate liver fibroblasts/KCs	Inhibit LF	104
Artificial exosomes	Macrophages	Exo-srIB	Inhibit fibrosis-associated gene	Inhibit LF	105
Hepatocytes	Macrophages	TNF-associated apoptosis-inducing ligands	Produce inflammatory cytokines	Promote LF	106
Hepatocytes	Macrophages	miR-192-5p	Rictor/Akt/FoxO1 pathway	Promote LF	109
Hepatocytes	Macrophages	miR-155	M1 polarization	Promote LF	110
Hepatocytes	Macrophages	miR-21 and Arg-1	M1 polarization	Promote LF	112
HSCs	Macrophages	EDA mRNA	Improves macrophage migration	Promote LF	117

Arg-1, arginase-1; CLD, clodronate; CTSB, cathepsin B; EDA, ectodysplasin-A; HSCs, hepatic stellate cells; hucMSCs, human umbilical cord MSCs; KC, Kupffer cell; LF, liver fibrosis; LSCs, liver stem cells; MSCs, mesenchymal stem cells; NIN, nintedanib; RBP-J-ODNs, RBP-J hairpin decoy oligonucleotides.

exosomes and macrophages in LF. Figure 1 depicts exosomes as messengers between macrophages, hepatocytes, KCs and MSCs. It is interesting to note that, according to the currently available data, hepatocyte-derived exosomes that target macrophages primarily have a promoting influence on LF. In contrast, macrophage-derived exosomes that target hepatocytes primarily have an inhibiting effect. Various exosome-mediated intercellular exchanges exhibit distinct activities based on variations in exosome origin, composition, and pathological conditions. However, there is still much to be understood about the exact contribution of exosomes to the development of LF as different exosomes originating

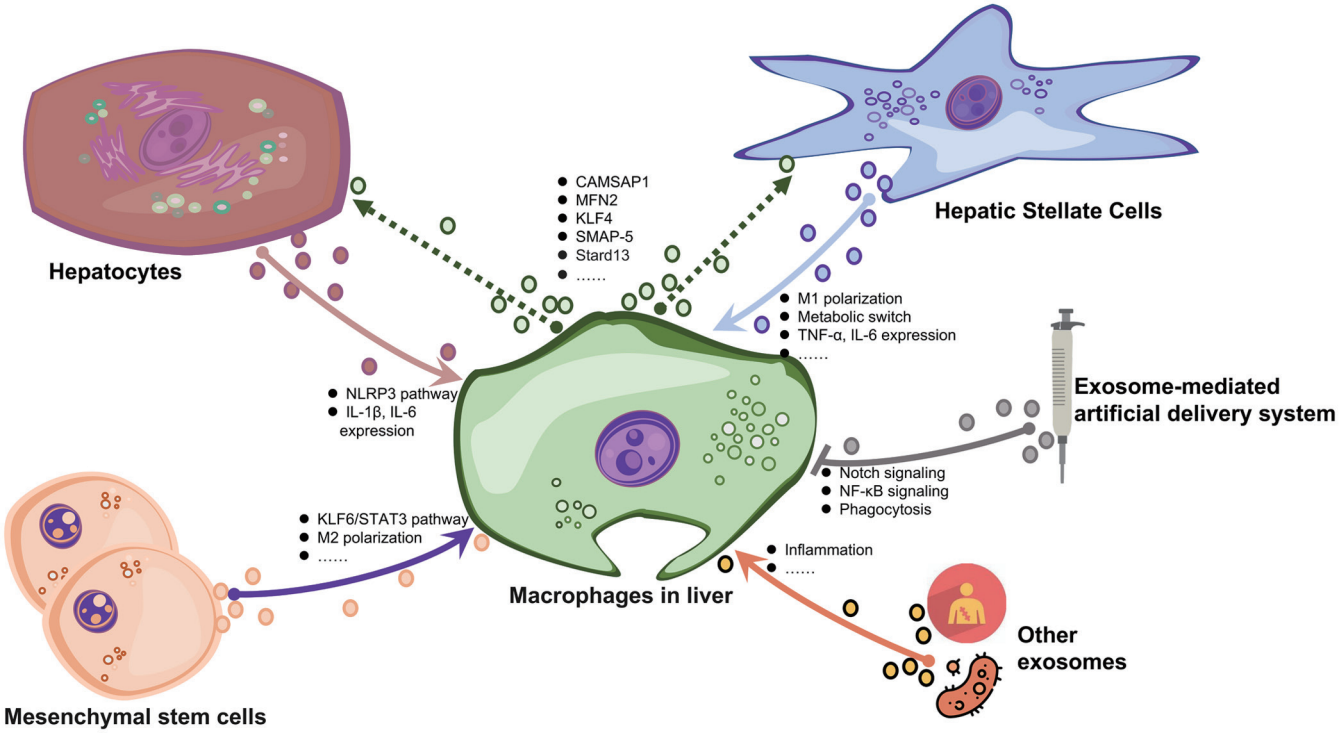

Fig. 1. Role of macrophage-associated exosomes in liver fibrosis. Damaged hepatocytes, activated HSCs, and MSCs regulate the functional state of macrophages via exosomes, while macrophages regulate hepatocytes and HSCs via exosomes. The opening of the arrow is the cell that releases the exosome, and the arrowhead points to the cell where the exosome acts. CAMSAP1, calmodulin-regulated spectrin associated protein 1; MFN2, mitofusin-2; KLF4, Krüppel-like factor 4; SMAP-5, small acidic protein-5; Stard13, StAR-related lipid transfer protein 13; IL1β, IL6, interleukin-1 beta; TNF-α, tumor necrosis factor-alpha.

from various biological sources may have distinct roles in the progression of the illness at different stages.

Hepatocellular carcinoma is the outcome of LF, and numerous studies have shown exosomes to play crucial roles in this disease, including promoting tumor growth and spread, preventing the immune system from recognizing and attacking tumors and enhancing tumor drug resistance.[117,118] However, LF is reversible, and irreversible cirrhosis and hepatocellular cancer only appear later in the disease course. Exosome composition and function will alter in line with this process. For instance, miR-122 levels in exosomes derived from hepatocellular carcinoma were lowered. They may be linked to the proliferation of cancer cells, but miR-122 levels in serum exosomes were enhanced in individuals with LF.[119] The pathological pathway from LF to hepatocellular carcinoma will be aided by an additional understanding of this transition and its molecular mechanism. This may also lead to the discovery of new treatment targets and approaches for reversing LF and preventing hepatocarcinogenesis.

Exosomes have been extensively studied as a potential treatment for various illnesses, including LF. However, there are still several issues that still need to be solved. Firstly, most current techniques for isolating and purifying exosomes are still in the laboratory stage. Therefore, developing efficient, cost-effective, and standardized methods for isolating and preparing exosomes for clinical application is crucial. Secondly, delivering exosomes to specific tissues or organs remains a challenging task. Factors such as population, cell source, and health status must be carefully considered. Lastly, determining the optimal therapeutic dose of exosomes is still a research topic. Depending on the disease and population, personalized treatment regimens may be necessary as exosomes have varying therapeutic dosage requirements. With the rapid advancement of knowledge about exosomes and the emergence of new targeted delivery technologies, it is anticipated that exosomes will soon replace other therapy options for LF.

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Conflict of interest

LC has been an associate editor of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Conceived and designed the article and critically revised the manuscript (BH, YL, LC), obtained funding and provided administrative, technical, and material support (LC), performed literature searches and wrote the manuscript (BF, HX), updated the text of the manuscript (QT, QL), and performed critical revision of the manuscript for important intellectual content (QT, QL, TG).

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