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Review Article

Role of Small Extracellular Vesicles in Liver Diseases: Pathogenesis, Diagnosis, and Treatment

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Abstract

Extracellular vesicles (EVs) are vesicular bodies that bud off from the cell membrane or are secreted virtually by all cell types. Small EVs (sEVs or exosomes) are key mediators of cell-cell communication by delivering their cargo, including proteins, lipids, or RNAs, to the recipient cells where they induce changes in signaling pathways and phenotypic properties. Tangible findings have revealed the pivotal involvement of sEVs in the pathogenesis of various diseases. On the bright side, they are rich sources of biomarkers for diagnosis, prognosis, treatment response, and disease monitoring. sEVs have high stability, biocompatibility, targetability, low toxicity, and are immunogenic in nature. Their intrinsic properties make sEVs an ideal delivery vehicle to be loaded with cargo for therapeutic interventions. Liver diseases are a major global health problem. This review aims to focus on the roles and mechanisms of sEVs in the pathogenesis of liver diseases, liver injury, liver failure, and liver cancer. sEVs are released not only by hepatocytes but also by stromal and immune cells in the microenvironment. Early detection of liver disease determines the chance for curative treatment and high survival of patients. This review focuses on the potential of circulating sEV cargo as specific and sensitive noninvasive biomarkers for the early detection and prognosis of liver diseases. In addition, the therapeutic use of sEVs derived from various cell types is discussed. Although sEVs hold promise for clinical applications, there are still challenges to be overcome by further research to bring utilization of sEVs into clinical practice.

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Keywords: Small extracellular vesicles; Liver injury; Liver fibrosis; Liver failure; Hepatocellular carcinoma; Biomarkers.

Abbreviations: ACHBLF, acute-on-chronic hepatitis B liver failure; ACLF, acute-on-chronic liver failure; AFP, alpha fetal protein; ALF, acute liver failure; AMSC, adipose mesenchymal stem cell; BMSC, bone marrow mesenchymal stem cell; CXCL8, CXC motif chemokine ligand 8; EV, extracellular vesicle; HCC, hepatocellular carcinoma; HMGB1, high mobility group box protein 1; HSC, hepatic stellate cell; hucMSC, human umbilical cord mesenchymal stem cell; LPS, lipopolysaccharide; MVB, multivesicular body; NID1, nidogen 1; NK, natural killer; PD-L1, programmed death ligand 1; pIgR, polymeric immunoglobulin receptor; PKM2, pyruvate kinase M2; RBC, red blood cell; TPI1, triosephosphate isomerase 1; UCB, umbilical cord blood; UHRF1, ubiquitin like with PHD and ring finger domains 1.

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Introduction

Liver failure is a serious liver injury caused by a variety of factors, resulting in serious dysfunction or decompensation of synthesis, detoxification, metabolism, and biotransformation. A group of clinical syndromes with jaundice, coagulation dysfunction, hepatorenal syndrome, hepatic encephalopathy and ascites are the main manifestations.[1,2] Liver failure progresses rapidly, its treatment is difficult, treatment-related medical expenses are high, and the overall prognosis is poor. Another dreadful liver disease, hepatocellular carcinoma (HCC), accounts for 75–85% of primary liver tumors, and the incidence rate is increasing worldwide. In 2018, HCC was the sixth most common cancer and fourth leading cause of cancer death worldwide.[3] Although new breakthroughs have been made in immunotherapy, chemotherapy, interventional radiology and surgical technology in recent years,[4–8] the prognosis of patients with advanced liver cancer is still poor, and there is no effective treatment so far. It is thus urgently needed to explore the molecular mechanism of these life-threatening liver diseases and advance the technologies for early detection and curative treatments.

In recent years, more and more studies of the function of extracellular vesicles (EVs) in the pathophysiology of human diseases and potential clinical applications have been performed. EVs are released by all cell types, and can be found in the culture medium of cell lines and body fluids such as blood, lymph, saliva, and urine. A particular population of EV, small EVs (sEVs, also known as exosomes) has been recognized as pivotal players to mediate cell-cell interactions in the microenvironment. sEVs deliver their cargo to recipient cells in local and distant tissues and induce alterations in signaling cascade and behaviors of cells.[9] sEVs widely exist in various body fluids including blood, lymph, saliva, urine, semen, and milk. The components of sEVs determine the functionality and reflects the origin of the releasing cells. From the clinical perspective, sEVs have great potential in the diagnosis and treatment of liver diseases.[10] Here, we review studies on the involvement of sEVs in liver diseases and the potential of sEV cargos in the diagnosis and treatment of liver failure and liver cancer.

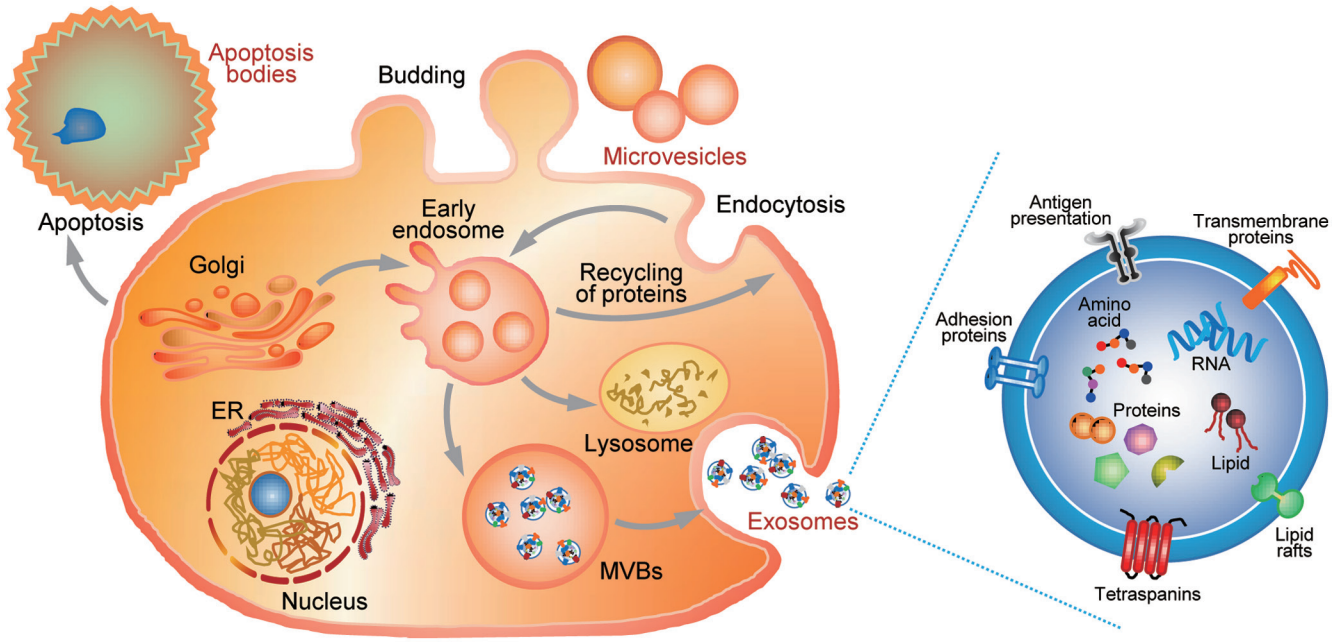

Fig. 1. Formation, release, structure, and cargo of EVs. Endosomes are formed by capturing extracellular molecules through endocytosis. Endosome can directly fuse with plasma membrane to release microvesicles (MVs) or they can carry intracellular molecules through material exchange with Golgi apparatus. The final form multivesicular bodies (MVBs) that reach the plasma membrane and lead to the release of small extracellular vesicles (sEVs) by exocytosis. Schematic diagram of exosome formation. An exosome carries various cargos, including proteins, RNAs and lipids.

Distinct populations of EVs

EVs are vesicular bodies with a bilayer membrane structure that bud off from the cell membrane or are secreted by cells. EVs are mainly composed of apoptotic bodies larger than 1,000 nm, microvesicles (MVs) with a size range of 200–1,000 nm and sEVs with a size range of 50–150 nm. MVs and apoptotic bodies are vesicles that bud off from the cell membrane after cell activation, injury, or apoptosis.[6] sEVs are formed as intraluminal vesicles within the endosomal multivesicular bodies (MVBs) and are released by the fusion of MVBs and the cell membrane (Fig. 1). sEVs widely exist in cell culture supernatant and various body fluids. They carry a variety of proteins, amino acids, lipids, DNA, and different types of RNA and participate in the processes of intercellular communication, cell migration, angiogenesis, and immune regulation.[11–13] Integrins and immunomodulatory proteins are found in the membrane of sEVs. Tetraspanin families, CD63, CD81, and CD9, are regarded as molecular markers of sEVs and flotillin, which is involved in the transport of sEVs are also enriched in sEVs.[14] Recently, sEVs have been further classified as large exosome vesicles (Exo-L) of 90–120 nm and small exosome vesicle (Exo-S) of 60–80 nm in diameter. New, distinct populations of nonmembranous nanoparticles composed of a 35 nm exomere and a supermere have been isolated.[15,16] They have been implicated in carcinogenesis, their applications in clinical application are yet to be revealed. In this review, we focus on the role of sEVs or exosomes in liver diseases.

Oncogenic properties of sEVs in HCC

The content of EVs comprises a variety of proteins, lipids, RNA, and other molecules. sEVs transmit their biocomponents between cells and alter signaling pathways, cellular function, and metabolism of the recipient cells. Here, we review the latest findings about the functional role of sEV biocomponents, noncoding RNAs and proteins, in tumor progression, metastasis, immune escape and drug resistance of HCC (Fig. 2).

Effects of sEVs on the cancerous properties of HCC

Accumulating studies have shown that sEVs deliver different RNAs to promote the growth and motility of HCC through multifaceted mechanisms. Tian *et al.*[17] found that acidic microenvironment induced exosomal miR-21 and miR-10b to promote HCC cell proliferation and metastasis. Coincidentally, miR-21 carried by sEVs has been shown to induce p-Akt and downregulate PTEN, PTENp1, and Tet methylcytosine dioxygenases (TETs), resulting in the enhanced growth of the recipient HCC.[18] Other studies showed that cargos of sEVs, miR-92a-2-5p, circRNA-100,338, and linc00511 augmented angiogenesis and invasiveness of HCC.[19–21] In addition, circRNA-SORE, transported by sEVs, prevented the degradation of the main oncoprotein YBX1 by binding to YBX1 in the cytoplasm, resulting in sorafenib resistance in HCC.[22] This finding provides a new strategy for improving the effect of HCC chemotherapy by silencing circRNA-SORE.

In addition to RNA, proteins are functional components of sEVs that contribute to the oncogenic activity of HCC EVs. Using proteomic profiling comparing proteins of sEVs derived from normal liver cells and metastatic HCC cells, nidogen 1 (NID1) was found to be highly expressed in the sEVs of metastatic cells.[23] The study revealed that EV-NID1 promoted tumor-cell colonization and extrahepatic metastasis of HCC by facilitating pre-metastatic niche formation. EV-NID1 induced pulmonary vascular permeability-activated fibroblasts that secreted tumor necrosis factor receptor 1, which promoted lung colonization, growth, and motility of disseminated HCC cells. Polymeric immunoglobulin receptor (pIgR)-enriched sEVs collected from patients with advanced

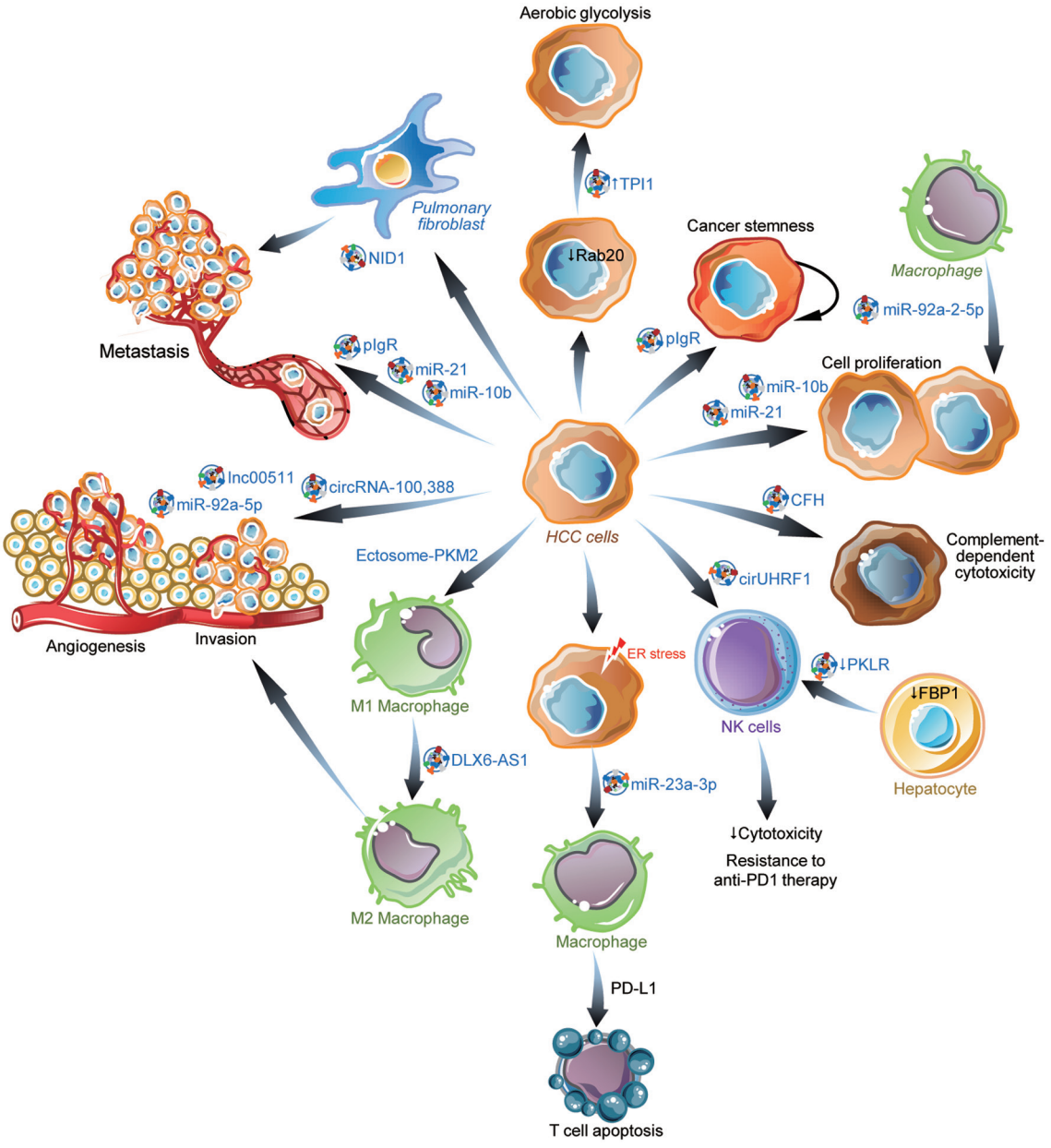

Fig. 2. Effects of sEVs on proliferation, invasion, metastasis, and immune regulation of HCC. sEVs derived from HCC cells deliver proteins (e.g. TPI1, pIgR, or CFH) to promote aerobic glycolysis and cancer stemness, and inhibit complement-dependent cytotoxicity of HCC cells. HCC sEVs also transfer microRNAs (e.g. miR-21, or miR-10b) that promote proliferation of HCC cells. Different RNAs such as miR-21, miR-10b, miR-92a-5p, circRNA-100,388, and lnc00511 promote metastasis by enhancing angiogenesis and invasiveness of cancer cells. EV-NID1 facilitates premetastatic niche formation by activating fibroblasts. In addition to cancer cells, HCC EVs decrease resistance of NK cells to immunotherapy by cirUHRF1. Hepatocytes with attenuated FBP1 release sEVs with reduced levels of PKLR to activate NK cells. HCC cells under ER stress release miR-23a-3p, carried by sEVs, to macrophages, resulting in the release of PD-L1 to induce apoptosis of T cells. Ectosome-PKM2 and EV-DLX6-AS1 are released by HCC cells to induce M2 polarization of macrophages. Inversely, macrophages transfer EV-miR-92a-2-5p to induce HCC cell proliferation. HCC, hepatocellular carcinoma; TPI1, triosephosphate isomerase 1; pIgR, polymeric immunoglobulin receptor; CFH, complement factor H; PKLR, pyruvate kinase L/R; FBP1, fructose-1, 6-bisphosphatase 1; NK, natural killer; PD1, programmed cell death protein 1; PD-L1, programmed death ligand 1; ER, endoplasmic reticulum; PKM2, pyruvate kinase M2; NID1, nidogen 1; UHRF1, ubiquitin like with PHD and ring finger domains 1; NLRP3, NOD-LRR- and pyrin domain-containing protein 3.

HCC have been reported to promote cancer stemness, tumorigenesis, and metastasis of HCC, by a mechanism that involved an EV-pIgR-activated PDK1/Akt/GSK3β/β-catenin signaling cascade.[24] Using anti-pIgR antibody, the study showed that blockade of EV-pIgR-mediated intercellular communication in tumor microenvironment might provide a new therapeutic strategy for cancer patients. Rab GTPases are involved in the biogenesis of exosomes. A study has revealed the unrecognized role of Rab20 in determining cargos of sEVs. HCC cells with Rab20 underexpression released sEVs with reduced triosephosphate isomerase 1 (TPI1) expression. sEVs with reduced TPI1 resulted in an enhanced aerobic glycolysis in recipient HCC cells.[25] Metabolic transition from oxidative phosphorylation to glycolysis may become an independent diagnostic marker for predicting poor prognosis in patients with HCC.[26]

Effects of sEVs on immune regulation of HCC

Tumor-associated macrophages (TAMs) occupy a prominent position in the tumor microenvironment (TME) and have an important role in the development of HCC. Pyruvate kinase M2 (PKM2) carried by sEVs of HCC cells not only induced reprogramming in monocytes, but also induced STAT3 phosphorylation in the nucleus to upregulate differentiation-related transcription factors, resulting in monocytes differentiation into macrophages and tumor microenvironment remodeling.[27] During HCC progression, polarization of M1 macrophages to M2 phenotypes promotes tumor progression.[28] It has been found that HCC sEVs induced M2 macrophage polarization to accelerate cell migration and invasion.[29] DLX6-AS1-enriched EVs derived from HCC cells stimulated CXCL17 to induce M2 macrophage polarization by competitively binding mir-15a-5p, so as to promote HCC migration and invasion. Programmed death ligand 1 (PD-L1) was found to modulate the immune microenvironment by the transfer of sEVs. HCC cells with high levels of Golgi membrane protein 1 released exosomal PD-L1 that was transferred into tumor-associated macrophages, leading to an immunosuppressive environment.[6] The expression of PD-L1 in macrophages was upregulated by uptake of exosomal miR-23a-3p released by liver cancer cells under endoplasmic reticulum stress.[30]

Under normal circumstances, natural killer (NK) cells are nonspecific tumor killing cells mediated by antibody-dependent cell-mediated cytotoxicity (ADCC). Dysfunction of NK cells greatly weakens immunity against cancer cells. Liu *et al.*[31] found that livers deficient in fructose-1, 6-bisphosphatase 1-deficient showed a decrease in NK cells and an acceleration of tumor growth.[31] Another study also reported that circUHRF1 carried by sEVs of HCC cells induced NK cell dysfunction and led to resistance to PD1 immunotherapy in HCC.[32]

After the complement system is activated, membrane attack complex (MAC) is formed on the surface of target cells, leading to the dissolution of target cells. This effect is called complement-dependent cytotoxicity (CDC), and it protects the host by eliminating damaged or changed pathogens and cells.[33] However, a recent study revealed that cancer cells hijacked this defense mechanism. The complement factor H, which is carried by sEVs released from metastatic HCC cells, has been shown to promote the growth, migration, invasion, and tumor growth in mice by protecting HCC cells from complement mediated cytotoxicity.[34]

Potential role of sEVs as biomarkers of HCC diagnosis and treatment

HCC is usually diagnosed at an advanced stage; most patients are excluded from curative surgical resection. Therefore, early diagnosis of HCC is crucial to improve the 5 year survival rate of patients. In spite of the detrimental properties of sEVs released by cancer and stroma cells in the tumor microenvironment in cancer progression and metastasis, the biocomponents of sEVs are promising indicators of cancers and treatment response (Table 1).[17,19–24,27,32,35,36] Von *et al.*[35] showed that unannotated small RNA clusters associated with circulating sEVs have great prospects in the early detection of liver cancer. A number of exosomal RNAs have been shown to functionally promote HCC by augmenting cell proliferation, invasiveness, angiogenesis, and metastasis. The noncoding RNAs, miR-21, miR-10b, miR-92a-2-5p, circRNA-100338, and linc00511 are also candidates as promising prognostic markers and therapeutic targets of HCC.[17,19–21] In addition to RNAs, sEV proteins have also been found to be sensitive and specific markers of HCC. In

Table 1. Potential biomarkers of sEVs for liver HCC

Biomarker	Origin of sEVs	Clinical application	Function	Reference
miR-21 and miR-10b	HCC	Prognosis evaluation and treatment	Promote cell proliferation and metastasis	17
linc00511	HCC	Prognosis evaluation and treatment	Augment angiogenesis and invasiveness	19
miR-92a-2-5p	Macrophages	Prognosis evaluation and treatment	Augment angiogenesis and invasiveness	20
circRNA-100,338	HCC	Prognosis evaluation and treatment	Augment angiogenesis and invasiveness	21
circRNA-SORE	HCC	Treatment	Overcome sorafenib resistance	22
NID1	Metastatic HCC	Early diagnosis and prognosis evaluation	Promote pre-metastatic niche formation	23
pIgR	HCC	Early diagnosis and prognosis evaluation	Promote cancer stemness and cancer properties	24
PKM2	Macrophages	Early diagnosis	Discriminate HCC patients and healthy control	27
circUHRF1	HCC	Treatment	Overcome anti-PD1 resistance	32
Unannotated small RNA clusters	Blood	Early diagnosis	High sensitivity and specificity	35
circTMEM181	HCC	Treatment	Overcome anti-PD1 resistance	36

HCC, hepatocellular carcinoma; NID1, nidogen 1; pIgR, polymeric immunoglobulin receptor; PKM2, pyruvate kinase M2; TME, tumor microenvironment.

the DEN/CCl4-induced HCC mouse model, plasma ectosomal PKM2 was detected before tumor foci had developed in the liver, implicating the application of plasma ectosomal PKM2 for early diagnostic of HCC. In HCC patients, the level of plasma ectosomal PKM2 is higher in HCC patients compared with healthy controls.[27] Circulating EV-NID1 has been shown to increase in a step-wise manner from control subjects to patients with early and advanced HCC. The level of EV-NID1 was able to discriminate HCC patients and control subjects. It was also noted that combined EV-NID1 and alpha fetoprotein (AFP) had better sensitivity and specificity than AFP alone in diagnosis.[23] Frequent up-regulation of cellular and serum EV-pIgR was detected in HCC patients. The level of pIgR in circulating sEVs was reduced in a majority of patients after surgical resection.[24] The studies underscore the potential of those sEV proteins as diagnostic and prognostic markers of HCC. The translation of the research findings to clinical application of these sEV proteins requires further validation of their expression in a large cohort of patients.

Sorafenib is a first-line chemotherapy drug for patients with unresectable HCC. However, many HCC patients have a poor response to sorafenib or develop drug resistance after several months of treatment.[37] Xu *et al.*[22] found that circRNA-SORE was transported by sEVs and resulted in sorafenib resistance in HCC. Sorafenib resistance was dampened by silencing circRNA-SORE.[22] In addition to chemotherapy, immune checkpoint inhibitors have garnered much attention for their effectiveness in treating cancers. However, resistance to immunotherapy such as anti-PD1 resistance has been reported. Exosomal circTMEM181 has been shown to upregulate CD39 in macrophages, resulting in impaired antitumor immunity. Clinically, exosomal circTMEM181 level is elevated in HCC patients poorly responded to anti-PD1 treatment and those with poor prognosis after operation.[36] A study demonstrating that inhibition of the ATP-adenosine pathway by targeting CD39 on macrophages compromised HCC resistance to PD1 therapy provided insights into new treatment strategy for overcoming drug resistance of HCC.[36]

Role of sEVs in inducing liver failure and injury

In spite of the compelling evidence of the functional impact of sEVs in cancer, studies related to sEVs in liver failure and injury are limited. Liver injury provokes death of hepatocytes. A study reported that heat stress influences the composition of cargos of sEVs released by hepatocytes.[38] Heat-stroked sEVs induced apoptosis and necroptosis of hepatocytes leading to liver injury. It was found that decreased autophagy sensitized hepatocytes to TNF/IL-1β cytotoxicity. TNF/IL-1β-treated hepatocytes secreted exosomal DAMPs that activated macrophages to cause inflammation and liver injury. That observation was corroborated by increased hepatic cell death and detection of inflammatory cells following Atg5 knockout and IL-1β/TNF co-administration.[39] The livers of alpha-1 antitrypsin-deficient individuals are characterized by hepatic injury and inflammation. Compared with normal controls, sEVs released by alpha-1 antitrypsin-deficient individuals carry altered compositions of cytokines and miRNAs that are profibrogenic. The sEVs activate human hepatic stellate cells (HSCs) in which genes related to the development of fibrosis are significantly upregulated.[40] Crosstalk between HSC with immune cells contributes to the initiation and development of liver fibrosis. Lipopolysaccharide (LPS)-treated macrophages release exosomes with increased levels of miR-500 that promote HSC activation. Consistent with the *in vitro* observation, serum exosomal miR-500 is elevated in CCl_4-induced liver fibrosis mouse model.[41] These studies are summarized in Figure 3.

Therapeutic effectiveness of sEVs in ALF and acute liver injury

sEVs derived from various types of stem cells

Stem cell therapy has attracted much attention as a potential treatment for a variety of diseases, including liver failure. MSCs are the most commonly used stem cells. Human umbilical cord mesenchymal stem cell (hucMSC)-sEVs have been shown to promote the recovery of liver oxidative damage by delivering glutathione peroxidase1 that can reduce oxidative stress and apoptosis.[42] hucMSC-sEVs also repair damaged liver tissue and alleviate acute liver failure (ALF) *in vivo* by inhibiting the activation of NLRP3 inflammatory bodies and caspase-1 and decreasing the expression of IL1β and IL6 in a mouse ALF model to reduce the inflammatory response.[43] In another study, TNF-α pretreatment of hucMSC-sEVs have been shown to alleviate the inflammatory injury caused by ALF and promote the repair of liver tissue by inhibiting activation of the NLRP3 pathway.[44] Inflammatory response can also by elicited by miRNA delivered by EVs. hucMSC-sEVs have been shown to alleviate IL6-induced acute liver injury via miR-455-3p, which reduces macrophage infiltration and the level of serum inflammatory factors, and improves liver histology and systemic diseases.[45] In addition, Wu *et al.*[46] reported that hucMSC-sEVs alleviated acetaminophen-induced ALF by inhibiting oxidative stress-induced apoptosis via the activation of the ERK1/2 and PI3K/AKT signaling pathways.[46] It had antioxidative activity *in vitro* and *in vivo* and protected against acetaminophen injury that induced ALF in humans. The studies suggest that hucMSC-sEVs induce anti-inflammatory factors and inhibit hepatocyte apoptosis in the treatment of ALF.

Bone marrow mesenchymal stem cell (BMSC)-sEVs also have great potential in the diagnosis and treatment of liver diseases. BMSC-sEVs have been shown to reduce D-galactosamine/TNF-α–induced hepatic inflammation and hepatocyte injury *in vivo* and improved the survival of mice with liver failure. BMSC-sEVs rich in lncRNA Y-RNA-1 have been shown to protect hepatocytes from apoptosis *in vitro*.[47] Zhao *et al.*[48] reported that BMSC-sEVs protected hepatocytes from damage and treated ALF by promoting autophagy and decreasing production of proapoptotic proteins Bax and cleaved caspase-3.

Apart from hUCMSC and BMSC, other stem cells also have potential curative effectiveness in ALF. sEVs derived from human adipose stem cells (hASCs) have been shown to promote the regeneration of damaged tissues in rats with ALF by releasing lncRNA H19.[49] Chen *et al.*[50] carried out a cross species study in which sEVs derived from female menstrual blood improved the survival rate of mice with ALF, which supports the use of sEVs obtained from stem cells obtained from menstrual as an alternative treatment of ALF.

sEVs derived from macrophages

Macrophages, as the main phagocytes in the inflammatory stage, are responsible for removing necrotic fragments and pathogens of damaged tissues and cells[51–53] that have an important regulatory role in the process of repairing. EVs released by macrophages regulate concanavalin A-induced hepatitis by inhibiting macrophage cytokine production.[54] High mobility group box protein 1 (HMGB1)-loaded sEVs released by LPS-induced macrophages was shown to trigger hepatocyte pyroptosis, a mechanism associated with the activation of the NLRP3 inflammasomes.[55] The level of sepsis serum sEVs-HMGB1 was positively correlate with clinical liver damage. The finding offers a rationale to de-

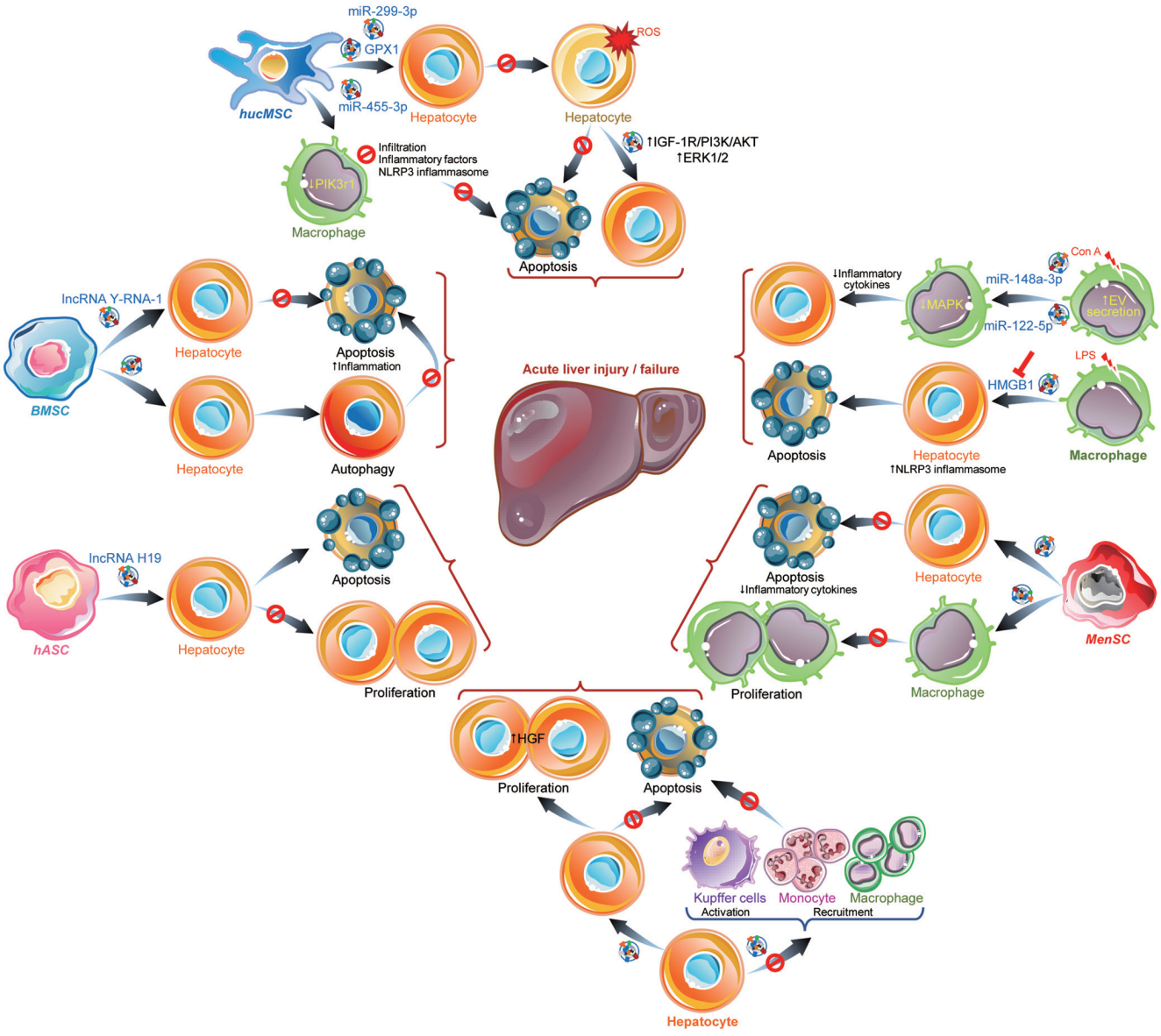

Fig. 3. Therapeutic effectiveness of sEVs in acute liver injury and ALF. sEVs derived from various sources of stem cells, such hucMSC, BMSC, hASC, and MenSC have been shown to reverse acute liver injury by inhibiting apoptosis and promoting hepatocyte proliferation. hucMSC-derived sEV-miR-455-3p inhibits PIK3r1 signaling in macrophages resulting in decreased inflammation. MenSCs-derived EVs suppress apoptosis of hepatocytes and proliferation of macrophages. sEVs of hepatocytes are known to inhibit apoptosis of hepatocytes and dampen inflammatory responses, including inhibition of Kupffer cell activation and recruitment of monocyte and macrophage. sEVs released by Con A-treated macrophages inhibit cytokine production by macrophages. LPS-treated macrophages release HMGB1-containing EVs that induce apoptosis of hepatocytes by the activation of NLPR3 inflammasomes, and blockade of HMGB1 is thought inhibit apoptosis of hepatocytes. hucMSC, human umbilical cord mesenchymal stem cell; GPX1, glutathione peroxidase1; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; BMSC, bone marrow mesenchymal stem cell; hASCs, human adipose stem cells; HGF, hepatocyte growth factor; MenSC, menstrual blood-derived stem cells; HMGB1, high mobility group box protein 1; Con A, concanavalin A; LPS, lipopolysaccharide.

velop new diagnostic and therapeutic strategies for acute liver injuries.

sEVs derived from hepatocytes

The therapeutic effectiveness of sEVs derived from normal liver is mediated by various mechanisms. It was reported that sEVs from normal liver cells weaken the pathway of apoptosis induced by CCl_4, induce the proliferation of hepatocytes, and accelerate the recovery of damaged liver necrosis induced by CCl_4.[56] The results showed that the recovery of liver injury may have been promoted by increased levels of HGF secreted by activated HSCs. That is, the liver sEVs promoted the activation of HSCs and HGF secretion to restore damaged tissues. Another study found that CCl_4 inhibited recruitment of monocytes by down-regulating chemokine receptors in bone marrow and neutrophil recruitment by reducing the expression levels of CXC motif chemokine ligand 1 (CXCL1) and CXCL2 in the liver, resulting in acute liver injury. In the mouse model, hepatocyte-derived sEVs effectively alleviated CCl_4-induced acute liver injury.[57] The

mechanisms of therapeutic sEVs in acute liver injury are summarized in Figure 3.

Therapeutic effectiveness of sEVs on chronic liver failure or liver fibrosis

Fibrosis is a wound healing response that produces and accumulates extracellular matrix (ECM) proteins including collagen fibers, resulting in scar tissue. Hepatic fibrosis is recognized as the main driving force leading to cirrhosis and CLF.[58] sEVs in human umbilical cord blood (UCB) plasma (UCB-sEVs) have been shown to suppress HSCs activity *in vitro* by suppressing transforming growth factor-β/inhibitor of DNA binding 1 signaling. The study also revealed that UCB-sEVs improved liver function and reduced the degree of fibrosis by increasing matrix metalloproteinase/tissue inhibitor of metalloproteinase degradation.[59] A recent study reported that silencing dihydrofolate reductase (DHFR) in HSCs reduced Lx-2 activation and M1 polarization of M0 macrophages, with subsequent delay of the development of liver fibrosis both *in vitro* and *in vivo*. The study results support DHFR as a potential therapeutic target for liver fibrosis.[60] The results may herald novel cell-free antifibrotic therapies.

Therapeutic effects of sEVs on acute-on-chronic liver failure (ACLF)

ACLF is a syndrome in patients with liver cirrhosis that often includes acute decompensation, organ failure, and high short-term mortality. There are no established diagnostic criteria for ACLF.[61–63] A recent study by Zhang *et al.*[64] reported simultaneous reduction in the level of miR-20a-5p in hepatocytes and their exosomes and upregulation of the CXC motif chemokine ligand 8 (CXCL8; interleukin 8) is upregulated. Coculture with BMSCs resulted in an upregulation of miR-20a-5p expression in the exosomes and hepatocytes of ACLF mice, with concomitant down-regulation of CXCL8 in hepatocytes. The study points to a potential mechanism of hepatocyte exocytosis regulated by MSCs to alleviate ACLF liver inflammation, and the potential use of CXCL8 as a target to reduce liver injury.

Potential role of sEVs as biomarkers for diagnosis and monitoring of liver fibrosis and liver failure

Various studies support the potential use of sEV cargos for the diagnosis of liver fibrosis and liver failure (Table 2).[41,65–71] miR-500 levels have been assayed in the circulating exosomes of patients with early and advanced stages of fibrosis. The exosomal miR-500 level was significantly higher in patients with advanced fibrosis than in patients at an early stage, suggesting the diagnostic value of exosomal miR-500 for advanced liver fibrosis.[41] Nakashiki *et al.*[65] found that the content of miRNA in bile sEVs was altered in patients with end-stage liver disease, and that the miRNA level was inconsistent with that in serum EVs. The study indicates the differential expression of miRNAs in bile sEVs between liver failure cells and normal cells may have high clinical diagnostic value. Furthermore, specific exosomes cargoes have diagnostic or prognostic potential in liver injury detection. Circulating sEVs in drug- and alcohol-mediated liver injury contain liver-specific proteins that can be used as a specific biomarker of hepatotoxicity.[66] Circulating sEVs carrying sphingolipids have been used in the diagnosis and dynamic risk analysis of alcoholic hepatitis.[67] They also may be use-

Table 2. Diagnostic and therapeutic applications of sEVs for liver failure

Biomarker	Origin of sEVs	Types of liver disease	Clinical application	Reference
miR-500	Macrophages	Liver fibrosis	Diagnosis	41
miRNA level	Bile	Liver failure	Diagnosis	65
liver-specific protein	Blood	Drug and alcohol-mediated liver injury	Diagnosis	66
sphingolipids	Blood	Alcoholic hepatitis	Diagnosis and dynamic risk analysis	67
lncRNA NEAT1	Blood	ACHBLF	Prognostic monitoring	68
sEVs spectra of ALB and VEGF	Hepatocyte	ACLF	Prognosis evaluation and early warning	69
quercetin and vitamin A	AMSCs	Acute liver injury	Treatment	70
microRNA-155	RBC	Acute liver failure	Treatment	71

ACHBLF, acute-on-chronic hepatitis B liver failure; ACLF, acute-on-chronic liver failure; AMSCs, adipose mesenchymal stem cells; HSCs, hepatic stellate cells; hUCMSC, human umbilical cord mesenchymal stem cell; RBC, red blood cell; UCB, human umbilical cord blood.

ful for monitoring prognosis in patients with liver failure. A prospective study discovered that the serum exosomal lncRNA NEAT1 was a better prognostic biomarker of 90-day mortality of acute-on-chronic hepatitis B liver failure compared with the MELD score.[68] Another study cohort found that in patients with ACLF, the sEVs spectra of ALB and VEGF were more accurate and specific than AFP, which may be very useful as an early warning in patients with ACLF.[69] In conclusion, early clinical studies provide encouragement for the development of sEVs as biomarkers for prognostic monitoring.

sEVs as a delivery vehicle in the treatment of liver failure

sEVs are ideal drug delivery carriers because of their high biocompatibility, free passage through biological barriers, and artificial modification.[72,73] Adipose mesenchymal stem cell (AMSCs)-derived sEVs were ideal drug carriers. sEVs derived from AMSCs loaded with quercetin and vitamin A were tested for the treatment of CCl_4-induced acute liver injury in mice. The results showed that quercetin enhanced the therapeutic efficacy of sEVs and that vitamin A enhanced the liver targeting of sEVs.[70] Similarly, drug-loaded red blood cell (RBC) sEVs have great potential for clinical applications in the treatment of liver disease. Zhang *et al*.[71] reported that RBC sEVs loaded with antisense oligonucleotides of microRNA-155 were effective in mice with ALF. Active exploration of the use of artificially modified sEVs for clinical treatment is ongoing.

Conclusions

sEVs have great potential for clinical application. They have the advantages of biocompatibility, low toxicity, and no immunogenicity. They can be targeted to specific organs, which facilitates their therapeutic use. They are widely distributed in the body and encapsulate various biological information molecules, which are helpful for diagnostic use. In recent years, the significant development and transformation of sEV-related clinical applications have progressed to preclinical and clinical research. In a clinical study, it has been observed that the serum exosomes of HCC patients have very high levels of miR-21 compared with noncancer patients, which has potential as a biomarker to discriminate healthy individuals and early-stage HCC patients.[74] In another clinical study, it was found that compared with patients with stage I–II HCC, serum exosome miR-638 in patients with stage III–IV HCC were significantly lower, and survival as poor, indicating that the down-regulation of serum exosome miR-638 could predict a poor HCC prognosis.[75]

Although sEVs have potential clinical value in various liver diseases, the field is still in the pre- or early clinical stage and there are many challenges to overcome before using EVs clinically. For example, most clinical trials have explored the potential of exosomes in detection and diagnosis and rarely involved therapeutic applications; most of the results are obtained from the mouse models of liver failure and HCC, similar phenomena caused by sEVs in human liver diseases await verification. Without an in-depth study of sEVs in a physiologically relevant model of liver disease, the efficacy and safety issues associated with the therapeutic use of sEVs have to be carefully considered. A number of potential biomarkers of liver diseases have been identified, but those candidates have been evaluated in small patient cohorts. Their diagnostic and prognostic abilities have to be validated in expanded cohorts of patients and controls to define their sensitivity and specificity in discriminating healthy individuals and patients. Because sEVs carry information about the functions and origins of the releasing cells, the circulating sEVs hold promise in the detection and monitoring of diseases. Given the tremendous potential of sEVs in clinical application, continual efforts should be made to translate findings of basic research to clinical settings.

Clinical trials of sEVs are ongoing. As of May 2022, https://www.clinicaltrials.gov lists 171 clinical trials related to exosomes or sEVs. Of the 171 trials, 89 are in cancers, including lung cancer (NCT05191849, NCT04939324), prostate cancer (NCT04167722, NCT03957252), pancreatic cancer (NCT04636788, NCT02393703), colorectal cancer (NCT04523389, NCT04394572) and breast cancer (NCT04530890, NCT04653740). Regrettably, clinical trials on liver cancer and liver failure have yet to be carried out. With time, technological advances, and more research, the remaining problems will be solved. We believe the application of sEVs in diagnosis and treatment strategy for liver failure and cancer is just around the corner.

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Conflict of interest

JWPY has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2021. TX has no conflict of interests related to this publication.

Author contributions

Conceptualization (TX, JWPY), writing-original draft preparation, (TX), writing, review and editing, supervision, and funding acquisition (JWPY). All authors have read and agreed to the published version of the manuscript.

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