REVIEW ARTICLE

Clinical implications of exosome-derived noncoding RNAs in liver

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Exosomes, one of three main types of extracellular vesicles, are ~30–100 nm in diameter and have a lipid bilayer membrane. They are widely distributed in almost all body fluids. Exosomes have the potential to regulate unknown cellular and molecular mechanisms in intercellular communication, organ homeostasis, and diseases. They are critical signal carriers that transfer nucleic acids, proteins, lipids, and other substances into recipient cells, participating in cellular signal transduction and material exchange. ncRNAs are non-protein-coding genes that account for over 90% of the genome and include microRNAs (miRNAs), long ncRNAs (lncRNAs), and circular RNAs (circRNAs). ncRNAs are crucial for physiological and pathological activities in the liver by participating in gene transcription, posttranscriptional epigenetic regulation, and cellular processes through interacting with DNA, RNA, or proteins. Recent evidence from both clinical and preclinical studies indicates that exosome-derived noncoding RNAs (ncRNAs) are highly involved in the progression of acute and chronic liver diseases by regulating hepatic lipid metabolism, innate immunity, viral infection, fibrosis, and cancer. Therefore, exosome-derived ncRNAs have promising potential and clinical implications for the early diagnosis, targeted therapy, and prognosis of liver diseases.

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INTRODUCTION

The pathogenesis of liver diseases is very complex and involves a variety of pathogenic factors, including hepatitis virus infection, immune dysregulation, drug abuse, excessive alcohol consumption, and obesity. The high prevalence of nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and viral hepatitis has become the most common cause of chronic liver disease[1]. Furthermore, more than 257 million people worldwide have chronic hepatitis B (CHB) virus infection[2]. If the pathogenic factors persist long-term, liver injury progresses to fibrosis, cirrhosis, and even cancer. Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, accounting for ~75–85% of primary liver cancer cases[3].

Exosomes can be secreted from parenchymal cells (hepatocytes) and nonparenchymal cells (including hepatic stellate cells (HSCs), bile duct cells, Kupffer cells, and hepatic endothelial cells) under physiological and pathological conditions[4]. Several studies have highlighted the key roles of exosomes in cell communication, as exosomes can transfer functional substances, especially noncoding RNAs (ncRNAs), into recipient cells to regulate cell biological properties[5].

In this review, we summarize the clinical significance and potential of exosomal ncRNAs, which provides us with a better understanding of the initiation of, development of, and interventions for liver diseases.

BIOLOGICAL CHARACTERISTICS OF EXOSOMES

Exosomes are small vesicles with a phospholipid bilayer membrane and a diameter of 30–100 nm[6]; exosomes can be released by B lymphocytes, T cells, mast cells, dendritic cells, tumor cells, endothelial cells, mesenchymal stem cells, and other cells[7]. They are widely distributed in serum, urine, bile, saliva, semen, cerebrospinal fluid, and breast milk[8]. Exosomes are mainly derived from intracellular multivesicular bodies (MVBs) by the "endocytosis-fusion-efflux" process. The process is as follows[9]: (1) The cell membrane invaginates to form endosomes, which fuse to form early endosomes; (2) the microparticle membranes of early endosomes are invaginated to form late endosomes, also called MVBs, enclosing intracellular fluids or biomolecules; and (3) these multiple intracavitary vesicles are degraded by fusion with lysosomes or cell membranes and are finally released into the extracellular matrix (ECM) (Fig. 1).

Exosomes contain a large number of biomacromolecules, including proteins (heat shock proteins and tetraspanins), nucleic acids (mRNA, ncRNA, and DNA), and lipids (cholesterol)[10].

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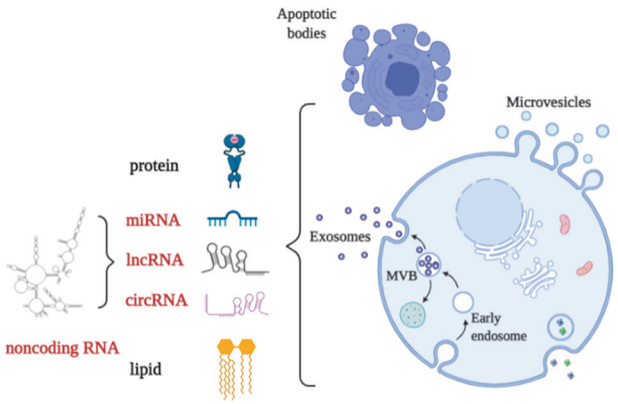

Fig. 1 The biogenesis, secretion, and composition of exosomes. Early endosomes are formed by endocytosis, then develop to multivesicular bodies (MVBs), enclosing the intracellular fluid and biomolecules. Some MVBs fuse with lysosome to be degraded are called exosomes, containing bioactive cargoes such as proteins, noncoding RNA and lipids.

Exosomes can directly interact with signaling receptors on target cells, and plasma membrane fusion then promotes the delivery of bioactive components into the cytoplasm[5,10], a process called receptor-mediated endocytosis and phagocytosis.

BIOLOGICAL CHARACTERISTICS OF NCRNAS

Protein-coding sequences account for <2% of the human genome; however, more than 90% of other sequences are located in noncoding regions[11]. These non-protein-coding genes are replicated to produce thousands of RNA molecules named ncRNAs, including microRNAs (miRNAs), long ncRNAs (lncRNAs), and circular RNAs (circRNAs). NcRNAs, which are crucial for physiological and pathological activities in the liver, can regulate gene transcription, posttranscriptional epigenetic modifications, and cellular processes by interacting with DNA, RNA, or proteins[12].

The length of miRNAs is ~20–24 nucleotides, and miRNAs mainly regulate protein translation by inhibiting posttranscriptional processes[13]. MiRNAs can target a complementary sequence in the 3′UTRs of mRNAs by base pairing (bp). MiR-122 is the most abundant microRNA in the liver, accounting for ~70% of the total miRNA population[14]. Some studies have shown that miR-122 strongly participates in liver homeostasis[15].

LncRNAs are ncRNAs with a transcript length of more than 200 bp. LncRNAs were initially, albeit wrongly, considered a byproduct of RNA polymerase II transcription, i.e., the "noise and garbage" of gene transcription with no biological function. However, recent studies have shown that lncRNAs can affect gene expression in the development of liver disease at various levels through DNA methylation, histone modification, posttranscriptional regulation, and RNA interference[16,17].

CircRNAs originate from precursor mRNA transcripts and are produced via a special alternative splicing mechanism called "backsplicing"[18]. Unlike lncRNAs, circRNAs are covalently closed loops. Due to their lack of typical terminal structures (3′- and 5′-terminal regions), they are more stable than lncRNAs. CircRNAs can also act as molecular sponges of miRNAs, negatively regulating the expression of miRNAs and participating in splicing, transcriptional, and posttranscriptional events, as well as host gene expression, in the initiation and progression of liver disease[18].

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

With the prevalence of obesity and other components of metabolic syndrome, NAFLD has become a common chronic liver disease. Recent studies have shown that exosomes play an important role in regulating injury, amplifying inflammation, and promoting fibrosis in NAFLD[19]. Exosomes from hepatocytes were enriched with an abundance of miR-122 and miR-192 after mice were fed a choline-deficient L-amino acid diet[19]. After miR-155 knockout mice were fed a methionine–choline-deficient or a methionine–choline-sufficient diet for 5 weeks, miR-155 deficiency was found to alleviate hepatic steatosis[20]. These studies indicate that miRNAs can be used as potential candidate markers and therapeutic targets for NAFLD.

ALCOHOLIC FATTY LIVER DISEASE (AFLD)

Alcohol addiction is a social health issue that directly leads to AFLD, which progresses to fibrosis, cirrhosis, and even cancer. AFLD progression has been suggested to be associated with exosome-derived ncRNAs. The miR-155 level was found to be increased in ALD patients and chronic alcohol-fed mice and can promote exosome release from both macrophages and hepatocytes by downregulating autophagic proteins, including mTOR, Rheb, LAMP1, and LAMP2[21]. Compared with that in healthy volunteers, the miR-155 level in circulating exosomes was found to be elevated in patients with ALD[21]. In addition, the expression of miR-let7f, miR-29a, and miR-340 in exosomes released from hepatocytes was increased in a mouse model of mild alcoholic steatohepatitis induced by treatment with ethanol for four weeks but not in other mouse models, including bile duct ligation, nonalcoholic steatohepatitis, and obese mouse models. These results indicate that the above miRNAs can be used as a "barcode" to distinguish ALD due to their specificity[22].

Furthermore, in a mouse model of alcohol-induced steatohepatitis, the number of circulating exosomes was increased significantly after intragastric administration of alcohol[23]. Through microarray screening of exosomes, the miR-192, miR-122, and miR-30a levels in the serum of chronic alcohol-fed mice were found to be increased. Moreover, increased levels of miR-192 and miR-30a were observed in humans with alcoholism and hepatitis, indicating their diagnostic value for alcoholic hepatitis[23]. A previous study showed that an increase in circulating miR-122 is associated with liver injury regardless of the cause of liver injury[24]. Serum exosome-derived miR-122 from hepatocytes can be absorbed by macrophages after alcohol drinking or ethanol ingestion; this event can reprogram monocytes and induce sensitization to lipopolysaccharide, thus promoting the inflammatory response in ALD[25].

Moreover, alcohol-induced monocytes can secrete exosomes to deliver miR-27a into non-alcohol-induced monocytes, which can subsequently differentiate into M2 macrophages with expression of inflammatory IL-10 and TGF-β1[26]. Exosomes from alcohol-treated hepatocytes expressing the CD40 ligand can promote macrophage M2 activation in a caspase-dependent manner, which can also lead to ALD-associated inflammation[27]. These studies indicate that exosome-derived ncRNAs play an important role in the process of alcohol-induced inflammatory responses and liver injury.

VIRAL HEPATITIS

Exosomes are highly involved in the pathogenesis of viral hepatitis through virus transmission, immune regulation, antiviral response control, and microenvironmental manipulation (including intracellular material secretion as well as intercellular exchange of material and information)[28–30].

Hepatitis C virus (HCV)

By isolating exosomes from individuals infected with hepatitis C virus (HCV), these exosomes were found to contain replication-competent viral RNA in a complex with Ago2-miR122-HSP90. More importantly, exosomes loaded with an miR-122 inhibitor can

inhibit HCV transmission mediated by exosomes from hepatocytes[28]. This indicates that targeting exosome-derived miRNAs can inhibit HCV transmission.

Hepatitis B virus (HBV)

Hepatitis B virus (HBV)-infected hepatocytes can activate NK cells by releasing exosomal miR-21 and downregulating IL-12 expression, which might be critical for viral escape from the host innate immune response[29]. The expression of exosome-derived HBV-miR-3 is positively correlated with the serum HBV titer in patients with HBV infection at the acute stage. HBV-miR-3 inhibits the expression of HBsAg and HBeAg as well as HBV replication by targeting a 3.5 kb region of the core protein mRNA[30]. The levels of exosome-derived miR-192-5p, miR-193b-3p, miR-194-5p, miR-122, and miR-22 are significantly higher in HBeAg-positive patients and are correlated with the HBV DNA level and HBsAg titer[31,32]. Therefore, exosome-derived ncRNAs can also be used as candidate biomarkers for HBV infection.

AUTOIMMUNE LIVER DISEASES (AILDS)

Autoimmune liver diseases (AILDs) are a group of chronic inflammatory hepatic disorders that includes autoimmune hepatitis (AIH), primary biliary cholangitis, and primary sclerosing cholangitis[33]. Recent investigations of ncRNA expression profiles indicate that ncRNAs have important impacts on some autoimmune diseases. MiR-223 is highly expressed in bone marrow-derived mesenchymal stem cells (BMSCs). The expression of cytokines, NLRP3 and caspase-1 is downregulated by BMSC-derived exosomal miR-223 (+) in a mouse model of AIH induced by the liver antigen S100, and this downregulation might protect against liver injury from immune-inflammatory dysregulation[34]. Interestingly, treatment with exosomes containing miR-223-3p successfully attenuates inflammatory cytokine release in both the liver and macrophages by regulating STAT3 expression[35].

DRUG-INDUCED LIVER INJURY (DILI)

Drug-induced liver injury (DILI) has gradually become a global public health problem and is characterized by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Exosome-derived ncRNAs might be better diagnostic biomarkers than the current biomarkers. A previous study suggested that acetaminophen (APAP) administration results in a robust increase in miR-122 expression in hepatocytes and a modest increase in the expression of inflammatory miRNAs such as miR-155, miR-146a, and miR-125b in plasma. These miRNA signatures could potentially represent biomarkers for APAP-induced liver injury[24]. The elevated levels of liver-specific miRNAs such as miR-122, miR-192, and miR-155 in exosomes in APAP-induced liver injury are attenuated by treatment with the antioxidant N-acetylcysteine[36]. Serum exosome miR-370-3p might be the key factor enhancing the cytotoxic effect of fructus meliae toosendan by elevating p21 and Cyclin E expression[37]. In summary, profiling circulating exosome-derived ncRNAs could contribute to deepening the understanding of DILI.

BILIARY ATRESIA (BA)

Biliary atresia is a progressive biliary disease of unknown etiology. It often occurs in newborns, resulting in intrahepatic and extrahepatic bile duct occlusion and cholestasis[38]. Exosome-derived ncRNAs have been reported to participate in the development of biliary atresia. For example, the level of serum exosomal lncRNA H19 is correlated with liver fibrosis severity in biliary atresia patients, especially in those with severe liver fibrosis (sixfold higher than in patients with mild liver fibrosis). LncRNA H19 plays a vital role in cholangiocyte proliferation and cholestatic liver injury in biliary atresia by regulating the S1PR2/SphK2 and let-7/HMGA2 axes[39].

CHOLANGIOCARCINOMA (CCA)

Cholangiocarcinoma is a highly heterogeneous malignant epithelial tumor whose early diagnosis is very difficult[40]. To date, an increasing number of exosomal miRNAs have been used in the diagnosis of cholangiocarcinoma. For instance, a novel miRNA-based panel (including miR-16, miR-486-3p, miR-484, miR-1274b, and miR-191) from biliary exosomes exhibited a sensitivity of 67% and specificity of 96% for CCA diagnosis. A diagnostic panel based on bile duct carcinoma-associated miRNAs showed potential clinical utility[41]. Furthermore, 38 miRNAs in CCA cell-derived exosomes are significantly upregulated. The miR-205-5p and miR-200 family members in CCA cell-derived exosomes are markedly upregulated (600–1500-fold). However, CCA cell invasion and migration are reduced by silencing miR-205-5p expression. The observed upregulation of miR-205-5p in CCA cells supports the oncogenic role of miR-205-5p in cholangiocarcinoma[42].

LIVER FIBROSIS

Liver fibrosis is caused by excessive production and accumulation of insoluble collagen and ECM components after induction of chronic liver injury by multiple pathological factors. Activation of (HSCs) is the central event in hepatic fibrosis development. The number of exosomes released from healthy hepatocytes is limited, while stress can promote the release of exosomes enriched with ncRNAs by hepatocytes[43]. These substances regulate transcriptional processes in adjacent hepatocytes and nonparenchymal cells in response to chronic hepatic inflammation, acute injury, and fibrosis[44].

For example, miR-214 exported from HSCs via exosomes can be delivered to adjacent cells and then downregulate connective tissue growth factor (CCN2) expression[45]. CCN2 expression is increased in activated HSCs and directly regulates the activation of HSCs, including the processes of mitosis, chemotaxis, and fibrosis[46]. It was further found that an E-box in the miR-214 promoter binds to the basic helix–loop–helix transcription factor Twist1, which drives the expression of miR-214 and leads to CCN2 inhibition[47]. Moreover, exosome-mediated toll-like receptor 3 (TLR3) activation in HSCs aggravates liver fibrosis by enhancing IL-17A expression in TLR3-positive γδ T cells. During liver injury, hepatocytes secrete exosomes that contain diverse types of self ncRNAs, which have been recognized as activators of TLR3[48].

Exosomes from HCV-infected hepatocytes transfer miR-192 into HSCs, which leads to the activation of HSCs and their transdifferentiation into myofibroblasts via upregulation of TGF-b1[49]. In addition, HCV-infected hepatocytes release exosomal miR-19a to activate the STAT3-mediated TGF-β signaling pathway by targeting SOCS3 in HSCs, which promotes HSC activation and liver fibrosis[50]. miR-155-knockout mice exhibit reduced expression of collagen and α smooth muscle actin (αSMA)[51]. In summary, exosomal miRNAs can activate HSCs through a variety of signaling pathways in liver fibrosis.

HEPATOCELLULAR CARCINOMA (HCC)

Liver cancer is the sixth most common malignant tumor and the fourth leading cause of cancer-related death worldwide[3]. Serum alpha-fetoprotein (AFP) is used for early diagnosis of liver cancer, but its specificity is very low[52]. Recently, an increasing number of studies have focused on ncRNAs in exosomes as candidate biomarkers for liver cancer, as shown in Fig. 2. NcRNAs, which perform biological functions in cancer cell processes such as

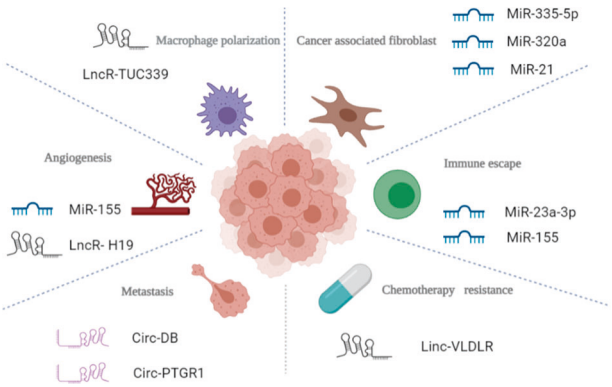

Fig. 2 The interaction between HCC cells and microenvironment via exosomal ncRNAs. Exosomal ncRNAs which relate to cancer-associated fibroblasts are miR-335-5p, miR-320a, and miR-21. Exosomal ncRNAs could escape from immune surveillance (miR-23a-3p, miR-155), mediate chemotherapy resistance (linc-VLDLR), promote tumor metastasis (circ-DB, circ-PTGR1), promote tumor angiogenesis (miR-155, lncR-H19), regulate macrophage polarization (lncR-TUC339).

tumorigenesis, tumor metastasis, angiogenesis, immune regulation, and drug resistance, can be selectively enriched in exosomes[53].

MiRNAs and diagnosis and prognosis

MiRNAs are encapsulated in the lipid bilayer membrane of exosomes and are not affected by RNases. MiRNAs exist widely in various body fluids. They can not only be used as markers for early diagnosis of liver cancer but also as promising biomarkers for liver cancer prognosis. Compared with those in healthy volunteers and patients with chronic liver disease, the serum miR-30e and miR-223 levels are decreased in HCC patients, which includes HCV-infected patients with HCC, HBV-infected patients with HCC and patients with non-viral infection-associated HCC[54]. In addition, an increasing number of studies have indicated that exosome-derived miRNAs can be excellent biomarkers for clinical diagnosis and prognosis. For instance, the levels of miR-18a, miR-221, miR-222, and miR-224 in plasma exosomes are significantly higher in patients with HCC than in patients with CHB or liver cirrhosis. However, plasma exosomal miR-101, miR-106b, miR-122, and miR-195 levels in patients with HCC are significantly lower than those in patients with CHB[43]. In addition, a high serum miR-103 level is associated with a higher metastatic potential of HCC; a higher level of miR-103 in exosomes is also positively associated with HCC metastasis[55]. The expression of miR-10b, miR-21, miR-122, and miR-200a is a more significant indicator than the expression of AFP in the early stage of HCC. Different combinations of AFP and exosomal miRNAs can predict HCC better than AFP can[56]. In one study, the level of miR-665 in patients with HCC was significantly higher than that in healthy subjects, and the survival time in the high expression group was significantly shorter than that in the low expression group, suggesting that serum miR-665 may be a new minimally invasive biomarker for the diagnosis and prognosis of HCC[57]. The level of exosomal miR-125b in HCC patients is lower than that in CHB and LC patients. The level of miR-125b in exosomes is related to the number of tumors and TNM stage. Patients with a low level of miR-125b have shorter recurrence-free survival and overall survival times. MiR-125b can be used as a promising prognostic marker for HCC[58]. Circulating exosomal miR-21 is associated with more advanced TNM stage, portal vein thrombosis, and other unfavorable prognostic factors. The overall survival and progression-free survival times are significantly shorter in patients with higher circulating exosomal miR-21 levels[59].

MiRNAs and cancer associated fibroblasts (CAFs)

In cancer, HSCs can be hijacked to transform into cancer-associated fibroblasts (CAFs). CAFs promote cancer metastasis. HSC-derived exosomes can transport miR-335-5p to recipient liver cancer cells in vitro and in vivo, inhibiting the proliferation and invasion of liver cancer cells and promoting HCC tumor shrinkage in vivo[60]. Sequencing of exosomes released from fibroblasts from HCC patients and the corresponding paracancerous fibroblasts showed that the miR-320a level in CAF-derived exosomes was significantly decreased. MiR-320a can inhibit the proliferation, migration, and metastasis of HCC cells by binding to its downstream target PBX3[61]. The miR-320a-PBX3 axis can inhibit tumor progression by blocking MAPK pathway activation[61]. HCC cell-secreted exosomal miR-21 activates PDK1/Akt signaling to induce the transformation of HSCs into CAFs by directly targeting PTEN, which promotes cancer progression via the secretion of angiogenic cytokines, including VEGF, MMP2, MMP9, bFGF, and TGF-β[62].

MiRNAs and angiogenesis

The expression of miR-155 in the exosomes of 40 patients with HCC. MiR-155 is significantly upregulated in both cells and exosomes under hypoxic conditions. miR-155 knockout in HCC cells attenuates the effect of exosomes on angiogenesis in human umbilical vein endothelial cells (HUVECs) under hypoxia, suggesting that exosomal miR-155 may affect angiogenic activity in HCC[63].

MiRNAs and immune escape

Alcohol increases the release of exosomes containing miR-155 from hepatocytes and Kupffer cells by reducing LAMP1 and LAMP2 expression in the ALD liver[21]. Again, upregulating miR-155 can increase exosome release from Kupffer cells and hepatocytes[21]. Tumor-associated macrophages further promote dysfunction of tumor-infiltrating lymphocytes by expressing the ligands of the inhibitory receptors programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte antigen 4, leading to immune evasion of cancer cells[64]. Under endoplasmic reticulum stress conditions, the expression of PTEN is inhibited in HCC cells via transfer of miR-23a-3p to macrophages, thus increasing the protein levels of phosphorylated Akt and PD-L1 in macrophages and inhibiting the function of T cells, finally leading to tumor cell escape immune surveillance[65].

LncRNAs and diagnosis and prognosis

Circulating exosomal lncRNA ATB is associated with more advanced TNM stage, portal vein thrombosis, and other unfavorable prognostic factors. The overall survival and progression-free survival times are significantly shorter in patients with higher circulating exosome lncRNA ATB levels[59]. The lncRNA FAL1 is upregulated in serum exosomes of patients with HCC; FAL1 can be transferred into HCC cells and accelerate HCC cell proliferation and metastasis by competitively binding miR-1236[66]. In patients with HCV-related HCC, the expression of lncRNA-HEIH in serum and exosomes is increased, but the expression of lncRNA-HEIH in serum is lower than that in exosomes, indicating that exosome-derived lncRNA-HEIH is more important than lncRNA-HEIH in serum[67]. Again, higher levels of serum exosomal lincENSG00000258332.1 and Linc00635 in HCC are associated with portal vein tumor embolization, lymph node metastasis, more advanced TNM stage, and lower overall survival. More importantly, the AUC for lincENSG00000258332.1 and Linc00635 binding to serum AFP is 0.894, indicating that these lincRNAs are good biomarkers for HCC[68].

LncRNAs and angiogenesis

Moreover, exosomes from CD90 + liver cells and hepatoma cell lines can regulate endothelial cells and promote angiogenesis and intercellular adhesion, unlike those released from parental

Table 1. The biological function of exosome-derived Noncoding RNA in liver diseases.

Disease	ncRNAs	Source	Model	Biological function	Ref
HCC	miR-335	Serum/Cell culture supernatants (MHCC97H, MHCC97L, HepG2, Huh7 and LX2 cell lines)	Human	Inhibit HCC cell proliferation and invasion by targeting CDC42	Wang et al.[60]
	miR-320a	Serum/Cell culture supernatants (MHCC97H, SMMC7721 and Huh7 cell lines)	Human	Suppress HCC cell proliferation, migration and metastasis by binding PBX3	Zhang et al.[61]
	miR-103	Serum/Cell culture supernatants (QGY-7703, HepG2, MHCC97H, LO2 and HEK293T cell lines)	Human、Mice	Promote HCC cell migration by repressing p120-catenin expression	Fang et al.[55]
	miR-21	Serum/Cell culture supernatants (MHCC97H, LM3, Huh7, HUVEC, LX2 and LO2 cell lines)	Human	Promote cancer progression by targeting PTEN-PDK1/AKT	Zhou et al.[62]
	miR-155	Serum/Cell culture supernatants (PLC/PRF/5, Huh7, HUVEC cell lines)	Human	Promote tube formation of endothelial cells	Matsuura et al.[63]
	miR-23a-3p	Serum/Cell culture supernatants (mThp-1 and RAW264.7 cell lines)	Human	Tumor cells escape from antitumor immunity by miR-23a-PTEN-AKT pathway	Liu et al.[65]
	miR-665	Serum/Cell culture supernatants (MHCC97H, MHCC97L, LO2 and SMMC-7721 cell lines)	Mice	Promote cell proliferation and tumor growth through the MAPK/ERK pathway	Qu et al.[57]
	lncR-FAL1	Serum/ Cell culture supernatants (LO2, SMMC7721, Huh7, HepG2 and HepG2.2.15cell lines)	Human	Accelerate cell proliferation and metastasis by binding to miR-1236	Li et al.[66]
	lncR-H19	Serum/Cell culture supernatants (HUVECs, Huh7 and SkHep cell lines)	Human	Influence tumor microenvironment by promoting angiogenesis	Conigliaro et al.[69]
	lncR-TUC339	Serum/Cell culture supernatants (THP-1,U937,HL-7702 and PLC/PRF/5 cell lines)	Human	Regulation of macrophage M1/M2 polarization	Li et al.[70]
	linc-VLDLR	Serum/Cell culture supernatants (HepG2, Hep3B, PLC/PRF5, Huh7 and HepG2.ST cell lines)	Human	Reduce cell death by chemotherapeutic drugs	Takahashi et al.[71]
	circ-DB	Serum/Cell culture supernatants (HepG2, Hepa 1-6, 3T3L1 cell lines)	Human	Promote tumor growth by suppressing miR-34a and activating the USP7/Cyclin A2 signaling pathway	Zhang et al.[72]
	circ-PTGR1	Serum/Cell culture supernatants (HepG2, 97L and LM3 cell lines)	Human	Promote HCC metastasis via the miR449a–MET pathway	Wang et al.[73]
	miR-122	Adipose-derived mesenchymal stem cells	Human	Increase chemosensitivity of HCC	Lou et al.[80]
CHB	miR-21	Serum/Cell culture supernatants (HepG2 and Huh 7 cell lines)	Tree shrew	Counteract innate immune response by suppressing IL-12p35	Kouwaki et al.[29]
	miR-3	Serum/Cell culture supernatants (Huh 7 cell line)	Human	Inhibit HBc protein expression by targeting the 3.5-kb transcript of HBV	Yang et al.[30]
CHC	miR-122	Serum/Cell culture supernatants	Human	Transmit HCV infection with Ago2-miR122-HSP90	Bukong et al.[28]
	miR-192	Serum/Cell culture supernatants (Huh 7 cell line)	Human	Activation and transdifferentiation of hepatic stellate cells by TGF-b1upregulation	Kim et al.[49]
	miR-19a	Serum/Cell culture supernatants (Huh7.5 cell line)	Human	Activate hepatic stellate cells by modulating SOCS-STAT3 axis	Devhare et al.[50]
Hepatic fibrosis	miR-214	Serum	Mice	Anti fibrosis by suppression the activity of CCN2 3'UTR; CCN2 suppression by binding Twist1	Chen et al.[45,47]
	miR-181-5p	Adipose-derived mesenchymal stem cells	Mice	Prevent liver fibrosis via autophagy activation	Qu et al.[78]
	miR-122	Adipose-derived mesenchymal stem cells	Mice	Suppress the activation of hepatic stellate cells and alleviate collagen deposition	Lou et al.[79]
AIH	miR-223	Bone marrow derived mesenchymal stem cells	Mice	Protect liver injury by downregulating cytokines, NLRP3 and caspase-1	Chen et al.[34]
	miR-223-3p	Bone marrow derived mesenchymal stem cells	Mice	Attenuate inflammatory responses and inflammatory cytokine release	Lu et al.[35]

Table 1. continued

Disease	ncRNAs	Source	Model	Biological function	Ref
ALD	miR-122	Serum/Cell culture supernatants (Huh7.5 cell line)	Human、Mice	Promote inflammation by inhibiting HO-1 pathway and stimulating	Momen-Heravi et al.[25]
	miR-27a	Plasma	Human	Modulate phagocytosis by targeting CD206 expression on monocytes	Saha et al.[26]
	miR-155	Serum	Human、Mice	Regulate the autophagy-exosome axis	Babuta et al.[21]
DILI	miR-370-3p	Serum	Mice	Aggravate liver injury by decreasing the level of miR-370-3p	Zheng et al.[37]
ALF	miR-17	Adipose-derived mesenchymal stem cells	Mice	Alleviate ALF by reduction of TXNIP/NLRP3 inflammasome activation	Liu et al.[81]
BA	LncR-H19	Serum	Human	Involve in the proliferation of cholangiocyte and cholestatic liver injury via regulating S1PR2/SphK2 and let-7/HMGA2 axis	Xiao et al.[39]
Liver regeneration	miR-124	Human umbilical cord blood mesenchymal stem cell	Mice	Enhance liver regeneration via inhibiting Foxg1	Song et al.[75]
	miR-10a	Serum	Mice	Accelerate liver regeneration through downregulation of EphA4	Luo et al.[76]

miR microRNA, *HCC* hepatocellular carcinoma, *CDC42* cell division control protein 42 homolog, *PBX3* pre-B-cell leukemia homeobox 3, *PTEN* phosphatase and tensin homolog deleted on chromsome ten, *AKT* vakt murine thymoma viral oncogene homolog, *MAPK* mitogen-activated protein kinase, *ERK* extracellular signal-regulated kinase, *lncR* lincRNA, *circ* circular RNA, *USP7* ubiquitin-specific protease 7, *MET* hepatocyte growth factor receptor, *IL* Interleukin, *CHB* chronic hepatitis B, *HBc* hepatitis B viral core, *HBV* hepatitis B virus, *CHC* chronic hepatitis C, *HCV* hepatitis C virus, *AGO2* argonaute2, *HSP90* the heat shock protein 90, *TGF-b1* transforming growth factor b1, *SOCS* suppressor of cytokine signaling, *STAT3* signal transducer and activator of transcription 3, *CCN2* connective tissue growth factor 2, *AIH* autoimmune hepatitis, *NLRP3* the nucleotide-binding and oligomerization domain-like receptor 3, *ALD* alcoholic liver disease, *HO-1* heme oxygenase-1, *DILI* drug-induced liver injury, *ALF* acute liver failure, *TXNIP* thioredoxin-interacting protein, *BA* biliary atresia, *S1PR2* sphingosine 1-phosphate receptor 2, *SphK2* sphingosine kinase 2, *HMGA2* high-mobility group AT-hook 2, *Foxg1* forkhead box G1, *EphA4* erythropoietin-producing hepatocellular receptor A4.

hepatoma cells. LncRNA H19 is enriched in exosomes released from CD90 + cells and can affect the tumor microenvironment by promoting angiogenesis[69].

LncRNAs and macrophage polarization

LncRNA-TUC339 derived from hepatoma cells plays a key role in macrophages by promoting M2 polarization and reduces the production of proinflammatory cytokines, the expression of costimulatory molecules, and the phagocytic capacity, finally promoting the progression of HCC[70].

LncRNAs and chemotherapeutic resistance

Exosomal ncRNAs have been found to be associated with chemotherapeutic resistance. For example, when hepatoma cells are exposed to anticancer agents such as sorafenib, camptothecin, and doxorubicin, the release of exosomal lincRNA-VLDLR (Linc-VLDLR) from HCC cells is obviously increased. Linc-VLDLR increases the expression of ATP binding box and subfamily G member 2 (ABC-g2) and then reduces chemotherapeutic agent-induced apoptosis[71].

CircRNAs and HCC metastasis

The exosomal content of the circRNA circ-deubiquitination (exo-circ-DB) is increased in HCC patients with high body fat percentages. In addition, in vitro and in vivo experiments have shown that exo-circ-DB promotes the growth of HCC cells by inhibiting miR-34a and activating deubiquitination-related USP7, effects that can be reversed by circ-DB knockout[72]. Sequencing of the circRNAs in exosomes from nonmetastatic (HepG2), low-metastatic (97 L), and high-metastatic (LM3) hepatoma cells showed that circPTGR1 is specifically expressed in exosomes from 97 L and LM3 cells. Exosomes from LM3 hepatoma cells can enhance the migration and invasion potential of HepG2 and 97 L cells by carrying circPTGR1. The prognosis of HCC patients with low exosomal circPTGR1 levels is better than that of HCC patients with high expression of circPTGR1[73]. In summary, circRNAs can absorb miRNAs to regulate the progression of liver cancer.

LIVER REGENERATION

Liver regeneration refers to the process of proliferation, migration, and differentiation of various hepatocytes to restore normal liver volume and function through a variety of biologically effective cellular signaling pathways. ncRNAs have been reported to be regulatory players in various cellular processes of liver regeneration. MiRNAs regulate the expression of proproliferative and antiproliferative genes to precisely coordinate the proliferation of hepatocytes[74]. For example, human umbilical cord blood mesenchymal stem cell (hUCB-MSC)-derived exosomal miR-124 enhances liver regeneration by inhibiting Foxg1 in rats after partial hepatectomy[75]. MiR-10a can accelerate liver regeneration through downregulation of erythropoietin-producing hepatocellular receptor A4 (EphA4)[76]. These findings can be utilized to develop novel therapies for liver regeneration.

INTERVENTION AND PERSPECTIVES

Understanding how exosomes promote the initiation and progression of liver diseases can help to develop exosome-based therapeutics for hepatic treatment. Although ncRNAs lack the potential to encode proteins, they can affect the expression and functions of other genes through a variety of mechanisms, as shown in Table 1. In some cases, their mechanisms of action are well known, and strategies for controlling their activity by knockin or knockout are well established. Currently, miravirsen (SPC3649), which can bind to the stem–loop structure of pri- and pre-miR-122 with nanomolar affinity and inhibit both Dicer- and

Table 2. Exosome-derived noncoding RNA as biomarkers in liver diseases.

Function	NcRNAs	Source	Expression	Model	Disease	Ref
Diagnosis	miR-let-7f	Serum	Up	Human、Mice	ALD	Eguchi et al.[22]
	miR-29					
	miR-340					
	miR-192	Serum	Up	Human、Mice	ALD	Momen-Heravi et al.[23]
	miR-30a					
	miR-122	Serum	Up	Mice	ALD	Bala et al.[24]
	miR-155					
	miR-155	Serum	Up	Human、Mice	ALD	Babuta et al.[21]
	miR-192-5p	Serum/Cell culture supernatants (HepG2 and HepG2 2.2.15 hepatoma cell lines)	Up	Human	CHB	Arataki K et al.[31]; van der Ree et al.[32]
	miR-193b-3p					
	miR-194-5p					
	miR-122					
	miR-22					
	lncR-H19	Serum	Up	Human	BA	Xiao et al.[39]
	miR-16	Biliary	Up	Human	CCA	Li et al.[41]
	miR-486-3p					
	miR-484					
	miR-1274b					
	miR-191					
	miR-205-5p	CCA cell lines and normal human cholangiocyte cells	Up	Human	CCA	Kitdumrongthum et al.[42]
	miR-200					
	miR-122	Serum	Up	Mice	DILI	Bala et al.[24]
	miR-155					
	miR-146a					
	miR-125b					
	miR-122	Plasma	Up	Mice	DILI	Cho et al.[36]
	miR-192					
	miR-155					
	miR-18a	Serum	Up	Human	HCC	Sohn et al.[43]
	miR-221					
	miR-222					
	miR-224					
	miR-101	Serum	Down	Human	HCC	Sohn et al.[43]
	miR-106b					
	miR-122					
	miR-195					
	miR-30e	Serum	Down	Human	HCC	Bhattacharya et al.[54]
	miR-223					
	miR-10b	Serum	Up	Mice	HCC	Liu et al.[56]
	miR-21					
	miR-122	Serum	Down	Mice	HCC	Liu et al.[56]
	miR-200a					
	miR-665	Serum/Cell culture supernatants (MHCC97H, MHCC97L, LO2 and SMMC7721 cell lines)	Up	Mice	HCC	Qu et al.[57]
	lncR-HEIH	Serum	Up	Human	HCC	Zhang et al.[67]
Predicting poor Prognosis	miR-665	Serum/Cell culture supernatants (MHCC97H,MHCC97L,LO2 and SMMC7721 cell lines)	Up	Mice	HCC	Qu et al.[57]
	miR-125b	Serum	Down	Human	HCC	Liu et al.[58]
	miR-21	Serum	Up	Human	HCC	Lee et al.[59]
	lncR-ATB					

Table 2. continued

Function	NcRNAs	Source	Expression	Model	Disease	Ref
	lncR-ENSG00000258332.1	Serum	Up	Human	HCC	Xu et al.[68]
	linc00635					

ALD alcoholic liver disease, *CHB* chronic hepatitis B, *BA* biliary atresia, *CCA* cholangiocarcinoma, *DILI* drug-induced liver injury, *HCC* hepatocellular carcinoma.

Drosha-mediated processing of miR-122 precursors, is in a stage II clinical trial for the treatment of HCV infections[77]. Targeting ncRNAs may become a novel strategy for the treatment of hepatic diseases.

Exosomes have great potential as delivery vehicles due to their natural substance transport ability, inherent long-term circulation ability, and excellent biocompatibility. They are suitable for delivering ncRNAs to improve their targeting accuracy and provide opportunities for diagnosis and treatment innovation. Qu et al. found that exosomes secreted by adipose-derived mesenchymal stem cells (ADSCs) can be used to transport miRNAs into HSCs. Exosomes from ADSCs have been engineered to overexpress miR-181-5p, which activates autophagy by down-regulating STAT3 and Bcl-2 in hst-t6 cells. Subsequently, upregulation of TGF-β1-induced fibrotic gene expression in HST-T6 cells is inhibited, which significantly downregulates type I collagen, vimentin, α-SMA, and fibronectin in the liver and reduces the occurrence of liver fibrosis[78]. MiR-122-modified exosomes from AMSCs (amsc-122) can enhance the therapeutic effect of AMSCs on CCl4-induced liver fibrosis by inhibiting the activation of HSCs and reducing collagen deposition. Amsc-122 can effectively inhibit proliferation and collagen maturation in HSCs[79]. Exosomes carrying miR-122 can improve sorafenib resistance in HCC[80]. Moreover, exosomes carrying miR-17 significantly ameliorate acute liver failure in mice[81]. Therefore, exosomes loaded with miRNAs might be a new potential strategy for the treatment of liver diseases.

DISCUSSION

Exosome-derived ncRNAs have been shown to have some potential for early diagnosis and prognosis (Table 2). For example, plasma exosome-derived miR-122 has diagnostic sensitivity in multiple hepatic diseases and might be valuable in replacing AFP detection. Some specific ncRNAs need to be further clarified for clinical application by evaluation in larger clinical sample sizes, for example, miR-let7f, miR-29a, and miR-340 for mild AFLD[22]. Studies on circRNAs should be given more attention, as exosome-derived circRNAs are more stable for diagnosis than miRNAs and lncRNAs due to their lack of a typical terminal structure. Again, as molecular sponges for miRNAs, circRNAs can target miRNAs involved in the progression of liver diseases. More importantly, targeting exosome-derived ncRNAs and exosomes as effective carriers loaded with ncRNAs or siRNAs for precision medicine is an extremely valuable and revolutionary approach for the future.

DATA AVAILABILITY

Some or all data used during the study are available from the corresponding author by request.

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AUTHOR CONTRIBUTIONS

Z.Z.W. and Z.W. wrote the paper; X.Z., Y.C.S., Y.Q.Q., W.S.H., X.C.A., W.W.H., H.T.C., W.Q.Q. and Z.L.Y. revised the tables and figures; X.K.Y. and P.H.Y. deigned and revised the paper; all authors have read and approved the final version of this paper.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL/CONSENT TO PARTICIPATE

All authors read and approved the final paper.

ADDITIONAL INFORMATION

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