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REVIEW ARTICLE

Dynamic role of exosomal long non-coding RNA in liver diseases: pathogenesis and diagnostic aspects

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Abstract

Background Liver disease has emerged as a significant health concern, characterized by high rates of morbidity and mortality. Circulating exosomes have garnered attention as important mediators of intercellular communication, harboring protein and stable mRNAs, microRNAs, and long non-coding RNAs (lncRNA). This review highlights the involvement of exosomal lncRNA in the pathogenesis and diagnosis of various liver diseases. Notably, exosomal lncRNAs exhibit therapeutic potential as targets for conditions including hepatic carcinoma, hepatic fibrosis, and hepatic viral infections.

Method An online screening process was employed to identify studies investigating the association between exosomal lncRNA and various liver diseases.

Result Our study revealed a diverse array of lncRNAs carried by exosomes, including H19, Linc-ROR, VLDLR, MALAT1, DANCR, HEIH, ENSG00000248932.1, ENST00000457302.2, ZSCAN16-AS1, and others, exhibiting varied levels across different liver diseases compared to normal liver tissue. These exosomal-derived lncRNAs are increasingly recognized as pivotal biomarkers for diagnosing and prognosticating liver diseases, supported by emerging evidence. However, the precise mechanisms underlying the involvement of certain exosomal lncRNAs remain incompletely understood. Furthermore, the combined analysis of serum exosomes using ENSG00000258332.1, LINC00635, and serum AFP may serve as novel and valuable biomarker for HCC. Clinically, exosomal ATB expression is upregulated in HCC, while exosomal HEIH and RP11-513I15.6 have shown potential for distinguishing HCC related to HCV infection.

Conclusion The lack of reliable biomarkers for liver diseases, coupled with the high specificity and sensitivity of exosomal lncRNA and its non-invasive detection, promotes exploring their role in pathogenesis and biomarker for diagnosis, prognosis, and response to treatment liver diseases.

Keywords LncRNA · Exosomes · Liver disease · Hepatocellular carcinoma · Liver fibrosis · Hepatitis C virus · Non-invasive biomarker · Exosomal-derived lncRNA · Hepatitis B virus · Hepatic stellate cell

Abbreviations

MVBs	Multivesicular bodies
NASH	Non-alcoholic steatohepatitis
MEG3	Maternally expressed gene 3
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
DANCR	Differentiation antagonizing non-protein-coding RNA
HCC	Hepatocellular carcinoma

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ADH4	Alcohol dehydrogenase 4 enzymes
Linc-RoR	Regulates reprogramming of long intergenic noncoding RNA
Lnc-ISG15	Interferon-stimulated genes
ISR2	Interferon-stimulated long noncoding RNA -2
ISR8	Interferon-stimulated long noncoding RNA-8
BISPR BST2	Interferon-stimulated positive regulator
IFN	Interferon
HULC	Hepatocellular carcinoma upregulated long non-coding RNA
XIST	X Inactive-specific transcript
HEIH	High expression in hepatocellular carcinoma lncRNA
MSC	Mesenchymal stem cell

Introduction

Exosomes are nano-vesicles of a phospholipid bilayer, released from different cells across different organs. It serves as a typical intercellular exchange means involving the encapsulation and conveyance of diverse molecules from one cell to another. Moreover, exosomes play a role in eliminating undesired and toxic products, including chemotherapeutic drugs, from cells [1].

LncRNA, a transcript exceeding 200 nucleotides without coding potential, has been established to play a fundamental regulatory role in both physiological and pathological conditions. Significantly, exosomes safeguard their lncRNA content from degradation by RNase, enabling efficient transfer from one cell to another. Accumulating evidence suggests that various disease conditions, including cancer, diabetes, liver disease, and other complex disorders, are associated with exosomal lncRNAs [2, 3]. The type and level of lncRNAs in exosomes exhibit variability across different diseases, underscoring their significance in disease diagnosis, progression, and treatment.

Within the liver, this intercellular transfer of lncRNAs can alter gene expression and cellular behavior, playing a significant role in both physiological and pathological processes. Moreover, the type and level of exosomal lncRNAs exhibit variability across different liver diseases [4], suggesting their potential as disease-specific biomarkers.

Liver disease poses a significant global health challenge, with high rates of morbidity and mortality. While traditional diagnostic and therapeutic approaches have advanced, therefore, a deeper understanding of the complex mechanisms underlying liver disease progression is crucial for developing more effective interventions. In the liver, diverse cell types such as hepatocytes, Kupffer cells, liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and others engage in the release or receipt of exosomes as part of intercellular communication [5]. The importance of exosomal lncRNAs stems from their unique ability to influence gene expression and cellular function within the complex microenvironment of the liver. The lack of reliable biomarkers for liver diseases, coupled with the high specificity and sensitivity of exosomal lncRNA and its non-invasive detection, promotes exploring the role of lncRNA in pathogenesis and the hunt for specific exosomal lncRNA markers for diagnosis, prognosis, and response to treatment for various hepatic diseases.

This review focuses on the emerging role of exosomal lncRNAs in the context of liver diseases to summarize the current knowledge and highlight the involvement of exosomal lncRNAs in the development and progression of various liver diseases, including hepatocellular carcinoma (HCC), infections such as hepatitis B virus (HBV) and hepatitis C virus (HCV), as well as liver fibrosis and cirrhosis. In addition, it explores the diagnostic potential by assessing the utility of exosomal lncRNAs as biomarkers for the early detection and monitoring of liver disease and identifies therapeutic opportunities by examining the potential of targeting exosomal lncRNAs for the development of novel therapeutic interventions.

Exosome

Exosome definition

Exosomes are spherical to cup-shaped membranous vesicles, typically ranging from 30 to 100 nm. They originate from the inward growth of the endosomal membrane, leading to the formation of multivesicular bodies (MVBs). Subsequently, these MVBs merge with the plasma membrane, releasing exosomes. While initially perceived as cellular waste disposal, exosomes are now recognized for their fundamental role in intercellular communication. They carry a diverse cargo, including proteins, lipids, DNAs, and various RNA species such as mRNA, miRNA, transfer RNA, lncRNA, and viral RNA, facilitating their transport to different cells [6] (Fig. 1). Recent evidence has highlighted the presence of common proteins in all exosomes, serving as markers for their detection. These include heat shock proteins (HSP70 and HSP90), endosome-specific tetraspanins (CD9, CD63, CD81, and CD82), MVBs biogenesis-related proteins (Alix and TSG101), and membrane trafficking and fusion proteins (GTPase, flotillin, and Annexin) [7, 8].

Exosome function

The fundamental roles of exosomes include intercellular communication and the exchange of molecules. Exosomes

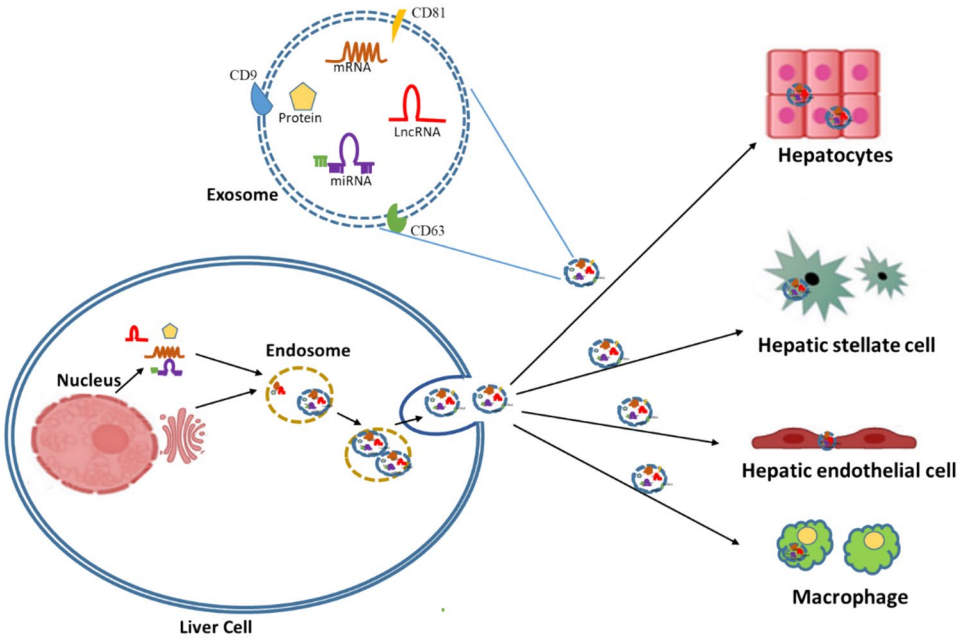

Fig. 1 Biological function and transcellular communication of exosomes

play a crucial role in influencing the progression of various diseases by facilitating the delivery of information and molecules for intracellular communication. This information transfer from exosomes to recipient cells occurs through mechanisms such as membrane fusion [9], ligand–receptor interactions [10], or cellular endocytosis of exosomes [11]. Under normal and disease conditions, exosomes are actively secreted by different cells [12–14] and they play a significant role in numerous human diseases, including liver diseases [12, 15]**.** Given their pivotal roles, exosomes and their contents have potential applications as novel biomarkers for disease diagnosis and prognosis. They can also serve as carriers for drug mediation and RNA interference therapy, and offer promising targets for treating various diseases. Examples include applications in Alzheimer's disease [16], myocardial remodeling [17], breast cancer [18, 19], and other cancers [20–22].

Exosome in liver disease

The liver, as a multicellular organ, comprises hepatocytes, Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), lymphocytes, and biliary cells [23, 24]. Intercellular signaling is essential to regulate their functions. While liver cells secrete exosomes, they regulate both hepatic and extrahepatic exosomes (Fig. 1). Understanding the role of exosomes is crucial in normal and abnormal liver conditions. For example, liver cholangiocytes and hepatocytes release exosomes, which regulate intracellular signaling and cell proliferation in cholangiocytes [15, 25–28]. During liver disease, exosomes have been identified in various biological fluids, including serum, plasma, urine, and bile. Serum and urine exosomes hold potential as a liquid biopsy, reducing the need for invasive tissue samples in the analysis and diagnosis of liver diseases. In the NASH model, liver-derived circulating exosomes showed a progressive increase, correlating with liver neoangiogenesis, apoptosis, and fibrosis [29]. In addition, a study by Garcia-Martinez et al. revealed an increase in hepatocyte-derived circulating exosomes containing mitochondrial DNA in the NASH model [30].

In viral hepatitis, exosomes play various roles, including facilitating viral transmission and modulating the immunity of the infected liver. Moreover, they hold potential as carriers for antiviral drug delivery. Specifically in HBV, exosomes transfer interferon-α (IFN-α) from non-parenchymal cells to HBV-infected hepatocytes, thereby stimulating antiviral activity and inhibiting viral replication [31, 32].

Hepatic stellate cells play a significant role in liver fibrosis through the excessive accumulation of insoluble collagen in fibrogenic pathways [33]. Exosomes derived from endothelial cells contribute to liver fibrogenic pathways by enhancing HSC activation and migration. They achieve this by promoting the binding of exosomes containing fibronectin to αVβ1-integrin on target cells. In addition, these exosomes exhibit angiogenic and procoagulant functions [11, 34–36]. Research has revealed multifactorial roles for exosomes in HCC pathogenesis, progression, and metastasis. Exosomes released from innate immune cells transfer integrin αMβ2 to HCC cells and regulate their invasion, migration, and metastasis [37]. Conversely, exosomes enriched with Linc-VLDLR upregulate in response to anticancer drugs, contributing to chemoresistance in HCC [38, 39].

LncRNA

LncRNAs are genetically encoded RNA with more than 200 nucleotides in size, as was explained by Okazaki et al. [40]. Despite their specific cellular and tissue expression, they exhibit low molecular expression at the cellular level [41]. Based on the genomic localization, lncRNAs are classified into sense, antisense, bidirectional, intronic, intergenic, and enhancer lncRNAs [42, 43]. Functionally described lncRNAs have been found to exert a diverse range of effects on gene expression control, influencing processes such as gene transcription, post-transcriptional processes, chromatin remodeling, protein function, and intercellular signaling.

The identification of many lncRNAs has been facilitated by large-scale genome-wide sequencing and next-generation sequencing techniques, with RNA sequencing and microarrays commonly used to broaden the scope of identified lncRNAs [44, 45]. While northern blots or quantitative PCR are typically employed to confirm putative lncRNAs, the lack of protein-coding capacity poses a significant challenge in achieving functional biological validation.

LncRNAs exhibit diverse tissue-specific distributions, and their intracellular localization varies, encompassing the cytoplasm, nucleus, mitochondria, and exosomes. Interestingly, a single lncRNA may be present in multiple intracellular regions simultaneously. The location of lncRNAs often provides insights into their potential functions [46, 47]. Based on their cellular localization, lncRNAs can be characterized as a signal, decoy, guide, and scaffold lncRNAs [43, 48]. Numerous studies have highlighted the regulatory roles of lncRNAs in various cellular processes, including cell development, differentiation, proliferation, regeneration, and apoptosis. The intricate involvement of lncRNAs in these fundamental cellular functions underscores their significance in shaping cellular behavior and responses [46, 47]

LncRNA and liver disease

The recent surge in research on lncRNAs in the liver has revealed their diverse and complex roles in the pathogenesis of various liver diseases. They actively participate in the cellular processes, influencing gene expression, signaling pathways, and disease progression (Table 1). This multifaceted nature of lncRNAs opens new avenues for understanding the pathogenesis of liver diseases and developing novel diagnostic and therapeutic strategies.

LncRNA could regulate gene expression, for example, MEG3 activates ADH4 [49] and the p53 pathway influencing cell proliferation and suppressing tumor growth in hepatocellular carcinoma (HCC). While at the post-transcriptional level, MEG3, for instance, sponges' miR-664, further contributes to HCC suppression [50, 51].

LncRNAs also modulate cellular processes and function; for example, the initially identified as highly expressed in liver cancer (HULC) can manipulate signaling pathways like the IRS2/AKT pathway, as seen with lncARSR in the context of nonalcoholic fatty liver disease (NAFLD) and HCC [52]. Also, MALAT1 downregulates in liver fibrosis and inflammation, influencing cellular functions, alternating splicing, and contributing to HCC development [53–55].

In the context of disease-specific roles, H19, a crucial regulator in the hepatic steatosis pathogenesis, regulates miR-130a and transcription factors involved in lipogenesis, highlighting its role in fat accumulation [56, 57]. Moreover, lncISG15, ISR2, ISR8, and BISPR are highly expressed in the liver with hepatitis C infection, suggesting their potential involvement in the viral response and inflammation [58, 59].

Table 1 Different lncRNAs and their target mechanism in various liver diseases

LncRNA	Liver disease	Function	Reference
MEG3	-HCC -Liver fibrosis	- Sponging miR-664 and activate ADH4 - P53-dependent pathway - Activate hepatic stellate cell	[49–51] [61]
MALAT1	-Liver fibrosis and inflammation -HCC	Oncogenic role	[53] [54, 55]
H19	Liver fibrosis	Bile acid homeostasis and profibrotic	[120]
XIST	HCC	PDCD4 miR-497-5p	[144]
HULC	HCC	Predictor for disease progression	[60]
ISR2, ISR8, lncISG15	HCV-infected liver	Upregulated in HCV-infected livers and cultured cells through the IFN signaling pathway	[58, 59]
FLRL2	NAFLD	Arntl-Sirt1 pathway	[62]
LncARSR	NAFLD and HCC	IRS2/AKT pathway by inhibiting YAP1 phosphorylation	[52]
AK054921 AK128652	Alcoholic cirrhosis	Serve as a survival predictor	[145]

The potential diagnostic and therapeutic applications of lncRNAs in liver diseases are promising. Their expression patterns could serve as biomarkers for disease progression and prognosis, as seen with HULC [60], and serum LncRNA AK054921 and AK128652 which act as survival predictors in patients with alcoholic cirrhosis. Furthermore, in liver fibrosis MEG3, while suppressing HCC, also activates hepatic stellate cells, potentially influencing fibrosis progression and suggesting its potential as a target for fibrosis treatment [61]. FLRL2 ameliorates NAFLD via the Arntl-Sirt1 pathway and presents an avenue for therapeutic intervention [62]. This expanding knowledge of lncRNAs in liver diseases offers exciting prospects for developing novel diagnostic tools and therapeutic strategies to improve patient outcomes.

Exosomal lncRNA in liver disease

Exosomes transport diverse lncRNAs to various local sites. Their lipid membrane bilayer safeguards lncRNAs from damage, impacting both physiological and pathological functions [63]. Various hepatic cells release exosomes, associated with transcriptomic and proteomic alterations in liver disease content [24]. These changes in exosome content hold promise as non-invasive biomarkers and therapeutic targets for liver diseases [27, 64, 65]. While liver cells contribute to exosome release, both extrahepatic and hepatic exosomes may influence their function. Therefore, understanding exosome functions is crucial for comprehending normal and abnormal liver function. Examining the expression patterns of circulating exosomal lncRNAs in different liver diseases provides molecular evidence of their involvement in vital processes, offering insights into regulatory pathways and networks [66, 67]. This knowledge can aid in identifying potential gene targets for diagnosis and therapy, facilitated by advanced RNA technologies capable of detecting differentially expressed exosomal lncRNAs in diverse liver diseases.

Exosomal lncRNA in hepatocellular carcinoma

HCC is a prevalent and highly lethal cancer with significant mortality rates. Early diagnosis challenges contribute to poor outcomes and limited treatment efficacy. Recent research has expanded its focus beyond mRNAs and proteins to include circulating exosomal lncRNAs, exploring their roles in the pathophysiology, progression, diagnosis, and treatment of liver cancer [68] (Table 2). Below is a potential example illustrating exosome-based signaling, involving lncRNAs in cellular processes associated with liver cancers (Fig. 2).

Exosomal lncRNA in the pathogenesis of HCC

Linc-RoR: a long intergenic noncoding RNA plays a crucial role in regulating cell reprogramming and provides essential evidence for the initiation of pluripotent stem cells [69]. In the context of hepatocellular carcinoma, exosomal linc-RoR undergoes significant changes, particularly during hypoxia. Previous research has demonstrated that linc-RoR regulates cell viability by acting as a sponge for miR-145, modulating HIF-1α during hypoxia stress, and enhancing cell survival [70]. To confirm the role of exosomal linc-RoR, it was extracted from hypoxic cells and incubated into tumor cells, resulting in improved tumor cell survival. In conclusion, exosomal linc-RoR plays a vital intracellular role in response to hypoxia, making it a potential predictor for HCC progression and treatment.

Linc-VLDLR: Linc-VLDLR emerges as a significant player in exosomal-based signaling, contributing to biologically active RNA genes involved in cellular processes in liver cancer. This long intergenic noncoding RNA is highly expressed in induced pluripotent stem cells, embryonic stem cells, and malignant human hepatocellular carcinoma cells [69]. In addition, Linc-VLDLR may participate in proliferative or chemotherapeutic stress responses in human cancer. Upon exposure to anticancer drugs like camptothecin, sorafenib, and doxorubicin, the level of Linc-VLDLR increases in both exposed cells and their exosomes. Among the effects of exosomal linc-VLDLR, it plays a role in the mechanism of chemoresistance and the expression of ABC

Table 2 Exosomal-derived lncRNAs and their contribution to the various liver diseases

LncRNA	Liver disease	Function	Reference
HEIH	HBV-related HCC	Suppresses the cell differentiation in G0/G1 phases and cancer-promoting lncRNA A novel biological marker	[101] [102]
Linc-RoR	HCC	Competing endogenous with miR-145, then modulate HIF1α to regulate cell viability and survival	[70]
H19	HCC Liver fibrosis	Promote angiogenic phenotype and cell-to-cell adhesion, inhibiting the SHP and Regulating bile acid homeostasis Increase HSC proliferation and the profibrotic gene expression Ce-miRNA let-2/HMGA2 and stimulate the cholangiocyte proliferation	[76] [123] [120, 121]
VLDLR	HCC	Modulate ABCG transporter expression and promote chemoresistance	[38] [71]

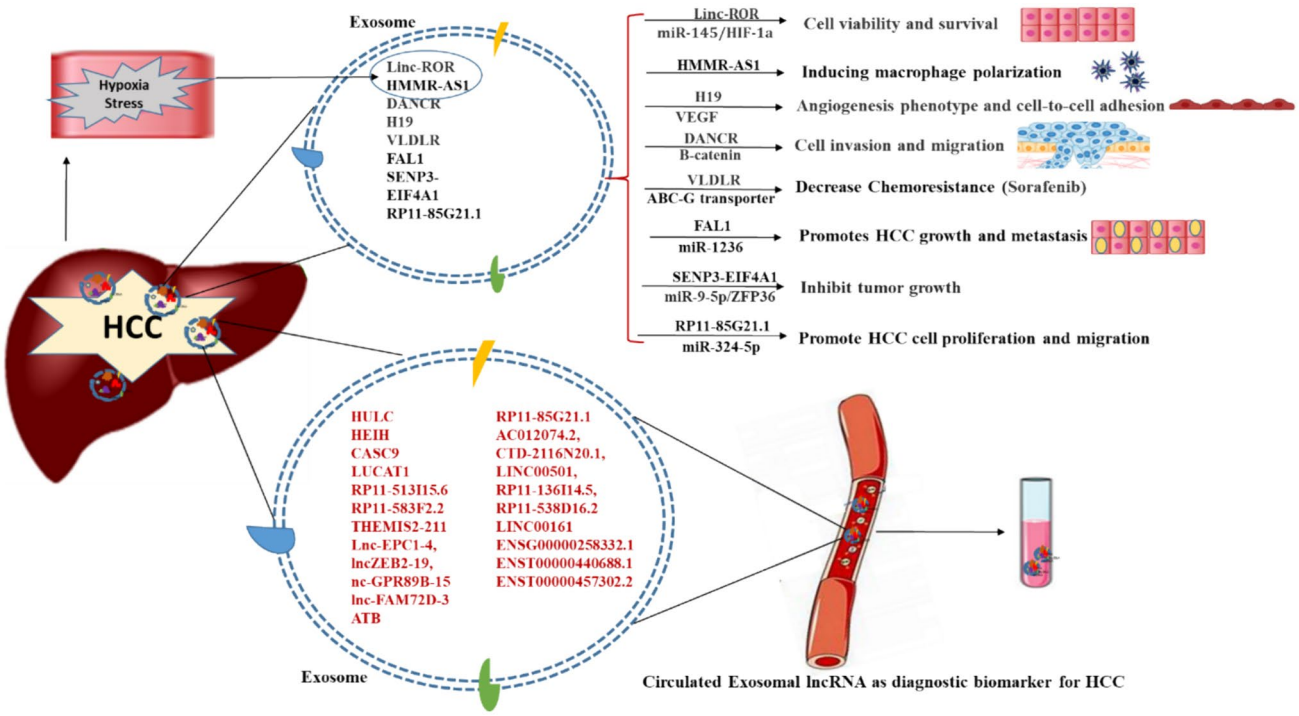

Fig. 2 Involvement of exosomal lncRNA in the pathogenesis and diagnosis of HCC

superfamily G transporter in HCC [71]. Knocking down linc-VLDLR1 leads to a reduction in ABCG2 and enhances sorafenib resistance. Simultaneously, the intracellular transfer of exosomal linc-VLDLR1 can confer resistance to neighboring tumor cells against anticancer treatments [38, 72].

H19 is a paternally imprinted gene located on chromosome 11p15.5 [73]. In line with recent articles, the lncRNA H19 functions as an oncogene in tumorigenesis and tumor progression [74, 75]. LncRNA H19 is enriched in exosomes released from the CD90 + cancer cell. Conigliaro et al. study found that silencing H19 in endothelial cells treated with exosomes will repeal the ability of endothelial cells to produce and release VEGF protein, in consequence, it modulates endothelial cells, and promote angiogenic phenotype and cell-to-cell adhesion. These functions could potentially represent new therapeutic targets for reducing the recurrence and metastasis of hepatocellular carcinoma [76]. Furthermore, H19 was found to be upregulated in serum exosomes during the progression of cholestatic injury and in the cirrhotic model [77, 78].

A recent study highlighted the role of H19 in HCC development, revealing that H19 acts as a molecular sponge for miR-107, thereby promoting CDK6 expression and cell-cycle progression [79]. However, the primary mechanism of H19 involves regulating the expression of microRNA-675 (miR-675), located in the first exon of the H19 gene. The RNA-binding protein human antigen R (HuR) controls the excision of miR-675 from H19. The H19/miR-675 axis impacts oncogenic and tumor-suppressive factors, as miR-675 targets numerous signaling pathways [80, 81]

DANCR: circulating differentiation antagonizing non-protein-coding RNA (DANCR), particularly in its exosomal form, exhibits a positive correlation with mesenchymal-associated β-catenin expression. This correlation is associated with enhanced cell invasion and migration in the context of HCC. Moreover, exosomal DANCR serves as a predictive factor related to the progression and recurrence of HCC [82]. DANCR primarily exerts its oncogenic effects by interacting with the heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), which regulates epithelial–mesenchymal transition in HCC. By directly binding to HNRNPA1, DANCR enhances its stability. In addition, DANCR acts as a molecular sponge for miR-140-3p, preventing miR-140-3p from inhibiting HNRNPA1 translation [83]. Moreover, DANCR has been reported to act as a sponge for miR-125b-5p, activating the MAPK pathway and thereby facilitating HCC progression [84]. miR-222-3p is another miRNA that is inhibited and sponged by DANCR, leading to increased expression of autophagy-related gene 7 (ATG7), which promotes cell proliferation and accelerates autophagy in HCC cells [85].

HULC: in HCC tissues, serum exosomes of HULC showed upregulation compared to healthy controls. The upregulated expression of HULC induced invasion and proliferation while suppressing apoptosis in HCC cells. The study revealed that HULC inhibited the expression of

miR-372-3p, with Rab11a identified as a downstream target of miR-372-3p [86]. In addition, HULC functions as a sponge for miR-186, thereby preventing miR-186 from suppressing oncogene high-mobility group A2 (HMGA2) [87]. Furthermore, in human liver cancer cells, HULC activates the AKT-PI3K-mTOR pathway by inhibiting PTEN, enhancing cellular proliferation and tumor progression, and accelerating autophagy [88]. HULC was reported to sequester miR-107, which normally targets E2F1 mRNA. E2F1, a transcription factor, promotes the expression of sphingosine kinase 1 (SPHK1), a key player in tumor angiogenesis [89]. Another mechanism by which HULC contributes to HCC pathogenesis via facilitating ZEB1-induced epithelial-mesenchymal transition (EMT), which is essential for tumor invasion and metastasis. It does this by targeting and sequestering miR-200a-3p, which inhibits ZEB1 expression [90].

FAL1: FAL1 was found to be overexpressed in the serum exosomes of HCC patients and could be transferred to HCC cells, thereby enhancing their proliferation and migration abilities. As a competing endogenous RNA, lncRNA FAL1 competitively binds with miR-1236 and promotes HCC growth and metastasis [91]. In HCC cells, miR-1236 is recognized as an inhibitor of alpha-fetoprotein (AFP). By targeting AFP, miR-1236 plays a crucial role in regulating the expression of this protein, which is often elevated in HCC and serves as a biomarker for the disease [92]. This mechanism highlights the potential role of FAL1 in driving the progression of HCC and suggests its significance as a therapeutic target and predictive biomarker for this disease.

SENP3-EIF4A1: primarily encapsulated by exosomes, exhibited a significant decrease in HCC tissues and plasma exosomes from HCC patients ($p < 0.05$) [93]. Moreover, exosomal SENP3-EIF4A1 demonstrated an ability to inhibit tumor growth in vivo and modulate ZFP36 expression through competitive binding with miR-9-5p [93]. These findings underscore the potential role of SENP3-EIF4A1 in HCC progression and highlight its interaction with miR-9-5p as a crucial regulatory mechanism in HCC.

TUG1: cancer-associated fibroblasts (CAFs) secreted exosomes promote HCC cell migration, invasion, and glycolysis by delivering TUG1. Silencing TUG1 attenuates these pro-metastatic effects. Our study reveals that TUG1 and SIX1 are transcription factors, targeted by miR-524-5p. Inhibition of miR-524-5p enhances the pro-metastatic effects of CAFs-secreted exosomes, which can be blocked by SIX1 knockdown. These findings suggest that the CAF-exosome/TUG1/miR-524-5p/SIX1 axis plays a crucial role in HCC metastasis, potentially serving as a novel therapeutic target [94].

CASC9 and LUCAT1: Gramantieri et al. study revealed that cancer sensitivity 9 or cancer susceptibility 9 (CASC9) and lung cancer-associated transcript 1 (LUCAT1) might play a regulatory role in suppressing invasion and modulating the epithelial-mesenchymal transition (EMT) process in HCC cells, where knockdown of CASC9 and LUCAT1 has been shown to enhance the invasion capability and cell motility in HCC cells. In addition, their knockdown affects the phenotypes associated with EMT [95]. Both CASC9 and LUCAT1 have been identified as being produced from exosomes. Moreover, a high level of CASC9 has been correlated with tumor growth and postoperative recurrence of HCC. This suggests that CASC9 may have the potential as a non-invasive predictive biomarker for HCC recurrence, highlighting its clinical significance in monitoring disease progression and recurrence in HCC patients [95]. The primary role of CASC9 is to activate the cytoplasmic kinase AKT. CASC9 interacts with the RNA-binding protein (HNRNPL) to form a functional long noncoding ribonucleoprotein (lncRNP) complex in the cytoplasm. However, the precise biochemical mechanism through which the CASC9-HNRNPL complex leads to the phosphorylation and thus activation of AKT was not clearly understood [96]. LUCAT1 inhibited the phosphorylation of Annexin A2 (ANXA2), which reduced the degradation of the ANXA2-S100A10 heterotetramer (AIIt). This stabilization of AIIt facilitated the conversion of plasminogen into plasmin, leading to the activation of metalloproteases. Consequently, this process enhanced metastasis by promoting the breakdown of extracellular matrix components, thereby aiding in cancer cell invasion and spread [97].

Exosomal lncRNA as a prognostic biomarker for HCC

RP11-817I4.1: acts as a prognostic marker for HCC patients and is incorporated into risk assessment models. Its suppression reduces HCC cell growth, movement, and invasiveness. RP11-817I4.1 upregulates lipid levels in HCC cells through the miR-3120-3p/ACLY pathway. RP11-817I4.1, identified as a prognostic marker for HCC patients, is implicated in the regulation of lipid metabolism in HCC cells. Its knockdown inhibits tumor growth, while its presence promotes lipid accumulation via the miR-3120-3p/ACLY axis [98]

HMMR-AS1: is significantly upregulated in HCC tissues and cell lines, indicating its involvement in HCC progression. In vitro studies demonstrate that high HMMR-AS1 expression promotes HCC cell migration, invasion, and proliferation. Furthermore, HMMR-AS1 contributes to the aggressive characteristics of HCC cells by modulating the miR-627-3p/HMGA2 axis. HMMR-AS1 interferes with the activity of miR-627-3p, which in turn enhances the expression of HMGA2, These results suggest that HMMR-AS1 plays a crucial role in the development and progression of HBV-associated HCC [99]. Under hypoxic conditions, the expression of exosomal HMMR-AS1 and ARID3A increased, facilitating their transportation and inducing macrophage polarization. Furthermore, evidence from the

study demonstrated that hypoxic exosomes promoted tumor cell proliferation [88].

RP11-556E13.1: is highly upregulated in HCC tissues compared to adjacent normal tissues ($p < 0.05$, fold change = 20.24), with this upregulation observed in 112 tissue pairs ($p < 0.05$). Increased RP11 expression correlates with worse clinical outcomes, including larger tumor size, poorer differentiation, and shorter survival (all $p < 0.05$). In vitro studies show that silencing RP11 in HCC cells inhibits its proliferation, promotes apoptosis, and induces G1/S cell-cycle arrest, accompanied by increased cleaved PARP1 and cleaved caspase-3 expression. Further analysis suggests that RP11 knockdown affects specific cellular pathways implicated in HCC development [100].

Exosomal lncRNA as a diagnostic biomarker for HCC

HEIH: also known as hepatocellular carcinoma upregulated EZH2-associated lncRNA, is highly expressed and specifically enriched in HCC. This lncRNA is associated with EZH2, a key player in the expression of EZH2-regulated target genes. Studies have revealed that lncRNA-HEIH plays a crucial role in cell-cycle regulation in HCC, suppressing cell differentiation in the G0/G1 phases [101]. LncRNA-HEIH is identified as a cancer-promoting lncRNA that enhances the carcinogenesis of hepatitis B virus-related hepatocellular carcinoma. It emerges as a potential target in the diagnosis and therapy of HCC. In addition, exosomal lncRNA-HEIH has been detected with high expression in HCC patient serum, suggesting its potential as a non-invasive biomarker for HCV-related HCC diagnosis [102].

RP11-513I15.6 and RP11-583F2.2: studies have shown that lncRNA-RP11-513I15.6 and RP11-583F2.2 possess excellent sensitivity and specificity in distinguishing HCC patients from those with chronic HCV infection and control subjects [103, 104]. Simultaneously measuring the levels of RP11-513I15.6, RP11-583F2.2, and AFP in serum exosomes significantly enhances diagnostic sensitivity and accuracy for early HCC detection, reducing false-negative errors compared to AFP alone. These findings indicate the diagnostic and therapeutic potential of these biomarkers in HCC patients.

THEMIS2-211: it was identified that the diagnostic superiority of a panel comprising exosomal lncRNA-THEMIS2-211 and PRKACA-202 over AFP in diagnosing HCC. In addition, exosomal THEMIS2-211 alone exhibited superior performance compared to AFP in diagnosing early-stage HCC patients (stage I) [105].

Lnc-EPC1-4, lncZEB2-19, lnc-GPR89B-15, and lnc-FAM72D-3: the study found these lncRNA exhibited differential expression patterns in HCC compared to patients with hepatitis, cirrhosis, and healthy controls. In addition, the expression levels of exosomal lnc-EPC1-4 and lnc-GPR89B-15 were correlated with serum AFP levels. Knockdown of lnc-FAM72D-3 promoted apoptosis and reduced cell viability, suggesting its oncogenic role in HCC. Conversely, overexpression of lnc-EPC1-4-induced cell apoptosis and suppressed cell proliferation, indicating its tumor-suppressive functions [106].

ATB: it was found that higher levels of lncRNA-ATB with miR-21 serve as independent biomarkers for disease progression and mortality in HCC patients. In addition, higher circulating exosomal lncRNA-ATB and miR-21 exhibited significantly lower OS and progression-free survival [66].

RP11-85G21.1: a novel plasma exosomal lncRNA, RP11-85G21.1, was found to promote HCC cell proliferation and migration by targeting and modulating miR-324-5p. In addition, the serum level of RP11-85G21.1 demonstrated high accuracy in distinguishing AFP-negative HCC from both liver cirrhosis and healthy controls [107]

Other exosomal LncRNAs in HCC

Several clinically significant exosomal lncRNAs have recently been identified as crucial predictors of HCC. Notably, exosomal levels of LINC00161 were found to be elevated in patients with HCC compared to healthy individuals [108]. Furthermore, blood exosomal ENSG00000258332.1 and LINC00635 exhibited upregulation in HCC patients compared to those with hepatitis B virus (HBV), and their levels decreased after tumor resection [109]. In addition, exosomal ENSG00000248932.1, ENST00000440688.1, and ENST00000457302.2 were observed to be increased in HCC [110]. Exosomes derived from HCC, namely AC012074.2, CTD-2116N20.1, LINC00501, RP11-136I14.5, and RP11-538D16.2, exhibited prognostic significance, with their expression levels correlating with overall survival outcomes. Specifically, RP11-538D16.2 and CTD-2116N20.1 were identified to exacerbate the prognosis of HCC patients by modulating the expression levels of proteins within exosomes [111]. In conclusion, the aforementioned exosomal lncRNAs hold promise as novel diagnostic biomarkers for the development of hepatic cancer. However, more in-depth studies are required to verify their potential mechanisms in HCC (Fig. 2).

Exosomal lncRNA in liver fibrosis

The accumulation of extracellular matrix (ECM) due to continuous healing of liver injury may progress to liver cirrhosis and hepatocellular carcinoma. Several studies have elucidated the significant role of lncRNAs in regulating liver fibrosis [53, 112, 113]. Exosomes, secreted upon hepatocyte damage, play a crucial role in the development of liver fibrosis by controlling important processes. Moreover, sinusoidal

epithelial cells and circulating cells also release exosomes, further influencing the progression of hepatic fibrosis [114–116]. In addition, there is growing evidence demonstrating the potential of exosomal lncRNAs in liver fibrosis, spanning pathogenesis, diagnosis, and treatment (Fig. 3).

MALAT1: it is among the extensively studied nuclear-limited lncRNAs due to its high expression levels and established roles in various pathological conditions [117]. Its upregulation is predominantly linked to inflammation and fibrosis in nonalcoholic steatohepatitis (NASH) patients [53]. Specifically, in fibrotic liver tissues characterized by activated hepatic stellate cells, MALAT1 expression inversely correlated with miR-101b levels [118]. Recent investigations have highlighted that arsenite-treated hepatic cells induce the release of exosomes containing MALAT1, which in turn regulate the expression of miR-26b/Col1α2 in stellate cells [119]. Moreover, fluctuations in circulating exosomal MALAT1 levels during the progression of hepatic fibrosis suggest its potential utility as a diagnostic biomarker.

H19: in various cholestatic liver fibrosis models, it has been observed that the serum level of exosomal H19 released from cholangiocytes correlates with the progression of hepatic fibrosis [78, 120, 121]. The expression of H19 in cholangiocytes and its release in exosomes are regulated by the ERK1/2 signaling pathway [78, 122]. Exosomal H19 released from cholangiocytes is taken up by hepatocytes and stellate cells; within hepatocytes, exosomal H19 modulates bile acid homeostasis by inhibiting the small heterodimer partner (SHP) [123]. A study by Liu et al. demonstrated that exosomal H19 derived from cholangiocytes induces cholestatic liver fibrosis, with exosomal H19 from hepatic stellate cells (HSCs) activating and enhancing HSC proliferation and increasing the expression of profibrotic genes [121]. In addition, in liver fibrosis, H19 functions as a competing endogenous RNA for the let-7 family, leading to increased levels of high-mobility group AT-hook 2 (HMGA2), which promotes cholangiocytes proliferation [120].

CYTOR: Xu et al. investigated the role of lncRNA CYTOR in liver fibrosis using an exosome-based model. Exosomes derived from normal and damaged liver cells were injected into both normal and fibrosis-induced mice. Exosomes isolated from normal liver cells demonstrated a therapeutic effect on liver fibrosis, suggesting a protective role in liver homeostasis. Silencing lncRNA CYTOR within these exosomes significantly attenuated fibrosis severity, highlighting its involvement in disease progression. Furthermore, the study revealed that lncRNA CYTOR expression modulated the levels of miR-125b, GDNF, and key liver fibrosis markers. These findings demonstrate that downregulating lncRNA CYTOR within exosomes effectively inhibits liver fibrosis development in vivo, suggesting a potential therapeutic target for this disease [124].

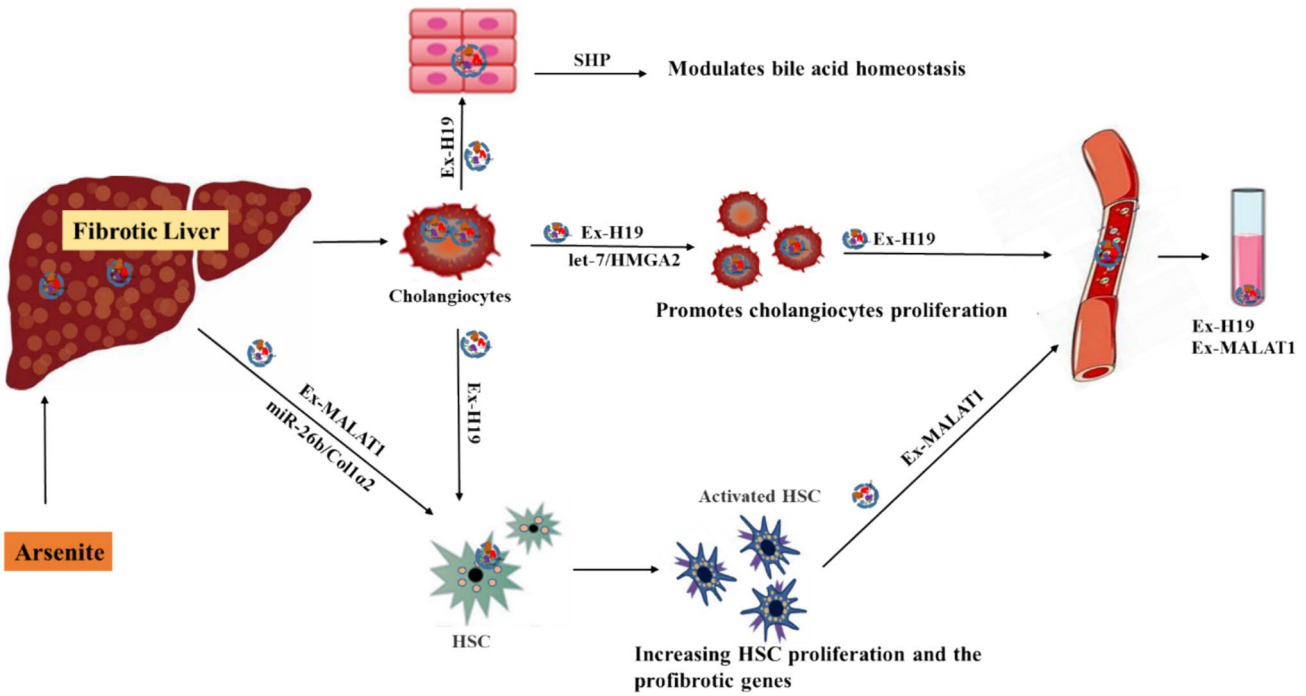

Fig. 3 Involvement of exosomal lncRNA in the pathogenesis and diagnosis of hepatic fibrosis

Exosomal lncRNA in viral hepatitis

In the context of viral hepatitis, variations in gene expression during liver infection are associated with susceptibility to and prognosis of hepatitis C virus (HCV)-induced hepatocellular carcinoma [125]. Studies on HCV patients and infected cells have revealed that plasma-derived exosomes contain full-length viral RNA and protein [126–130]. These exosomes facilitate HCV transmission between cells through intracellular communication, potentially influencing viral replication and transmission [127, 128, 131].

DANCR: in HCV infection, circulating exosomes derived DANCR are elevated and may serve as a biomarker for HCV-related HCC progression and recurrence [82]. Moreover, clinical examinations have shown upregulation of exosomal lncRNA-HEIH levels, indicating its potential as a novel biological marker for HCV-associated HCC [102].

In hepatitis B virus (HBV) infection, a newly identified exosomal lncRNA, ZSCAN16-AS1, is increased and positively correlates with liver injury biomarkers involved in the model for end-stage liver disease (MELD) score. However, further studies are needed to elucidate the exact mechanism of this lncRNA in HBV-infected liver [132].

Further investigations into exosomal-derived lncRNAs are necessary to elucidate their roles in the pathophysiology, transmission, and progression of HBV-infected liver.

Exosomal lncRNAs as clinical non-invasive biomarkers for liver disease

Exosomal content serves as a promising predictor for various diseases and their progression, specifically, the levels and types of exosomal lncRNA in circulation serve as unique biomarkers for several diseases, including liver disease. Blood serum, plasma, or urine are commonly utilized as sample sources for exosomal lncRNA when employed as non-invasive biomarkers (Fig. 4).

Clinically, in HCC patients, lncRNA-ATB holds potential as a prognostic biomarker for disease prognosis. Elevated levels of exosomal lncRNA-ATB in circulation are linked to a markedly reduced survival rate among patients. This biomarker enables non-invasive prognostic prediction, independent of age, sex, presence of liver cirrhosis, or etiology [66]

Clinically biomarker panel comprising lncRNA RP11-513I15.6, along with miR-1262 and RAB11A, exhibits excellent sensitivity and specificity, enabling the differentiation of HCC patients from those with chronic HCV infection and individuals with other health conditions [103].

The serum levels of lncRNA-HEIH are higher in HCV-related HCC patients compared to chronic HCV patients. Tests conducted on serum lncRNA-HEIH demonstrate its potential utility as a non-invasive biomarker for diagnosing and screening HCV-related HCC. In addition, serum exosomes can function as gene transfer vectors and exert regulatory effects on cancer metastasis [102]. Furthermore, the combined analysis of serum exosomes utilizing ENSG00000258332.1, LINC00635, and serum AFP may

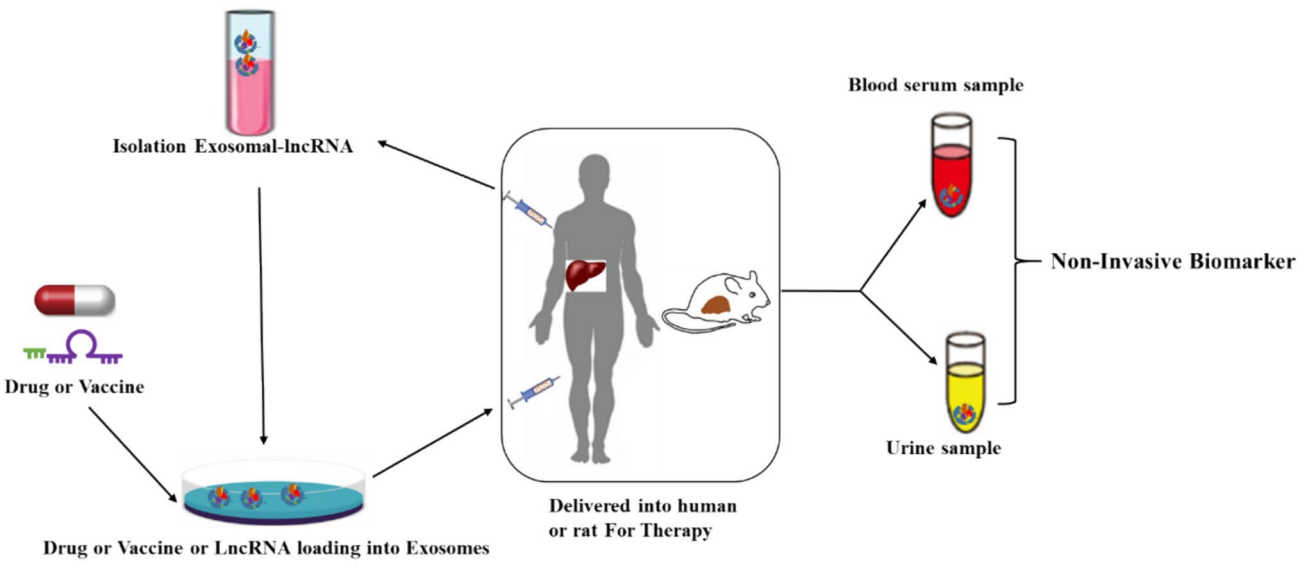

Fig. 4 Exosomes as a non-invasive potential biomarker and deliver therapeutic payloads including lncRNA, drugs, and vaccines

represent a novel and valuable biomarker for liver cancer [109].

Sensitivity, specificity, and reliability of exosomal lncRNAs as biomarkers

The specificity of lncRNAs in liver disease refers to their unique expression patterns, functions, and regulatory roles in the context of liver pathology. While some lncRNAs may have broad roles across different tissues or diseases, others exhibit specific regulation and activity in liver-related processes. One aspect of specificity in lncRNAs in liver disease is their tissue-specific expression. Certain lncRNAs are predominantly expressed in liver cells or tissues, indicating their potential involvement in liver-specific functions and diseases. Moreover, the dysregulation of specific lncRNAs in different liver diseases can serve as potential biomarkers for disease diagnosis, prognosis, or treatment response. The specificity of lncRNAs as biomarkers can be attributed to their unique expression profiles or functions that are associated with specific liver pathologies. From a clinical viewpoint, AFP serves as the primary serum biomarker used in the diagnosis of HCC [133]. By synthesizing data from various clinical studies—summarized in Table 3—that explore the potential of exosomal lncRNAs as biomarkers for HCC patients compared to AFP level. Some studies have shown promising biomarkers for HCC in contrast to healthy individuals including RP11-513I15.6, RP11-583F2.2, HOTAIR, BRM, ICR, MyD88, THEMIS2-211, lnc85, and CASC2 [103–105, 107, 134–137]. Furthermore, our findings demonstrate that these exosomal lncRNAs exhibit high specificity and sensitivity, bolstering their efficacy as diagnostic tools for HCC and thereby enhancing diagnostic sensitivity.

Despite the promising initial findings, several challenges remain in translating exosomal lncRNA biomarkers into routine clinical practice, further larger, well-designed clinical trials are needed to validate the diagnostic and prognostic utility of these biomarkers in diverse patient populations, ethnicities, ages, sexes, and co-morbidities that may influence exosomal lncRNA expression. This will help determine the generalizability of findings and establish the reliability of exosomal lncRNA biomarkers in real-world clinical settings.

Potentiality and challenges of exosomal lncRNAs as therapy and diagnostic biomarkers for liver disease

As a potential therapeutic delivery system, exosomes display several characteristics that make them attractive as therapeutic delivery vehicles. Their biocompatibility and low immunogenicity contribute to minimal rejection and adverse effects in treated subjects. Furthermore, exosomes efficiently transport bioactive substances, modulating cellular signaling pathways and promoting a favorable therapeutic outcome. Their ability to target specific cells enhances therapy precision and efficacy by facilitating the targeted release of genes, drugs, or bioactive substances [124]. Despite their promise, exosomes face limitations as a therapeutic modality. The complex and costly acquisition and purification process hinders their widespread clinical application. In addition, exosomes require stringent storage conditions to maintain stability and activity. Further clinical trials are needed to rigorously assess the safety and efficacy of exosome-mediated therapy for liver diseases.

As biomarker, exosomes hold significant potential as a source of biomarkers, including lncRNAs; however, their low expression levels combined with the challenges of exosomal purification pose significant hurdles. The low abundance of many lncRNAs, coupled with the inherent difficulties in isolating exosomes with high purity and yield, can significantly impact the sensitivity and reliability of lncRNA-based exosome diagnostics. Furthermore, the heterogeneity of exosomes within a sample adds another layer of complexity to the analysis. To overcome these challenges, researchers are exploring various strategies including: microfluidic platforms and nanomaterial-based

Table 3 Sensitivity and specificity of the current identified diagnostic exosomal lncRNA

LncRNA	AUC	Specificity	Sensitivity	*p* value	Reference
RP11-513I15.6	0.963	95%	96.7%	p < .01	[103]
RP11-583F2.2	0.934	96.7%	95%,	p = .000**	[104]
MyD88	0.776	80.0%	74.5%	0.000	[134]
CASC7	0.808	95.2%	63.8%	< 0.001	[135]
THEMIS2-211	0.818	82.8	70.8	p < .01	[105]
lnc85	0.883	76.5%	80.5%	p < .005	[107]
CASC2	–	60.5%	97.2%	–	[136]
HOTAIR	0.991	95.0	96.7	< 0.001	[137]
BRM	0.983	95.0	98.4	0.001	[137]
ICR	0.966	85.0	98.4	0.001	[137]

approaches, immunomagnetic beads, and covalent chemistry into exosome isolation protocols to provide a multidirectional approach for enhancing yield, purity, and preserving exosome bioactivity [138, 139]. These innovative strategies hold significant potential for overcoming the limitations of traditional methods and accelerating the clinical translation of exosome research. In addition, enhanced sensitive detection methods through advancements in next-generation sequencing (NGS), digital PCR, and other sensitive detection techniques are crucial for detecting low-abundance lncRNAs in exosomes. Sophisticated bioinformatics algorithms are being developed to analyze complex exosome data, identify lncRNA biomarkers, and distinguish between true signals and noise.

Despite these challenges, the potential benefits of using exosomal lncRNAs as vehicles for target delivery in therapy and potential biomarkers are considerable. Their involvement in diverse cellular processes, their stability in biological fluids, their potential to reflect the state of the cell of origin, their potential for engineering to enhance targeting specificity, and their protective capacity for delivering therapeutic cargo makes them attractive targets for disease therapy, diagnosis, and monitoring.

Anticipated advancement in the involvement of exosomal lncRNAs in liver diseases

Take advantage of leveraging the transport function of exosomes, drugs such as chemotherapeutics, proteins, or nucleic acids can be encapsulated within exosomes and precisely delivered to different target cells, thereby potentially reducing drug toxicity. Exosomes can be isolated from cell cultures derived from patients or plants, with the liver being the primary target for exogenous exosomes.

LncRNAs can serve as therapeutic agents when encapsulated and delivered via exosomes to target organs and cells, for example, exosomal H19 obtained from mesenchymal stem cells (MSCs) could enhance ulcer healing in diabetic foot ulcers by competitively binding with microRNA-152-3p [140]. Although exosomal targeted therapy has shown promise in numerous animal experiments [141] and many exosomal miRNAs have been utilized in therapy in animal experiments [142, 143], further researches were needed to validate the efficacy of exosomal lncRNAs in the treatment of liver diseases.

In the upcoming years, circulating exosomal lncRNAs hold promise as targets for liquid biopsy and non-invasive biomarkers facilitating early detection, diagnosis, and treatment of HCC; however, continued progress in exosome isolation and characterization techniques is needed to refine the extraction of exosomes from peripheral blood, paving the way for their broader use as biomarkers and liquid biopsy tools in preclinical and clinical settings.

Future research directions and potential therapeutic avenues of exosomal lncRNA could be directed toward pioneering personalized therapeutic approaches based on the unique exosomal lncRNA profiles of individual patients. In addition, modifying treatment strategies to target specific disease-associated lncRNAs encapsulated within exosomes could enhance therapeutic efficacy and minimize adverse effects. As well as utilized the natural nanocarriers characteristic of exosomes for delivering therapeutic cargoes, including small molecules, RNA-based therapeutics, or gene editing tools. Leveraging the inherent targeting capabilities of exosomes to deliver therapeutic agents specifically to diseased liver cells holds great promise. Furthermore, translating discoveries in exosomal lncRNA biology into clinically relevant diagnostic tools and therapeutic interventions will be crucial for improving patient outcomes.

In conclusion, this review paper has provided insights into the dynamic role of exosomal lncRNA in liver diseases, examining their involvement in pathogenesis and diagnostic aspects, thus underscoring their significant implications for liver disease management. However, further research is necessary to delve deeper into the complex interactions of exosomal lncRNAs in liver pathogenesis, and future clinical studies with a larger sample size are needed to enhance the reliability of findings.

This review sets the stage for future exploration and implementation of exosomal lncRNAs in liver diseases, representing a crucial advancement towards enhancing patient outcomes and propelling the field of precision medicine in hepatology.

Author contributions MI was responsible for manuscript writing, data curation, and literature search. MI and LS did manuscript conceptualization. MM, RT, and OS participated in manuscript writing and editing. ME and BA participated in manuscript editing and data curation. ZJ, LZ, and LS participated in research design and editing. The authors approved the final manuscript.

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Data availability The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

Declarations

Conflict of interest Mohammed Ismail, Missaa M. Fadul, Reham Taha, Orwa Siddig, Muhanad Elhafiz, Bashir A. Yousef, Zhenzhou Jiang, Luyong Zhang, and Lixin Sun declare no conflict of interest.

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