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# Advances and challenges of exosome-derived noncoding RNAs for hepatocellular carcinoma diagnosis and treatment

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ABSTRACT

Exosomes, also termed extracellular vesicles (EVs), are an important component of the tumor microenvironment (TME) and exert versatile effects on the molecular communications in the TME of hepatocellular carcinoma (HCC). Exosome-mediated intercellular communication is closely associated with the tumorigenesis and development of HCC. Exosomes can be extracted through ultracentrifugation and size exclusion, followed by molecular analysis through sequencing. Increasing studies have confirmed the important roles of exosome-derived ncRNAs in HCC, including tumorigenesis, progression, immune escape, and treatment resistance. Due to the protective membrane structure of exosomes, the ncRNAs carried by exosomes can evade degradation by enzymes in body fluids and maintain good expression stability. Thus, exosome-derived ncRNAs are highly suitable as biomarkers for the diagnosis and prognostic prediction of HCC, such as exosomal miR-21-5p, miR-221-3p and lncRNA-ATB. In addition, substantial studies revealed that the up-or down-regulation of exosome-derived ncRNAs had an important impact on HCC progression and response to treatment. Exosomal biomarkers, such as miR-23a, lncRNA DLX6-AS1, miR-21-5p, lncRNA TUC339, lncRNA HMMR-AS1 and hsa_circ_0004658, can reshape immune microenvironment by regulating M2-type macrophage polarization and then promote HCC development. Therefore, by controlling exosome biogenesis and modulating exosomal ncRNA levels, HCC may be inhibited or eliminated. In this current review, we summarized the recent findings on the role of exosomes in HCC progression and analyzed the relationship between exosome-derived ncRNAs and HCC diagnosis and treatment.

## 1. Introduction

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death worldwide and the most common subtype of liver cancer [1]. In addition, the lack of surveillance and therapeutic options is largely responsible for the high mortality rate of HCC patients [2]. The pathogenesis of HCC is associated with diverse factors, such as alcohol consumption, chronic hepatitis B (HBV) infection, aflatoxin B1 intake and non-alcoholic fatty liver disease (NAFLD) [3]. These factors contribute to the mutations of cancer-related genes, the silence of tumor suppressive genes and the activation of oncogenes and then promote HCC development [4]. Currently, several strategies for early-stage diagnosis and therapeutic targets have been developed in HCC management [4,5], but the therapeutic effect remains not ideal. Therefore, developing novel diagnostic/therapeutic biomarkers to improve the clinical outcomes of patients with HCC is still urgent.

Exosomes, also termed extracellular vesicles (EVs) and intraluminal vesicles (ILVs), are nano-scale membrane vesicles derived from nearly all types of cells and are distributed in various body liquids and organs [6–8]. Recently, a great number of studies have revealed that exosome-mediated molecule communications among live cells have significant impacts on the physiological state of recipient cells, and are attributed to the tumorigenesis of HCC [9,10]. Exosomes typically exert regulatory effects by transferring biologically functional molecules, including lipids, proteins, messenger RNAs (mRNAs), non-coding RNAs and DNA [11,12]. During sheep reticulocyte culture in 1986, Johnstone and coworkers discovered and harvested exosomes [13]. However, at that time, exosomes were considered as "garbage" resulting from the shedding of specific membrane functions, without much understanding of their structural and biological significance [13]. It wasn't until 1996 when that Raposo and colleagues made a significant discovery [14]. MHC class II molecules are secreted by both mouse and human B lymphocytes. In addition, exosomes transferred MHC molecules to the plasma membrane, inducing antigen-specific class II-restricted T cell

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responses [14]. Then, researchers first realized that exosomes might have significant biological functions. In 2007, Valadi et al. discovered that mRNAs and microRNAs derived from exosomes can be transferred to other cells, and the transferred mRNA molecules are capable of translation [15].

Subsequently, researchers found that both stromal and cancer cells in the tumor microenvironment (TME) secrete exosomes, which play a modulatory role in tumor progression through molecular exchange [16,17]. Researchers further analyzed function molecules derived from exosomes, and found that exosomes can also exert their effects by transferring proteins, DNA, peptides, lipids, and other molecules [18]. After that, functional exosomes have been detected in serum, gastric juice, lymph, bile, saliva, urine, plasma, breast milk, bronchial fluid, synovial fluid, amniotic fluid, semen and cerebral spinal fluid (CSF) [19–32]. Recently, growing evidence have revealed that exosomes and their cargos exert crucial effects on the tumorigenesis and progression of HCC [33–35].

Among the molecules carried by exosomes, non-coding RNAs (ncRNAs) are one type of the most abundant and stable molecules [7]. In addition, growing evidence have revealed the functional regulation of ncRNAs, such as microRNA, long non-coding RNA and circular RNA (circRNAs), in HCC pathogenesis and progression [36,37]. Recent findings found that many ncRNAs are specifically transferred into exosomes and exosome-derived ncRNAs could regulate the progression and treatment response in HCC [38–40]. For example, Zhang and coworkers found that exosome-depleted miR-148a-3p derived from activated hepatic stellate cells was able to accelerate HCC progression by regulating ITGA5/PI3K/Akt pathway [41]. Besides, LINC00511 regulated invasive behavior to further promote HCC progression by regulating exosome secretion [42]. Then, targeting exosomes or their cargo may provide a novel approach to the therapeutic strategy of HCC. In the current review, retrieval of relevant studies, published in English between 1990 and 2023, was conducted in PubMed and Web of Science online databases using the following keywords: “hepatocellular carcinoma” or “liver cancer” and “non-coding RNA” “exosome” or “extracellular vesicles” Eligible studies were screened by two investigators by going through the title, abstract, and full text of each article. we have summarized the occurrence and features of exosomes and analyzed their relevance to HCC. Additionally, we have explored the potential applications of exosomes in HCC diagnosis and treatment, aiming to provide new insights for the management and prevention of HCC.

## 2. Biological genesis and basis of exosome

Exosomes are membranous structures secreted by various cells, and the classification of these membranous structures is based on the size of their diameter [43–45], such as exosomes (with a 30–150 nm diameter), ectosomes (with a 50–10000 nm diameter), apoptotic bodies (with a 1000–5000 nm diameter), and other vesicles (Fig. 1). Although the exact mechanism of the biogenesis of exosomes remains not very clear, the involvement of several pathways, including the ESCRT mechanism, has been confirmed by numerous studies (Fig. 2) [46,47]. As the first identified pathway associated with exosome biogenesis in 2001 [48], the ESCRT pathway is consist of four ESCRT complexes (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III), disassembly and deubiquitylating enzymes and many proteins of Vps family, such as Vps2, Vps4, Vps22, Vps24 and Vps25 [46,49,50]. In addition to the ESCRT pathway, several other mechanisms, such as the syndecan-syntenin-Alix pathway [51] and lipid-mediated exosome biogenesis [52] were also revealed in recent studies (Fig. 2). In addition, Rojas-Gomez and coworkers recently revealed that the production of exosomes can be regulated by chaperonin chaperonin-containing TCP1 [53]. Therefore, in the future, it is possible to regulate the formation and secretion of exosomes through specific targets.

## 3. Characteristics and hallmarks of exosomes

Recent analyses of exosomal cargos and biomarkers showed that some trafficking effectors are responsible for the cargo loading of exosomes, and are associated with the biogenesis of exosomes [54]. In addition, there are some molecules are packaged into exosomes specifically [40]. Some widely distributed exosomal proteins can serve as markers for exosome quantification and identification, such as HRS, CD63, CD9, ALIX, CD81, tumor susceptibility gene 101 (TSG101), VPS4 and heat shock protein 70 (HSP70) [7,43,55,56]. In addition, calnexin is not expressed in exosomes, thus it is often used as a negative marker [57]. Typically, the presence of 3 positive markers and 1 negative marker is considered sufficient for the identification of exosomes, although there is still no definitive standard.

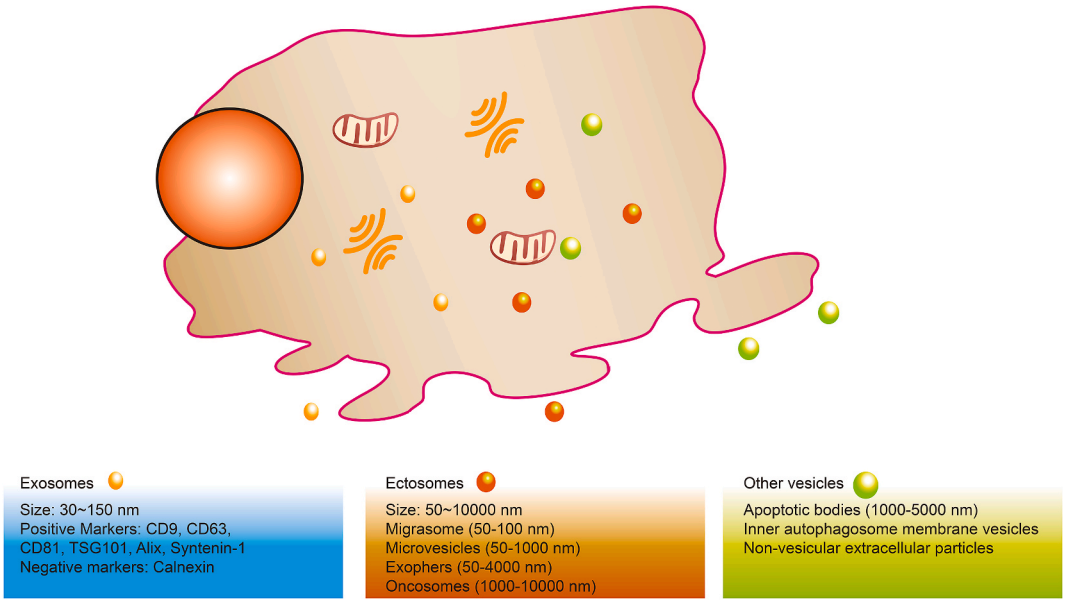


**Fig. 1.** The classification of these membranous structures based on the size of their diameter: exosomes are characterized by a 30–150 nm diameter, ectosomes are characterized by a 50–10000 nm diameter, apoptotic bodies and other vesicles are characterized by a 1000–5000 nm diameter.

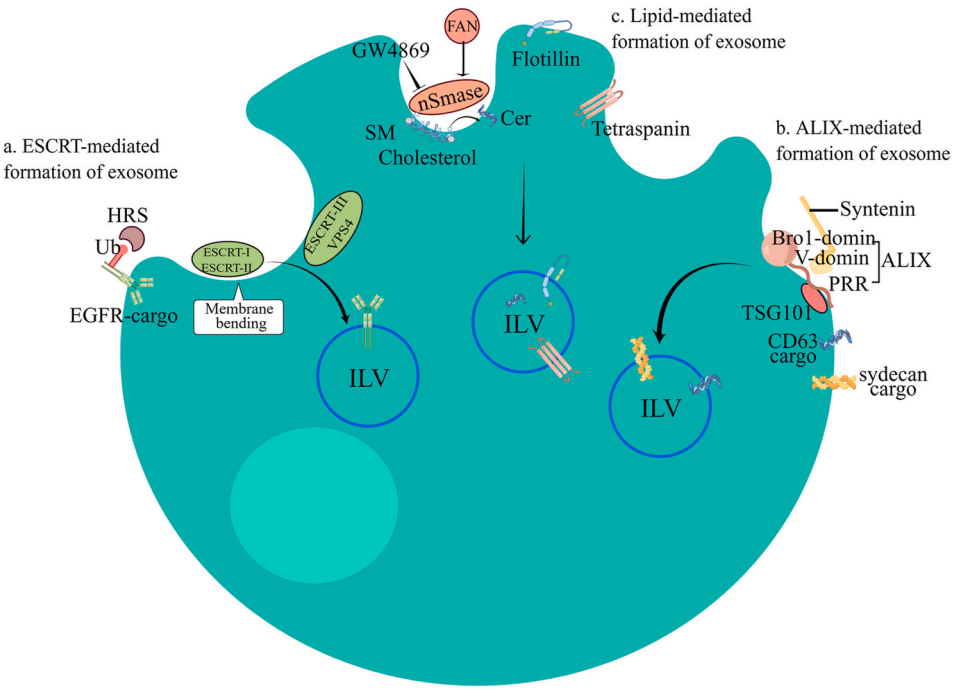


**Fig. 2.** The mechanisms of biological genesis of exosomes. (a) The ESCRT-0 complex (HRS–STAM dimers) and other ESCRT components cluster ubiquitylated cargoes and recruit flat clathrin domains on the endosome to incorporate them into ILVs. ESCRT-I and ESCRT-II are responsible for membrane bending, while ESCRT-III filaments facilitate membrane scission, working together with VPS4. (b) Syntenin interacts directly with ALIX through Bro1-domin and V-domin, similarly to retroviral proteins, and supports the intraluminal budding of endosomal membranes. Syntenin exosomes depend on the availability of heparan sulphate, syndecans, ALIX and impact on the trafficking and confinement of FGF signals. (c) Lipid-mediated formation of exosome: Lipids and membrane proteins may promote ILV generation by acting as cone-shaped wedges that bend the endosome membrane. nSMase2 removes the head group of SM to Cer, a cone-shaped lipid sufficient for in vitro ILV formation. nSMase2 is activated by factor associated with FAN and is pharmacologically inhibited by the small molecule GW4869.
ILVs: intraluminal vesicles; Dubs: deubiquitylating enzymes; Ub: ubiquitin; PRR: proline-rich region; nSMase2: neutral sphingomyelinase 2; SM: sphingomyelin; Cer: ceramide; FAN: factor associated with nSMase.

## 4. Exosomal ncRNAs and HCC

Over the past few decades, a growing number of studies have noticed the important role of ncRNAs in cancer development and progression, including HCC [58–61]. Several characteristics distinguish liver cancer from other cancers, including hypoxic microenvironment, HBV and HCV infections. These factors promote the initiation and development of HCC. For example, HBV infection and HCV infection remain the most common risk factors for HCC worldwide [62]. In 2009, Zhang and co-workers revealed that increased miRNA-143 transcribed by nuclear factor kappa B promoted hepatocarcinoma metastasis by repressing fibronectin expression [63]. Besides, other ncRNAs also exhibited a modulatory role in HBV infection and HCC progression [64–66].

Recently, increasing studies revealed that numerous miRNAs, lncRNAs and circular RNA can be transferred into other cancer cells through exosomes to regulate HCC progression and therapeutic efficacy [67–69]. In 2011, Kogure and colleagues first found that the intercellular nanovesicle-mediated microRNA transfer has an impact on the cell growth HCC by regulating the tumor microenvironment [70]. Subsequently, Singer et al. found that Nup98 was reduced in human HCC and correlated to p21 mRNA levels [71]. Further investigation showed that Nup98 protected p21 mRNA from degradation by the exosome and then inhibited HCC progression. After that, exosome-mediated transfer of lncRNA ROR was found to exert modulatory effects on chemosensitivity in human HCC [72]. After that, the role of exosomal ncRNAs in HCC attracted increasing attention among researchers.

### *4.1. Association between exosome-derived ncRNAs and HCC progression*

Recently, some studies have revealed the association between well-known risk factors with HCC and ncRNAs [73,74]. For example, Zhao et al. identified an HBV-encoded miRNA, miR-3, that restrained the HBV replication by targeting the HBV transcript [73]. Furthermore, miR-222 could mediate the promotion of liver fibrosis by exosomes derived from hepatitis B virus-infected hepatocytes [74]. In addition, previous studies also revealed an association among ncRNAs and alcohol consumption [75], NAFLD [76,77] and aflatoxin B1 [78,79]. This evidence indicated that exosome-derived ncRNAs play an important role in the tumorigenesis of HCC (Fig. 3).

After tumor formation, the invasion and migration of HCC cells are tightly associated with tumor progression [80]. Therefore, investigating the factors that are associated with tumor cell invasion and migration is important for a comprehensive understanding of HCC development, and then exploring potential therapeutic targets. Recently, a growing number of studies have identified that some exosome-derived ncRNAs were associated with HCC progression (Table 1) [38]. For example, HCC cell-derived exosomal miR-1247-3p promotes the lung metastasis of HCC by inducing cancer-associated fibroblast activation [38]. Exosomal lncRNA SENP3-EIF4A1 was significantly decreased in HCC tissues and exosome-transmitted SENP3-EIF4A1 weakened the invasive and migrative abilities of HCC cells [39]. Xu et al. found that macrophage-derived exosomes could facilitate HCC cell proliferation by transferring lncMMPA to HCC cells and activating the glycolysis pathway [81]. In addition, exosome-transmitted circ_MMP2 enhanced the migrative ability of HCC cells by upregulating MMP2 [33]. Exosomal miR92a-3p derived from High-metastatic HCC cells promoted the epithelial-mesenchymal transition and metastasis of low-metastatic HCC cells by activating the PTEN/Akt pathway in HCC [82]. Besides, the relationship between HCC progression and many exosome-derived ncRNAs, such as miR-21 [83], miR-628-5p [84], miR-122 [85], lncRNA ASMTL-AS1 [86], circ-DB [87], lncRNA DLX6-AS1 [88], lncRNA TUC339 [89], hsa_circ_0004658 [90], hsa_circ_0051443 [91],

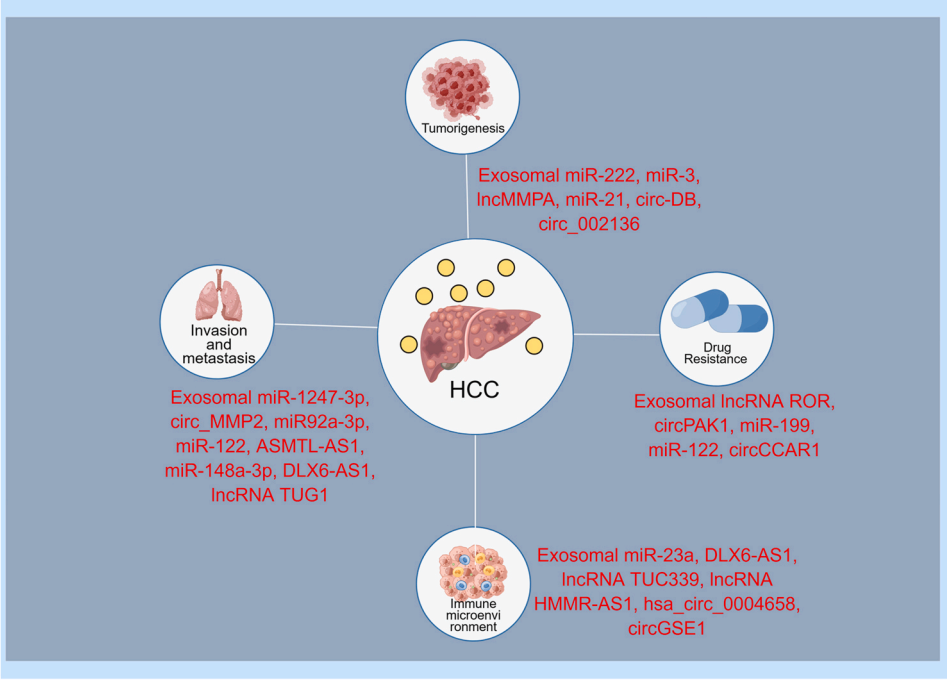


**Fig. 3.** Exosomal ncRNAs regulated the tumorigenesis, invasion and metastasis, drug resistance and immune microenvironment of HCC.

**Table 1**
Exosome-derived ncRNAs and HCC progression.

| NcRNA | Role in HCC progression | Refence |
|---|---|---|
| lncRNA ROR | Modulates chemosensitivity | [72] |
| miR-3 | Activates innate immunity to restrict HBV replication | [73] |
| miR-222 | Promote liver fibrosis | [74] |
| miR-1247-3p | Foster lung metastasis | [38] |
| lncRNA SENP3-EIF4A1 | Weaken the invasion and migration abilities of HCC cells | [39] |
| lncMMPA | Promote glucose metabolism and cell proliferation in HCC | [81] |
| circ_MMP2 | Promote HCC metastasis | [33] |
| miR92a-3p | Promote epithelial-mesenchymal transition and metastasis | [82] |
| miR-21 | Convert hepatocyte stellate cells to cancer-associated fibroblasts | [83] |
| miR-122 | Promote HCC progression | [85] |
| lncRNA ASMTL-AS1 | Aggravate the malignancy in residual HCC | [86] |
| circ-DB | Promote HCC growth and reduce DNA damage | [87] |
| miR-148a-3p | Impair the proliferation and invasiveness | [41] |
| lncRNA DLX6-AS1 | Promote migration and invasion | [88] |
| hsa_circ_0051443 | Suppress the malignant biological behaviors | [91] |
| lncRNA TUG1 | Promote HCC cell migration, invasion, and glycolysis | [92] |
| circ_002136 | Enhance cell viability and invasive ability | [93] |

lncRNA TUG1 [92], circ_002136 [93] and miR-638 [94], was also detected, and exploring targets on exosomal ncRNAs may pave a new approach to inhibit or eradicate HCC.

### *4.2. Association between exosome-derived ncRNAs and immune microenvironment in HCC*

With the development of single-cell technology and multiplex immune fluorescence (mIF), the cellular composition of tumor tissue is becoming increasingly clear. We noticed that tumor tissue contains a significant number of immune-infiltrating cells [95,96]. Furthermore, the identification of various immune subtypes has greatly enhanced scientists' understanding of tumors and has led to the development of immune-based therapeutic strategies, such as immune checkpoint inhibitors which achieved great success in cancer treatment recently [97–100]. Then, further understanding the tumor immune microenvironment and the development of correlated targets are also crucial in the progression and treatment of HCC.

Recently, increasing studies have revealed a modulatory role of exosome-derived ncRNAs in the immune microenvironment of HCC (Table 2). For example, HCC cells release miR-23a-carrying exosomes that could inhibit T-cell function through the PTEN-AKT pathway [101]. Exosomal lncRNA DLX6-AS1 and miR-21-5p, derived from HCC cells, can induce M2 macrophage polarization to enhance the invasion and migration of HCC cells [88,102]. In addition, HCC cell-derived exosomal lncRNA TUC339, lncRNA HMMR-AS1 and hsa_circ_0004658 can also regulate macrophage activation and polarization to impact the outcome of HCC [89,90,103]. Besides, exosomal circGSE1 induces more infiltration of regulatory T cells to support HCC progression by increasing immune escape [104]. Furthermore, these studies also revealed that engineering exosomes carrying these ncRNAs may modulate the tumor immune microenvironment to impact tumor progression. Therefore, these findings provide a novel insight into understanding the mechanism of how HCC cells escape from antitumor immunity.

**Table 2**
Role of exosomal ncRNA in regulating tumor immune microenvironment.

| NcRNA | Role in regulating immune infiltration | Refence |
|---|---|---|
| miR-23a | Up-regulate PD-L1 expression in macrophages | [101] |
| lncRNA DLX6-AS1 | Induce M2 macrophage polarization | [88] |
| miR-21-5p | Induce M2 macrophage polarization | [102] |
| lncRNA TUC339 | Elevated level in M(IL-4) macrophages | [89] |
| lncRNA HMMR-AS1 | Promote the M2 polarization of macrophages | [103] |
| hsa_circ_0004658 | Upregulated in RBPJ+/+ macrophage-derived exosomes | [90] |
| circGSE1 | Induce expansion of regulatory T cells | [104] |

### 4.3. Association between exosome-derived ncRNAs and treatment response

The patients' poor prognoses with HCC are caused by numerous factors, and resistance to treatment remains one of the primary factors contributing to short survival [105–107]. Then, developing precision medicine and biomarkers monitoring treatment response is crucial for improving the treatment outcomes and prognosis for patients with HCC [108]. Based on the important role of exosome-derived ncRNAs in drug delivery and cellular communication, exploring their applications in terms of therapeutic biomarkers is crucial. Furthermore, increasing evidence has revealed a regulatory role of exosome-derived ncRNAs in the response to treatment of HCC patients (Table 3). For example, a recent study found that circPAK1 could be delivered by exosomes from the lenvatinib-resistant HCC cells to sensitive cells, and then promoted resistance to lenvatinib of receipt cells [109]. Therefore, the expression levels of exosomal circPAK1 could be used as an indicator to stratify HCC patients and patients with low exosomal circPAK1 levels were recommended to receive lenvatinib treatment. In addition, Lou et al. revealed that adipose tissue-derived mesenchymal stem cells could improve chemosensitivity in HCC by delivering miR-199 through exosomes [110]. Recently, exosomal miR-200c-3p, miR-222-5p, and miR-512-3p were also confirmed to be correlated to treatment resistance in HCC [111]. Collectively, some specific exosomal ncRNAs are associated with treatment response in HCC and promising biomarkers for screening potential responders to treatment and then elevating therapeutic efficacy.

## 5. Clinical application

Due to the crucial role of exosome-derived ncRNAs in HCC development and treatment response, studies targeting their functions and modulatory mechanisms may be potential direction of clinical application. According to the current findings, we summarized two primary applications: diagnostic biomarkers and prognostic biomarkers.

### 5.1. As biomarkers for HCC diagnosis

Currently, alpha-fetoprotein (AFP) remains the main circulating biomarker for HCC diagnosis and the early diagnosis of HCC is still challenging, especially in a patient with negative AFP [113]. Delayed diagnosis is closely associated with poor prognosis, higher recurrence rates, chemotherapy resistance, and missed surgical opportunities in HCC [114]. Then, earlier detection of tumors may improve the therapeutic approaches, disease prognosis and treatment outcomes. Over the past several years, scientists have been increasingly interested in exosome-derived ncRNAs. The detection of exosome-related ncRNAs is more convenient since they are located in different body fluids. Furthermore, recent results showed that exosome-derived ncRNAs harbor promising effects as diagnostic biomarkers for HCC (Table 4). For example, the expression levels of exosome-derived miR-21-5p, miR-223-3p, miR-221-3p and miR-10b-5p were found significantly increased in HCC patients than chronic hepatitis [115]. ROC analysis revealed these biomarkers can effectively distinguish HCC from chronic hepatitis with an AUC of 0.86 (95% CI: 0.77–0.94), which is more accurate than currently clinically used biomarkers. In addition, Huang and coworkers revealed that plasma exosome-derived lnc85 was up-regulated in both AFP-negative and AFP-positive HCC patients, and then was able to distinguish all HCC patients from liver cirrhosis and healthy control (AUC: 0.869) [116]. In addition, early-stage HCC can be accurately predicted by a panel of miR-4661-5p and miR-4746-5p derived from exosomes (AUC: 0.947, 95% CI: 0.889–0.980) [117]. Thus, exosomal ncRNAs provide a novel effective approach for the diagnosis of HCC.

**Table 3**
Role of exosomal ncRNA in regulating treatment outcomes.

| NcRNA | Impact on treatment response | Refence |
|---|---|---|
| circPAK1 | Induce lenvatinib resistance | [109] |
| lncRNA ROR | Modulates chemosensitivity | [72] |
| miR-199 | Improve chemosensitivity | [110] |
| miR-122 | Increase chemosensitivity | [85] |
| circCCAR1 | Promote anti-PD1 resistance | [112] |

**Table 4**
As biomarkers for HCC diagnosis and prognosis.

| Exosomal NcRNA | Role | Refence |
|---|---|---|
| miR-21-5p<br>miR-10b-5p<br>miR-221-3p<br>miR-223-3p | Diagnostic biomarkers | [115] |
| lnc85 | Diagnostic biomarker | [116] |
| miR-4661-5p and miR-4746-5p | Biomarker for early-stage HCC | [117] |
| miRNA-21<br>lncRNA-ATB | Independent predictors of mortality and disease progression | [118] |
| miR-21 | Prognostic biomarker | [119] |
| miR-638 | Prognostic biomarker | [94] |
| lncRNA CRNDE | Diagnostic and prognostic biomarker | [120] |

### 5.2. As biomarkers for HCC prognosis

In addition to early diagnosis, developing biomarkers that predict treatment response and prognosis is also important to improve outcomes for patients with HCC, because drug-resistance, metastasis and recurrence are tightly associated with the poor prognosis of patients with HCC [121,122]. Using PCA and WGCNA analysis, Peng et al. constructed an exosome-derived lncRNA signature, which effectively predicted the prognosis, immune microenvironment, and response to immune checkpoint inhibitors [123]. In addition, Lee and colleagues revealed that higher miRNA-21 and higher lncRNA-ATB were independent risk factors of disease progression and mortality, also predicting larger tumor size and higher C-reactive levels [118]. In the serum of HCC patients, higher exosomal miR-638 expression was correlated to metastasis and harbored potential as a promising prognostic biomarker [94]. Recently, Nie et al. performed a meta-analysis and found that miR-21 was a risk factor for HCC patients with an HR of 2.48 (95% CI: 1.52–4.05), and might serve as a potential prognostic biomarker [119]. Collectively, exosome-derived ncRNAs can act as specific and sensitive non-invasive biomarkers for the prognosis of HCC patients.

### 5.3. Developing exosomal ncRNA-based therapeutic targets

Currently, the targeted therapy targets used in the clinical treatment of HCC still fail to achieve the desired therapeutic effects, and developing effective new targets remains a focus of HCC research. Given the critical role of exosomal markers in the tumor microenvironment and tumor progression, utilizing exosomal markers to develop corresponding targeted therapies holds tremendous potential [124]. For example, deleting exosomes carrying circPAK1 may be also an effective strategy for enhancing the efficacy of lenvatinib in HCC, as high levels of exosomal circPAK1 promote drug resistance [109]. Exosomes containing miR-199 can be used concurrently with chemotherapy to enhance efficacy [110]. In addition, all exosomal ncRNAs that promote HCC progression can be explored as novel therapeutic targets, though extensive basic research and clinical trials are required to screen for truly effective components. Taken together, developing exosomal ncRNAs-related target treatments may be an effective approach to enhance the efficacy of current chemotherapy and target therapy.

### 5.4. Enhancing immune remodeling and elevating efficacy of immunotherapy

Recently, immune checkpoint inhibitors, such as PD-1/PD-L1 inhibitors and CTLA4 inhibitors, have achieved great success in the treatment of various cancers [99,125,126], including HCC [127–129]. In addition, recent findings revealed an association between exosomal ncRNAs and immune remodeling induced by immunotherapy in HCC. As crucial mediators of intercellular communication, alterations in the physicochemical properties and distinct contents of exosomes may provide important clues for reshaping the immune microenvironment [130]. Then, exploring the relationship between changes in exosome composition and immune therapy biomarkers is of great significance for further understanding the mechanisms of immune therapy and identifying potential targets for enhanced efficacy. Recently, Wei and coworkers revealed a positive relationship between PD-L1+ exosomes and anti-PD-L1 immunotherapy response in HCC, which was regulated by an RNA-RNA crosstalk network involving HMGB1 and RICTOR [131]. This finding highlighted the association between exosome-cargos and immunotherapeutic targets. In addition, the immune regulatory role of numerous exosome-derived ncRNAs was also identified. For example, exosomal circCCAR1 derived from HCC cells contributes to immunosuppression by facilitating CD8 + T-cell dysfunction in HCC [112]. Therefore, exosome-derived circCCAR1 can enhance resistance to PD1 blockade in HCC. Fan et al. found that exosomes containing PCED1B-AS1 from HCC cells enhanced PD-Ls expression in receipt HCC cells by targeting miR-194-5p, while inhibited receipt T cells and macrophages to induce immunosuppression [132]. Then, exosomes with more PCED1B-AS1 may indicate an immunosuppressive tumor microenvironment and potential response to PD-L1 blockade. These findings proposed a new approach for blocking treatment resistance or enhancing therapeutic efficacy in HCC.

## 6. Conclusions and future perspectives

Exosome-carrying ncRNAs exert crucial modulatory effects on the complex communication between HCC cells and composition in the tumor immune microenvironment. Some specific exosomal ncRNAs play an important role in the direction of immune cell infiltration and differentiation, such as the expansion of regulatory T cells and M2 polarization of macrophages, and tumor development, suggesting that these exosome-derived ncRNAs might be important targets for blocking or eradicating HCC. In addition, it is well known and verified that exosomes can be released and internalized by nearly all types of cells. Then, the development of exosomes or exosomal ncRNAs-related strategy to inhibit or eradicate HCC may be a novel approach with promising efficacy. Developing engineering exosomes using ncRNAs and transmitting these engineered exosomes into HCC cells is also a potential strategy, as the application of exosomes loading miR-335-5p inhibitor have achieved success in blocking HCC [133]. In addition, modifying exosomal ncRNA function through RNA modifications is also a potential application method [134,135].

Benefiting from the membrane structure of exosomes, the levels of exosome-derived ncRNAs are more stable in body liquids, which contributes to effectively screen out HCC and serve as diagnostic biomarkers. An increasing number of findings suggest that exosomal RNA can serve as biomarkers for early tumor screening with high identification efficiency [7,8,136]. In addition, using exosomes as drug carriers for RNA targeted therapy offers higher specificity and therapeutic efficacy [137,138]. Therefore, developing exosome-based drug formulations represents a novel approach in tumor therapy using exosomes. Besides, given the crucial communication role of exosomes in regulating the tumor microenvironment, utilizing exosomal markers as synergistic agents in immunotherapy holds immense potential.

There are also several unique advantages of exosomes compared to other methods of liquid biopsies, such as the detection of circulating tumor DNA and circulating tumor cells. For example, the high detection rate of exosomes and their molecules. In addition, exosomes contain a plethora of tumor-related molecules, rendering them rich in information and facilitating large-scale, multi-molecular analyses. All these characteristics suggest that exosomes possess significant development advantages. However, the most effective and suitable exosomal ncRNA also needs further identification.

Although recent findings have achieved remarkable advances in understanding the biogenesis and function of exosomes, much information remain unclear. For example, the extraction methods for various bodily fluid samples are still not well-defined. As a result, there are variations in the detection results of exosomal ncRNAs depending on the different exosome extraction methods employed [139–141]. In addition, the expression data of control samples may vary depending on individual conditions and the time of sampling. Also, the impact of several clinical features, such as race, age and sex, on the contents of exosomes remains unclear. Thus, it is imperative to investigate the associations between these confounding factors and exosomal characteristics related to HCC. Conclusively, continued in-depth exploration to explore the roles and underlying molecular mechanisms of exosomal ncRNAs are important to develop exosome-related approaches for improving the diagnosis and treatment outcomes in patients with HCC.

## Data availability statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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## CRediT authorship contribution statement

**Min Shi:** Conceptualization, Data curation, Investigation, Writing – original draft. **Jun-Su Jia:** Data curation, Formal analysis, Methodology, Software. **Guo-Sheng Gao:** Formal analysis, Investigation, Software, Visualization. **Xin Hua:** Funding acquisition, Project administration, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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