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Review Article

Unlocking the Future of Hepatocellular Carcinoma Early Diagnosis: The Promise of Extracellular Vesicle Biomarkers

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Abstract

Hepatocellular carcinoma (HCC) is one of the most prevalent and aggressive malignant tumors globally, with a notably low five-year survival rate. Its high mortality is largely attributed to challenges in early detection. Extracellular vesicles (EVs) are naturally occurring nanoparticles secreted by nearly all cell types and carry a diverse array of bioactive molecules, including proteins, nucleic acids (particularly non-coding RNAs), and lipids. EVs play pivotal roles in remodeling the tumor microenvironment and driving cancer progression through intercellular communication. Accumulating evidence has established that EVs are critically involved in the pathogenesis of HCC and are emerging as promising biomarkers for its early detection. With advances in EV isolation technologies, these vesicles have garnered considerable attention in the field of liquid biopsy for HCC. This review provides a comprehensive overview of the diagnostic potential of EV-derived biomarkers in HCC, including DNA, RNA, proteins, and lipids. Additionally, it discusses the advantages of integrating multi-omics approaches for HCC diagnosis. Furthermore, the review highlights the technical challenges in EV isolation and characterization, as well as the crucial role of reference genes in the standardization of EV data. These insights underscore the potential of EVs as novel, minimally invasive liquid biopsy biomarkers for the early diagnosis of HCC.

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Introduction

Liver cancer is the sixth most common malignant neoplasm and the third leading cause of cancer-related fatalities worldwide, with an estimated 865,000 new cases and 758,000 deaths in 2022, as reported by the International Agency for Research on Cancer.[1,2] Hepatocellular carcinoma (HCC) accounts for 75%–85% of all primary liver cancers.[1,2] Primary risk factors include chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), non-alcoholic fatty liver disease (NAFLD), and excessive alcohol consumption.[3–5] Despite substantial progress in imaging, chemotherapy, interventional radiology, and surgical approaches, HCC patients continue to face high recurrence rates and poor overall survival. Early-stage HCC typically presents with few or no symptoms, leading to missed opportunities for effective treatment; the overall five-year survival rate for advanced HCC remains below 20%.[6,7] In contrast, early detection can improve the five-year survival rate substantially, from 60% to 70%.[8]

Current clinical guidelines recommend imaging techniques—such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound screening—along with serum alpha-fetoprotein (AFP) testing for early HCC diagnosis.[9] However, these methods have notable limitations in diagnostic accuracy and sensitivity. A meta-analysis indicated that the sensitivity of ultrasound for early-stage HCC screening was below 50%, increasing to only 63% when combined with AFP.[10] CT and MRI, the primary detection modalities for HCC with approximately 84% sensitivity,[10] demonstrate relatively low accuracy in diagnosing early-stage HCC or tumors smaller than 2 cm with atypical imaging features.[11] Histological biopsy, the gold standard for HCC diagnosis, is unsuitable for broad screening owing to its invasive nature. These limitations collectively underscore an urgent clinical need for novel, minimally invasive diagnostic approaches. In this context, extracellular vesicles (EVs) have emerged as highly promising candidates for the early detection of HCC.

EVs are membrane-derived nanoparticles that encapsulate proteins, nucleic acids, lipids, metabolites, and even organelles; they are broadly categorized as exosomes (typically <200 nm in diameter) and ectosomes/microvesicles (>200 nm in diameter), reflecting their distinct biogenetic origins in the endosomal system and plasma membrane, respectively.[12] EVs feature a lipid bilayer membrane and contain various bioactive molecules, including proteins, messenger RNAs (mRNAs), microRNAs (miRNAs), circular RNAs (circRNAs), and long non-coding RNAs (lncRNAs).[13] Given the lack

Keywords: Hepatocellular carcinoma; Extracellular vesicles; Biomarker; Liquid biopsy; Early diagnosis.

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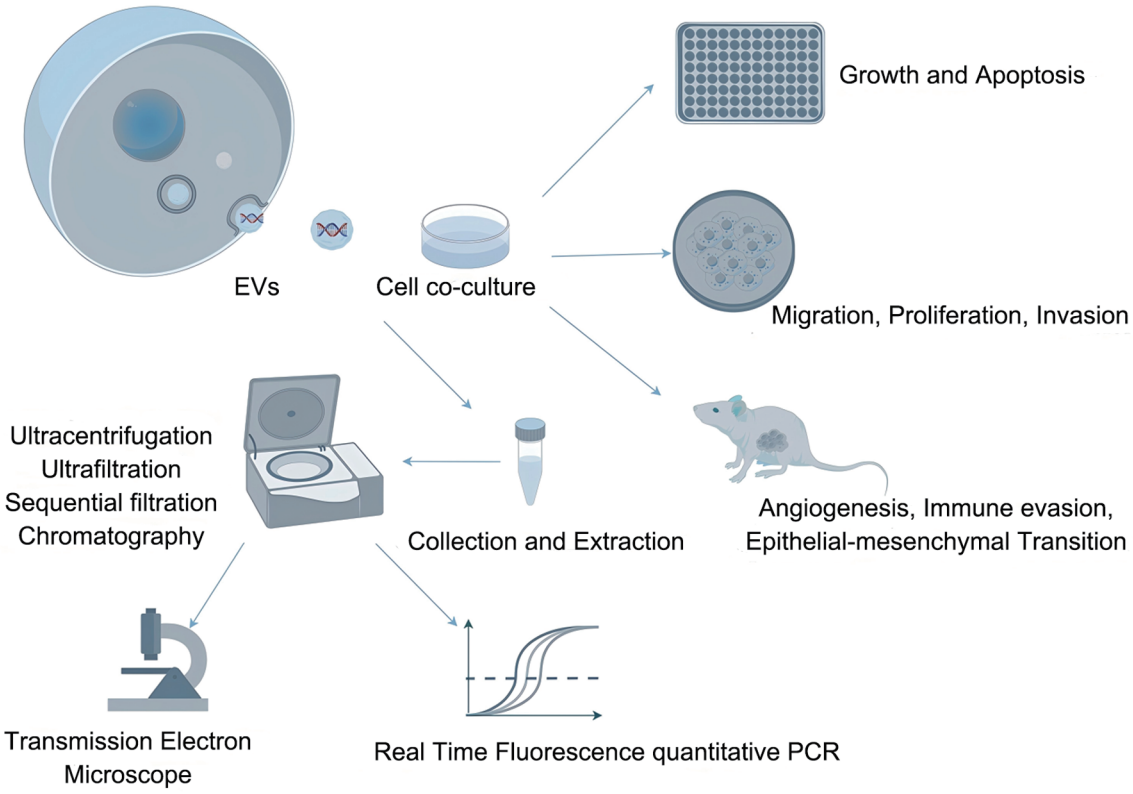

Fig. 1. The role of EVs in HCC progression and early diagnosis. HCC, hepatocellular carcinoma; EVs, extracellular vesicles; PCR, polymerase chain reaction.

of consensus on subtype-specific biomarkers, this review adopts the term 'EVs' as recommended by the International Society for Extracellular Vesicles (ISEV). Most studies referring to 'exosomes' or 'ectosomes/microvesicles' in the existing literature describe heterogeneous EV populations rather than vesicles defined by specific biogenesis pathways.[12] EVs play a crucial role in HCC development and progression by facilitating intercellular transfer of diverse molecular cargoes. Their unique lipid-raft structure protects EV contents from degradation by ribonucleases and proteases in the circulation. Studies have demonstrated significantly elevated EV levels in the blood of patients with HCC compared with healthy individuals,[14] making EVs a promising component of liquid biopsies for noninvasive early HCC screening (Fig. 1). This review aims to provide a comprehensive overview of current EV-derived molecular cargoes as potential biomarkers for early HCC diagnosis,[15] while also addressing existing technical challenges in EV isolation and characterization and critical gaps in the literature that must be resolved to realize the full clinical application potential of EV-based diagnostics.

The role of EVs in HCC

EVs are critical mediators of intercellular communication and play pivotal roles in the initiation, progression, and metastasis of HCC through multiple mechanisms. First, EVs regulate the HCC immune microenvironment in a context-dependent manner, exerting both tumor-promoting and tumor-suppressive effects through distinct molecular mechanisms. For example, miR-3129 is highly enriched in plasma-derived EVs from HCC patients; EV-mediated delivery of miR-3129 to recipient HCC cells enhances their malignant behavior.[16] Conversely, EVs can suppress HCC progression: overexpression of Vps4A in HCC-derived EVs inhibits the PI3K–Akt signaling pathway in recipient tumor cells, thereby suppressing HCC metastasis.[17] Second, EVs facilitate tumor growth, metastasis, and immune evasion. Mao *et al*. demonstrated that nidogen-1 carried by EVs from metastatic HCC cells promoted angiogenesis, enhanced lung endothelial permeability, activated cancer-associated fibroblasts to secrete TNFR1, and ultimately established pre-metastatic niches in the lung (Fig. 2).[18] Furthermore, tumor cell-derived EVs promote angiogenesis and metastasis by interacting with endothelial angiogenic factors.[19] Immune evasion is achieved through EV-mediated delivery of lysyl oxidase-like protein 4 (LOXL4) to macrophages, where it activates STAT1 signaling to induce PD-L1 expression, thereby suppressing $CD8^+$ T lymphocyte cytotoxicity against HCC cells. Third, EVs serve as therapeutic platforms and drug delivery vehicles. Their spherical lipid bilayer structure enables efficient loading and targeted transport of therapeutic agents to tumor cells. Zhang *et al*. demonstrated that red blood cell-derived EVs loaded with doxorubicin or sorafenib exhibited enhanced therapeutic efficacy in an orthotopic HCC mouse model via a macrophage-dependent mechanism.[20] Finally, EVs serve as biomarkers for HCC diagnosis and prognostic evaluation, carrying tumor-specific molecules such as miRNAs and proteins with high specificity and sensitivity.[21]

Collectively, the roles of EVs in HCC span immune microenvironment regulation, intercellular communication, tumor progression, and the development of diagnostic and therapeutic strategies. These findings provide critical mechanistic insights into the complex biology underlying HCC and highlight the potential of EVs as multifunctional tools for iden-

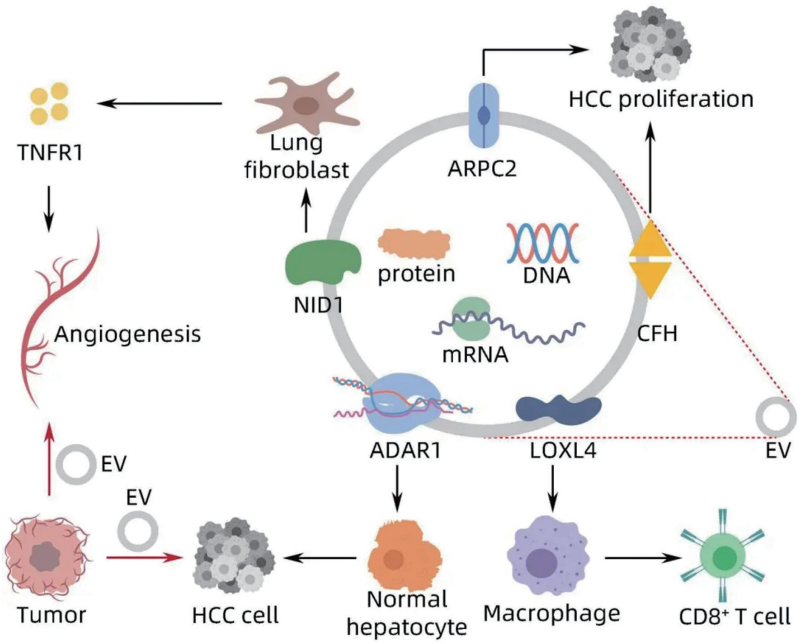

Fig. 2. Impact of HCC-EV on HCC (Reprinted from Shi *et al*., J Clin Hepatol, 2024, with permission). HCC, hepatocellular carcinoma; EV, extracellular vesicle; ADAR1, adenosine deaminase acting on RNA-1; LOXL4, lysyl oxidase-like protein 4; CFH, complement factor H; TNFR1, tumor necrosis factor receptor 1; ARPC2, actin-related protein 2/3 complex subunit 2; NID1; nidogen 1.

tifying cancer stages, monitoring treatment response, and delivering targeted therapies.

The diagnostic performance of EVs in HCC

EV-derived miRNAs

miRNAs are a class of small noncoding RNAs, typically 18–25 nucleotides in length, that regulate gene expression post-transcriptionally by targeting the 3′-untranslated regions of mRNA transcripts, leading to translational repression or mRNA degradation. This regulatory capacity confers critical roles in cell growth, differentiation, and metabolism.[22] EV-derived miRNAs have demonstrated considerable promise as biomarkers for the early detection of HCC. A meta-analysis encompassing 5,125 HCC patients and 6,561 controls reported pooled sensitivity and specificity of 0.80 and 0.73, respectively, for miRNAs in differentiating HCC from non-HCC cases.[23] Importantly, miRNAs encapsulated within EVs are significantly more stable and abundant than free miRNAs in serum or plasma,[24] supporting EV-derived miRNAs as a novel approach for early HCC liquid biopsy screening (Table 1).[25,26–38]

miR-21 is a liver-enriched miRNA. It plays a core role in the proliferation, invasion, and apoptosis of HCC cells by targeting multiple genes, including SMAD7,[39] kruppel-like factor 6 (KLF6),[40] and suppressor of cytokine signaling 6 (SOCS6).[41] Based on a meta-analysis of 1,589 subjects from 14 publications, Zhang *et al*.[42] reported that the pooled sensitivity, specificity, and area under the curve (AUC) of miR-21 were 0.83, 0.80, and 0.88, respectively, suggesting that miR-21 is a valuable biomarker for early HCC diagnosis. Nevertheless, several publications have indicated that miR-21 is mainly enriched in EVs, and its expression level is significantly higher than that in EV-depleted serum or plasma.[43] Wang *et al*. assessed the potential of EV-derived miR-21 as a diagnostic biomarker, and the results showed a powerful ability to diagnose early HCC with an AUC value of 0.912, which was significantly higher than those of AFP and serum-derived miR-21.[26] The sensitivity, specificity, and AUC of EV-derived miR-21 for the early diagnosis of HBV- or HCV-induced HCC in India were 0.74, 0.77, and 0.77, respectively.[27] Interestingly, many researchers have established new, more rapid, and sensitive detection methods for EV-derived miR-21 compared to quantitative reverse transcription PCR (RT-qPCR), thereby accelerating its clinical application.[44] It is worth noting that serum EV-derived miR-10b-5p had the best diagnostic performance (AUC of 0.968)[28] for differentiating HCC from healthy controls (HCs) among all reported EV-derived miRNAs, which was remarkably higher than that of miR-18a-5p (AUC = 0.842), miR-212 (AUC = 0.79), miR-1262 (AUC = 0.92), miR-93 (AUC = 0.825), miR-320a (AUC = 0.86), and AFP (AUC = 0.84).[27,29–32] However, Ghosh *et al*. reported that single miR-10b-5p may be difficult to demonstrate good sensitivity in distinguishing HCC from non-HCC.[27] Therefore, further large-scale prospective studies are required to assess their role in HCC. miR-122 is considered a key regulator of innate immunity, EMT, apoptosis, and proliferation of HCC and has powerful potential in the early diagnosis of HCC.[45] Recently, the diagnostic potential of EV-derived miR-122 was evaluated for early HCC screening in several different studies,[26,33,34] and the highest AUC value of 0.9 (sensitivity, 100%; specificity, 92%) was obtained. Other studies further indicated that miR-122 in EVs showed better accuracy than that in serum; thus, EV-derived miR-122 could be used as a new diagnostic biomarker for early HCC.

Chronic liver disease (CLD) is the primary cause of HCC, particularly due to conditions like liver cirrhosis (LC), hepatitis from HBV or HCV, alcoholic hepatitis, and NAFLD. The effectiveness of EV-derived miRNAs as diagnostic tools can differ among various types of CLD. Yang *et al*.[35] conducted a diagnostic evaluation of miR-26a, miR-29c, and miR-199a using 50 HCC patients, 50 LC patients, and 50 healthy subjects and found that plasma EV-derived miR-29c had the

Table 1. The diagnostic efficacy of EV-derived miRNA for HCC patients

Author	Country	Year	Sample size	Categories	Diagnostic efficacy	Reference controls
Wang *et al*.[26]	China	2020	50 NC 50 CLD 50 HCC	miR-21 miR-96 miR-122 miRNA panels	0.912 0.802 0.853 0.996	U6
Ghosh *et al*.[27]	India	2020	35 NC 98 CLD 38 HCC	miR-10b miR-221 miR-21 miR-223 miRNA panels albumin	0.65 0.69 0.78 0.63 0.80 0.75	miR-39
CHO *et al*.[28]	Korea	2020	28 NC 60 CLD 90 HCC	miR-10b miR-18a miR-215 miR-940 AFP	0.968 0.842 0.936 0.827 0.707	miR-1228
Zhang *et al*.[29]	China	2019	70 NC 153 CLD 78 HCC	miR-212 AFP CA125	0.793 0.84 0.777	U6
Asmaa *et al*.[30]	Egypt	2018	18 NC 42 CLD 60 HCC	miR-1262 RNA panels	0.92 0.883	U6
Xue *et al*.[31]	China	2018	23 NC 85 HCC	miR-93	0.825	miR-16
Hao *et al*.[32]	China	2020	50 NC 55 CLD 104 HCC	miR-320a	0.86	miR-39
Wang *et al*.[33]	China	2018	50 NC 80 CLD 50 HCC	miR-122 miR-148a miR-1246 AFP Combination	0.816 0.891 0.785 0.712 0.931	cel-miR-39
Xue *et al*.[34]	China	2019	30 NC 80 HCC	miR-122 miR-125b miR-145 miR-192 miR-194 miR-29a miR-17 miR-106a	0.746 0.65 0.535 0.752 0.738 0.703 0.85 0.704	miR-16
Yang *et al*.[35]	China	2022	50 NC 50 CLD 50 HCC	miR-26a miR-29c miR-199a Panels	0.890 0.917 0.850 0.994	U6
Cui *et al*.[36]	China	2019	50 NC 89 HCC	miR-224	0.91	let-7
Fründt *et al*.[37]	Germany	2021	20 NC 51 CLD 86 HCC	miR-146a miR-16 miR-221 miR-192 Panels	0.8 0.63 0.69 0.59 0.78	cel-miR-39 miR-484
Cho *et al*.[38]	Korea	2020	22 NC 50 CLD 72 HCC	AFP/miR-4661 AFP miR-4661/miR-4746	0.921 0.704 0.942	U6

APF, alpha-fetoprotein; CLD, chronic liver disease; HCC, hepatocellular carcinoma; miRNA, mi-croRNA; NC, negative control.

highest diagnostic value with an AUC of 0.917 for identifying early HCC, while miR-26a and miR-199a had AUC values of 0.890 and 0.850, respectively. Moreover, the EV-derived miRNA panel significantly differentiated patients with HCC from other CLD or HCs. Previous research also indicated that EV-derived miR-320a was significantly reduced in HCC, with sensitivity, specificity, and AUC values of 76.1%, 81.8%, and 0.829, respectively, for detecting HCC in patients with alco-

holic hepatitis.[32] EV-derived miRNAs may serve as more effective biomarkers than AFP for monitoring malignant lesions in patients with LC. For example, EV-derived miR-224 levels were linked to larger tumors (>3 cm), achieving an AUC of 0.910 for early HCC diagnosis.[36] Conversely, low levels of EV-derived miR-638 in HCC indicate more advanced stages (III/IV).[46] In Wang's study, the AUCs of serum EV-derived miR-122, miR-148a, and miR-1246 in detecting early HCC from LC were 0.816, 0.891, and 0.785, respectively, which were significantly higher than that of AFP (0.712).[33] This advantage was further supported by the validation group results (AUCs of 0.795, 0.86, and 0.861 compared to 0.665 for AFP). Additionally, Ghosh *et al*. found that EV-derived miR-21 was a more effective biomarker for distinguishing HCC from chronic hepatitis than HCs, with specificity and AUC values of 83% versus 68% and 0.83 versus 0.71, respectively.[27] The expression of EV-derived miR-215-5p increases with liver disease progression and is associated with tumor stage advancement and vascular invasion. Further studies indicated that serum EV miR-215-5p could be a promising biomarker for early HCC detection in healthy individuals, with an AUC of 0.936, thus outperforming AFP levels. However, its diagnostic performance (AUC of 0.7) in distinguishing early HCC at stages I or II from non-tumor patients (including those with LC and chronic hepatitis) was less impressive.[28]

Combining multiple markers can appreciably improve the specificity and accuracy of early HCC screening compared with a single indicator. For instance, the AUC values of EV-derived miR-21, miR-96, and miR-122 in distinguishing early HCC from HC were 0.912, 0.802, and 0.853, respectively, which were better than those of AFP and serum miRNAs. Their combination exhibited a better diagnostic performance with a sensitivity, specificity, and AUC of 96%, 98%, and 0.996, respectively. This study represents a preliminary investigation with a limited sample size, and the relevant conclusions need to be further verified by subsequent large-sample studies.[26] Further research found that the diagnostic AUC value of the EV-derived miRNA panel for tracking the progression of LC to HCC increased to 0.924.[26] In another study, EV-derived miR-221 showed low specificity (63%) and AUC (0.75) in HCC screening, whereas the combination of four miRNAs (miR-21, miR-10b, miR-221, and miR-223) led to a remarkable improvement, with 86% specificity and 0.86 diagnostic accuracy in differentiating early HCC from chronic hepatitis.[27] Fründt *et al*. reported that the specificity of plasma EV-derived miR-16, miR-221, and miR-96 for early diagnosis was 7.6%, 27%, and 10%, respectively, whereas the diagnostic efficiency of miRNA panels improved to 81% for sensitivity, 86% for specificity, and 0.78 for AUC.[37] Current evidence indicates that AFP alone is no longer recommended for the early diagnosis of HCC, owing to its limited sensitivity and specificity. Cho *et al*. reported that AFP (cut-off value 20 ng/mL) showed a low diagnostic value in detecting HCC in LC and HC patients, with a sensitivity of 15.9%, specificity of 71.4%, and AUC of 0.541, whereas combined AFP and serum EV-derived miR-4661-5p represented a promising diagnostic strategy with a sensitivity, specificity, and AUC of 95.4%, 72.4%, and 0.921, respectively. This study is among the first to report on the potential clinical significance of serum EV-derived miR-4661-5p in HCC.[38] Further analysis demonstrated the superiority of combined AFP and EV-derived miR-4661-5p (AUC = 0.91) over AFP alone (AUC = 0.604) for detecting early HCC. In another study, the diagnostic performance of HCC in early-stage CLD patients was significantly improved using a combination of serum EV-derived miR-122, miR-148a, and AFP, with a sensitivity of 87%, specificity of 90%, and AUC of 0.947.[33] Thus, EV-derived miRNA panels combined with AFP may be a more valuable assay for increasing the diagnostic performance of HCC.

High-throughput sequencing has identified numerous EV-derived miRNAs that are significantly differentially expressed between HCC patients and HCs, providing a rich repertoire of candidate biomarkers for HCC.[47] Multi-marker EV-derived miRNA panels, when combined with traditional biomarkers such as AFP, substantially enhance the efficiency and accuracy of early-stage HCC diagnosis. Nevertheless, several important gaps remain. The expression profiles of EV-derived miRNAs in HCC have shown inter-study inconsistencies and variable diagnostic performance across different ethnic groups and HCC etiologies; most existing studies have focused on Asian populations with HBV-related HCC, highlighting the need for larger, diverse prospective cohorts. Additionally, EV-derived miRNA expression correlates with Tumor–Node–Metastasis (TNM) stage and tumor size,[38] and differs between virus-related and CLD-induced HCC.[43] Systematic identification of the most discriminating miRNA categories or panels for different HCC etiologies remains an unmet priority for the field.

EV-derived lncRNAs

lncRNAs are a class of noncoding RNA transcripts exceeding 200 nucleotides in length, once regarded as transcriptional 'noise' without coding potential.[48] Advances in molecular biology have since revealed their indispensable roles in maintaining cellular homeostasis. lncRNAs participate in diverse biological processes, including transcriptional and post-transcriptional regulation (e.g., mRNA degradation, translational inhibition, competing endogenous RNA (ceRNA) networks, and m^{6}A methylation).[49–51] By acting as ceRNAs, lncRNAs function as miRNA sponges and thereby modulate downstream gene expression.[52] Recent evidence has demonstrated that EV-mediated transport of lncRNAs facilitates intercellular communication between tumor cells, promoting HCC proliferation, immune evasion, and drug resistance.[53] The dysregulation of EV-derived lncRNAs during HCC development and progression correlates with tumor stage and size, establishing EV-derived lncRNAs as promising novel biomarkers for early HCC screening (Table 2).[36,54,55,56–62]

Based on RNA sequencing, 8,572 plasma EV-derived lncRNAs were found to be differentially expressed between patients with HCC and HCs.[54] EV-derived lnc85, which showed the most significant expression, was further validated in HCC cell EVs, and its knockdown significantly affected the proliferation and migration of HCC cells by sponging miR-324-5p. Moreover, the AUC, sensitivity, and specificity of lnc85 for distinguishing HCC patients from HCs and LC patients were 0.873, 80.0%, and 74.5% (cut-off = 1.563), respectively.[54] Therefore, EV-derived lnc85 may be a promising biomarker for HCC diagnosis. Another study identified over 40,000 lncRNAs dysregulated in HCC and hepatitis patients compared to the control group, providing numerous candidate targets for HCC detection.[55] Additionally, the expression of serum EV-derived lncRNAs FAM72D-3 and EPC1-4 was found to be closely linked to the proliferation and apoptosis of HCC cells, showing potential diagnostic efficacy for HCC, with AUC values of 0.584 and 0.852, respectively.[55] The lncRNA CRNDE, recognized as a significant oncogene in cancer, is notably elevated in HCC tissues and can enhance the proliferation, migration, and invasion of HCC cells through various pathways involving the miR-217/MAPK1,[63] miR-33a-5p/CDK6,[64] and miR-136-5p/ILF2 axis.[65] A recent study indicated that high levels of serum EV-derived lncRNA CRNDE were associated with larger tumor sizes and more advanced stages of HCC, and diagnostic assessments revealed an AUC of 0.839 with 69.3% sensitivity and 85.0% specificity for EV-derived lncR-

Table 2. The diagnostic efficacy of EV-derived lncRNAs for HCC patients

Author	Country	Year	Sample size	Categories	Diagnostic efficacy	Reference controls
Huang *et al*.[54]	China	2020	52 NC 43 CLD 112 HCC	lnc85	0.873	GAPDH
Yao *et al*.[55]	China	2020	45 NC 90 CLD 45 HCC	lnc-EPC1-4 lnc-FAM72D-3 lnc-ZEB2-19 lnc-GPR89B-15	0.521 0.629 0.772 0.508	GAPDH
Wang *et al*.[56]	China	2021	100 NC 166 HCC	lnc-CRNDE	0.839	cel-miR-39
Tan *et al*.[57]	China	2019	14 NC/CLD 31 HCC	lnc-RN7SL1S	0.93	MIR1228
Kim *et al*.[58]	Korea	2020	29 NC 63 CLD 90 HCC	LINC00853 AFP	0.965 0.604	HMBS
Sun *et al*.[59]	China	2018	76 NC 76 HCC	LINC00161	0.794	cel-miR-39
Abd El *et al*.[30]	Egypt	2018	18 NC 42 CLD 60 HCC	lnc-RP11-513I15.6 miR-1262 RAB11A Combination	0.964 0.868 0.754 0.877	Beta-actin U6
Yao *et al*.[60]	China	2022	148 NC 101 HCC	H19-204 THEMIS2-211 PRKACA-202 AFP PRKACA-202+THEMIS2-211 H19-204+THEMIS2-211 THEMIS2-211+PRKACA-202+ AFP	0.67 0.816 0.812 0.824 0.826 0.825 0.947	U6
Xu *et al*.[61]	China	2018	120 NC 241 CLD 115 HCC	ENSG00000258332.1 LINC00635 AFP Combination	0.719 0.750 0.666 0.894	GAPDH
Lu *et al*.[62]	China	2019	200 NC 200 CLD 200 HCC	ENSG00000248932.1 ENST00000440688.1 ENST00000457302.2 AFP Panels Combination	0.530 0.632 0.883 0.400 0.818 0.833	Cel-miR-39

AFP, alpha-fetoprotein; CLD, chronic liver disease; GAPDH, glyceraldehyde-3-phosphate dehy-drogenase; HCC, hepatocellular carcinoma; HMBS, hydroxymethylbilane synthase; lncRNA, long noncoding RNA; NC, negative control.

NA CRNDE in early HCC screening.[56] Furthermore, lncRNA RN7SL1S was found to be highly expressed in both HCC serum and EVs, where it inhibited p53 translation by targeting and regulating TP53 expression.[57,66] Tan *et al*. reported that the diagnostic performance of the lncRNA RN7SL1S fragment in plasma (AUC = 0.87) was significantly higher than that of AFP (AUC = 0.712) and EV-derived RN7SL1S (AUC = 0.75), indicating that plasma-derived lncRNA RN7SL1S may be much more suitable for the screening of early stages of HCC.[57] Currently, serum EV-derived LINC00853 has the best diagnostic value among reported EV-derived lncRNAs, with an AUC of 0.934.[58] More importantly, EV-derived LINC00853 was found to have a sensitivity of 93.75% and specificity of 89.77% for the diagnosis of early-stage HCC (stage I), which was much better than that of AFP (9.38% sensitivity and 72.73% specificity). In particular, EV-derived LINC00853 showed excellent discriminatory ability in AFP-negative and AFP-positive early HCC, yielding positivity rates of 97% and 67%, respectively.[58] Thus, EV-derived LINC00853 detection is expected to be a new breakthrough in the early noninvasive screening of HCC. At present, the specific function of EV-derived LINC00853 in the progression of HCC remains unclear and deserves further investigation. LINC00161 has been identified as a promising and independent biomarker for HCC diagnosis and prognosis, and its increased expression promotes tumor migration and invasion in HCC, leading to poor survival.[67] Sun *et al*. found that serum LINC00161 expression was closely related to AFP concentration and TNM stage in HCC, and its AUC, sensitivity, and specificity were 0.794, 75%, and 73.2%, respectively, in early HCC detection.[59] Further analysis showed that EV-derived LINC00161 expression in patients with HCC was remarkably higher than that in HCs[68]; however, no statistical significance was detected between serum EV-free samples and control serum samples, demonstrating that LINC00161 is mainly present in EVs.[59] EV-carried LINC00161 can promote HCC tumorigenesis and metastasis by inhibiting the miR-590-3p/ROCK2 signaling pathway.[68]

Serum EV-derived lncRNA RP11-513I15.6 was significantly increased in the malignant group compared to the chronic HCV and HC groups, while EV-derived miR-1262 was downregulated.[30] Further studies showed that lncRNA RP11-513I15.6 could reduce the repression of RAB11A mRNA by competing with miR-1262, and that the sensitivity and specificity of lncRNA RP11-513I15.6 were 96.7% and 95%, respectively, in detecting HCC in chronic HCV patients and HC; however, when combined with EV-derived miR-1262 and AFP, the specificity dropped to 75%. Similarly, in early-stage HCC screening, lncRNA RP11-513I15.6 alone had a higher diagnostic accuracy (AUC = 0.964) than their combination (AUC = 0.877).[30] Therefore, the early diagnostic efficiency of EV-derived lncRNA RP11-513I15.6, combined with AFP, in HCC is still worth evaluating. Conversely, EV-derived lncRNA panels have been reported as a better choice for HCC diagnosis. Plasmatic EV-derived H19-204, THEMIS2-211, and PRKACA-202 were increased in patients with HCC, and their AUC values in HCC screening were 0.67, 0.816, and 0.812, respectively, which were inferior to that of AFP (AUC = 0.824).[60] In contrast, PRKACA-202 or H19-204 + THEMIS2-211 dramatically elevated the diagnostic efficiency (0.826 and 0.825); the combination of the above three markers had a better AUC value (0.887), and THEMIS2-211 + PRKACA-202 + AFP showed the highest diagnostic performance (AUC = 0.947) in discriminating HCC patients from normal controls. Crucially, Yao *et al*. found that H19-204, THEMIS2-211, and PRKACA-202 in plasma EVs were highly stable because of the strong protective effect of the bilayer membranes through treatment with RNase or low-temperature storage.[60] Hence, EV-derived H19-204, THEMIS2-211, and PRKACA-202 have been viewed as novel biomarkers with high stability for early-stage HCC diagnosis. Xu *et al*. reported that EV-derived ENSG00000258332.1 and LINC00635 expression was significantly upregulated in serum samples of patients with HCC, and their high levels were strongly associated with portal vein tumor emboli, lymph node metastasis, and TNM stage of HCC patients.[61] The AUC of ENSG00000258332.1 (cut-off = 1.345), LINC00635 (cut-off = 1.690), and AFP (cut-off = 20 ng/mL) were 0.719, 0.75, and 0.666, respectively, in distinguishing HCC patients from CLD controls, whereas their combination substantially improved the diagnostic efficiency with an AUC of 0.894, sensitivity of 83.6%, and specificity of 87.7%.[61] In addition, a recent study reported that upregulated plasma EV-derived ENSG00000248932.1, ENST00000440688.1, and ENST00000457302.2 played a crucial role in the development and progression of HCC, and the diagnostic AUC of three lncRNAs alone, AFP, their combination, and lncRNA panel combined with AFP were 0.794, 0.571, 0.538, 0.510, 0.838, and 0.905.[62] Accumulating evidence indicates that EV-derived lncRNAs can serve as key regulators of tumorigenesis and metastasis in HCC.[69] More importantly, EV-related lncRNAs are sufficiently stable and suffer minimal effects during repeated freezing and thawing.[60,62] Thus, EV-derived lncRNA panels combined with AFP represent a powerful diagnostic strategy for HCC screening.

EV-derived circRNAs

circRNAs are a distinct class of noncoding RNAs characterized by a covalently closed circular structure, which differentiates them from linear RNA species and confers exceptional resistance to exonuclease degradation and RNase activity.[70] This structural stability is a key advantage, as circRNA levels often exceed those of their corresponding linear mRNAs, making them highly attractive as diagnostic biomarkers. Functionally, circRNAs harbor multiple miRNA-binding sites and act as competitive ceRNAs, attenuating the suppressive effect of miRNAs on target mRNA expression.[71,72] Diverse ceRNA networks involving circRNAs, miRNAs, and mRNAs have been characterized in HCC.[73] Next-generation sequencing has revealed widespread dysregulation of circRNAs in blood, urine, saliva, and tumor tissues of HCC patients. Notably, EV-derived circRNA expression levels correlate closely with TNM staging and tumor size, reflecting their tissue specificity and potential as stage-specific diagnostic markers.[74]

circ-0072088, also known as circ-103809 and circZFR, is highly expressed in EVs isolated from HCC cell lines and the plasma of HCC patients, and its increase suggests tumor node metastasis and unfavorable prognosis of patients.[75,76] circ-0072088 is an important regulator of the proliferation, migration, invasion, and TMT of HCC cells by sponging miRNAs to affect downstream gene expression, including miR-1270/PLAG1-like zinc finger 2 (PLAGL2),[76] miR-377-3p/fibroblast growth factor receptor 1 (FGFR1),[77] and miR-620.[78] A recent study showed that upregulated EV-derived circ-0072088 secreted by HCC cells could promote cell invasion and migration by mediating the degradation of miR-375 to regulate MMP-16.[75] Plasma EV-derived circ-0072088 showed promising performance for HCC diagnosis, with an AUC of 0.899 (Table 3).[75,79–85] Similarly, Huang *et al*. reported that circANTXR1 was highly and stably expressed in serum EVs of HCC patients and could facilitate the proliferation and migration of HCC cells by being transported by EVs between cells to induce the miR-532-5p/X-ray repair cross-complementing 5 (XRCC5) axis. In clinical diagnostic evaluation, ROC curve analysis demonstrated that serum EV-derived circANTXR1 achieved an AUC of 0.76 for HCC diagnosis.[79] In contrast, circ-0051443 was dramatically decreased in the plasma EVs of patients with HCC, and its overexpression in normal cells via EVs significantly suppressed the proliferation of tumor cells by regulating the miR-331-3p/BAK1 axis.[80] More importantly, the AUC value for EV-derived circ-0051443 to differentiate patients with HCC from controls was 0.8089. In another study, EV-derived circ-0004277 was remarkably increased in the plasma of patients with HCC, and its AUC, sensitivity, and specificity for diagnosing HCC were 0.816, 58.3%, and 96.7%, respectively.[81] Further studies found that circ-0004277 could be transmitted from HCC cells to surrounding normal cells by EVs to induce its overexpression, thereby stimulating EMT progression and inhibiting ZO-1 expression.[81] These results demonstrated that EV-derived circ-0072088, circANTXR1, circ-0051443, and circ-0004277 have great potential as novel and independent diagnostic markers and therapeutic targets for HCC.

circRNA signatures combined with AFP tend to show higher diagnostic performance in distinguishing early-stage HCC from controls. Sun *et al*. reported that the accuracies of circ-0004001, circ-0004123, and circ-0075792 in HCC screening were 0.79, 0.73, and 0.76, respectively, while their circRNA signature contributed to increased sensitivity (90.5%), specificity (78.1%), and AUC values (0.89) for diagnosis.[82] circ-0070396 was highly overexpressed in the plasma of HCC patients, whereas its free expression level in HCC plasma samples was not significantly different from that in non-cancerous controls, indicating that circ-0070396 is mainly packaged into EVs.[83] The efficiency of EV-derived circ-0070396 (AUC = 0.857) in detecting HCC from HC was substantially higher than that of AFP (AUC = 0.781) and free circ-0070396 (AUC = 0.587), whereas the AUC, sensitivity, and specificity of the combination of EV-derived circ-0070396 and AFP were 0.938, 82%, and 100%, respectively. In addition, the combination had optimal diagnostic performance (AUC of 0.85 and 0.75) for discriminating HCC from LC and chronic hepatitis B.[83] In another study, 143 upregulated EV-derived circRNAs

Table 3. The diagnostic efficacy of EV-derived circRNAs for HCC patients

Author	Country	Year	Sample size	Categories	Diagnostic efficacy	Reference controls
Lin *et al*.[75]	China	2021	50 NC 50 HCC	circ-0072088	0.899	GAPDH
Huang *et al*.[79]	China	2021	50 NC 70 HCC	circANTXR1	0.76	GAPDH
Chen *et al*.[80]	China	2020	60 NC 60 HCC	circ-0051443	0.809	GAPDH
Zhu *et al*.[81]	China	2020	60 NC 60 HCC	circ-0004277	0.816	GAPDH
Sun *et al*.[82]	China	2020	40 NC 71 HCC	circ-0004001 circ-0004123 circ-0075792 Panels	0.79 0.73 0.76 0.89	GAPDH
Lyu *et al*.[83]	China	2021	54 NC 108 CLD 111 HCC	circ-0070396 AFP Combination	0.857 0.781 0.938	18s rRNA
Guo *et al*.[84]	China	2021	30 NC 87 HCC	circ-0006602 AFP CEA circ-0006602 +AFP	0.907 0.694 0.589 0.942	GAPDH
Wang *et al*.[85]	China	2021	56 CLD 104 HCC	circ-0028861 AFP Combination	0.82 0.76 0.86	GAPDH

HCC, hepatocellular carcinoma; circRNA, circular RNA; NC, negative control; CLD, chronic liver disease; AFP, alpha-fetoprotein; CEA, carcinoembryonic antigen; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

were identified by full transcriptome sequencing, in which EV-derived circ-0006602 showed the highest fold-change. Biological cellular experiments have shown that silencing EV-derived circ-0006602 expression dramatically suppresses the proliferation, invasion, and migration of HCC cells.[84] In clinical diagnosis, the AUC of plasma EV-derived circ-0006602 was 0.907, especially 0.956 for early-stage HCC, superior to that of serum tumor markers AFP (AUC = 0.694) and carcinoembryonic antigen (CEA) (AUC = 0.598), while it improved to 0.942 when combined with AFP.[84] Likewise, Wang *et al*. reported that serum EV-derived circ-0028861 exhibited an AUC of 0.79 for screening all HCC patients from HBV and LC patients, similar to AFP (AUC = 0.76); whereas the combination of EV-derived circ-0028861 and AFP had a better accuracy (0.86).[85]

Dysregulated EV-derived circRNAs play crucial roles in HCC development and malignant progression and show considerable promise as biomarkers for early-stage HCC owing to their distinctive structural properties. However, several important challenges hinder their clinical translation. For instance, EV-derived circRNAs including circ-0074854,[86] circAKT3,[87] and circFBLIM1[88] are all highly expressed in HCC serum and associated with poor survival and elevated recurrence risk, yet sufficient clinical data to rigorously assess their diagnostic capabilities remain unavailable. Moreover, the lack of standardized EV isolation and circRNA detection protocols across studies makes it difficult to directly compare diagnostic performance metrics. Large-scale, multicenter prospective studies enrolling diverse HCC patient cohorts are urgently needed to identify stable, high-specificity EV-derived circRNA signatures. Improvements in circRNA extraction, amplification, and quantification technologies will further facilitate rigorous diagnostic evaluation.

EV-derived mRNAs

Unlike noncoding RNAs, which are transferred to recipient cells via EVs to modulate downstream gene expression, EV-derived mRNAs can be translated in recipient cells to directly regulate molecular signaling pathways, thereby promoting tumor growth and migration in HCC and surrounding stromal cells.[89] Furthermore, key mRNAs encoding critical epigenetic information about HCC cells can be packaged into EVs and secreted into peripheral blood and ascites, exhibiting high heterogeneity and tissue specificity that may be exploited for diagnostic purposes.[89] Recent transcriptomic studies have begun to define the EV-derived mRNA landscape in HCC: Huang *et al*. identified 9,440 dysregulated mRNAs in HCC plasma EVs by RNA sequencing, although the majority showed relatively low tumor specificity.[54] Wu *et al*. further identified 159 differentially expressed mRNAs using an EV-related online database, suggesting that elevated SMARCA5, CDC42, and UBC mRNA levels in HCC patient EVs represent promising candidate biomarkers.[90] Detection of EV-derived mRNA thus offers new avenues for minimally invasive HCC diagnosis.

Cancer-testis antigen lactate dehydrogenase C (LDHC), a specific class of the lactate dehydrogenase (LDH) isoenzyme family, was first reported in human spermatozoa and spermatogenic cells.[91] LDHC, which has limited expression in normal tissues, is primarily involved in the maintenance of glycolysis and ATP homeostasis throughout spermatogenesis.[92] High-throughput sequencing results showed that LDHC is significantly expressed in some malignancies and participates in regulating tumor cell invasion and metastasis, such as lung adenocarcinoma[93] and breast cancer.[94] Interestingly, Cui *et al*. found that the expression level of LDHC mRNA was remarkably increased in HCC serum and serum EVs, with 68% and 60% positivity rates, respectively. ROC analysis

showed that the AUC of LDHC mRNA in serum EV (0.945) was significantly higher than that in free serum (0.838), suggesting that EV-derived LDHC mRNA is a suitable peripheral screening tool for HCC.[95] Iron metabolism is a vital pathway in the promotion and progression of HCC. Abnormal iron accumulation is mainly enriched in hepatocytes, which in turn promotes mitochondrial DNA damage, lipid peroxidation, and protein denaturation.[96] HEPCIDIN plays a crucial role in iron metabolism by affecting intestinal absorption pathways. Sasaki *et al*. found that the HEPCIDIN mRNA variant was more abundant in HCC serum EVs and hepatoma-derived cell lines than in controls; meanwhile, the copy number of the EV-derived HEPCIDIN mRNA variant could be used to discriminate HCC patients from CLD controls, with a sensitivity of 52.2% and specificity of 100%.[97] Similarly, heterogeneous nuclear ribonucleoprotein H1 (hnRNPH1) gene was listed for the first time as a new and promising biomarker for early-stage HCC diagnosis. The hnRNPH1 gene is an important RNA-binding protein involved in all key events in cellular RNA biogenesis, and its expression is upregulated in multiple cancer cells.[98] Quantitative analysis of serum EV-derived contents in Xu's study revealed that increased EV-derived hnRNPH1 mRNA indicated advanced Child-Pugh classification and TNM stage.[99] In further diagnostic evaluation, serum EV-derived hnRNPH1 mRNA displayed satisfactory efficiency in differentiating HCC patients from CHB patients (AUC 0.865, sensitivity 85.2%, specificity 76.5%), while serum AFP had an AUC of 0.785. However, both markers yielded similar AUC values in diagnosing HCC from LC patients (0.647 for hnRNPH1 mRNA vs. 0.674 for AFP). Notably, EV-derived hnRNPH1 mRNA in combination with AFP increased the diagnostic accuracy with 0.891 AUC, 87.5% sensitivity, and 84.8% specificity.[99]

RNA expression profiling is widely used to construct predictive signatures for cancer diagnosis and progression. For instance, myosin light chain 6 B (MYL6B) is a class of light chains of the myosin family, which is mainly responsible for material transport and cell adhesion processes.[100] A previous study showed that MYL6B could induce ubiquitination and degradation of the p53 protein to suppress HCC cell apoptosis.[101] The THO complex subunit 2 gene (THOC2) is located on chromosome Xq25 and encodes a subunit of the Transcription-Export (TREX) complex.[102] THOC2 plays an essential role in nuclear mRNA export and maintaining highly conserved properties. Dysregulated THOC2 or mutations result in abnormal retention of mRNA in the nucleus, which can cause neurodevelopmental disorders and tumorigenesis.[103] Zhu *et al*. found that EV-derived MYL6B and THOC2 expression were closely related to clinical features of HCC patients and HCC cell migration. They established a highly effective model for diagnosing early-stage HCC: P = −23.0370 + (2.2566 × expression level of EV-derived MYL6B) + (3.8247 × expression level of EV-derived THOC2). This model had an AUC of 0.879, sensitivity of 76.0%, and specificity of 82.0%.[104] Sun *et al*. constructed a diagnostic model with 10 specific plasma-EV mRNAs for early detection of HCC, with an AUC value of 0.93 (sensitivity = 94.4%, specificity = 88.5%).[105] However, these gene signatures require further clinical data to verify their practical application.

EV-derived proteins and lipids

Although traditional serum protein markers remain the primary minimally invasive approach for HCC screening, their clinical utility is limited, particularly for early-stage disease or for monitoring disease progression.[106] Growing evidence indicates that EV-derived proteins and lipids play dual roles: they serve as essential structural and functional components for EV biogenesis and cargo transport, while simultaneously carrying substantial molecular information that reflects the physiological and pathological state of the originating cells.[107–109] Compared with conventional serum protein markers, which are subject to dilution in blood and interference from complex protein interactions, EV-derived proteins and lipids are highly concentrated and can be enriched through EV purification procedures.[108] Multiple studies have now established the diagnostic and prognostic utility of EV-derived proteins and lipids across various cancer types, including gastric cancer,[110] HCC,[111] pancreatic cancer,[112] and colorectal cancer.[113]

Data-independent acquisition (DIA) is a robust and precise proteomic quantification technology based on mass spectrometry that can be used to provide highly reproducible measurements of complex protein samples.[114] Recently, DIA was developed for protein biomarker discovery and validation through bottom-up proteomics.[115] By performing DIA analysis, Zhao *et al*. clustered a spectral library containing 1,251 proteins from the serum EV-derived proteomic profiling of HCC patients and identified ten EV-derived protein panels as candidate biomarkers, such as hemopexin (HPX).[111] HPX glycan has been verified as a promising marker for distinguishing HCC patients from negative controls, with a sensitivity and specificity of 79% and 93%, respectively.[116] Chaperonin containing TCP1 subunit 8 (CCT8) is widely known to play an essential role in maintaining the correct folding of various oncoproteins and tumor suppressors. Studies have found that CCT8 is significantly overexpressed in HCC cells and blood samples. It correlates directly with the histological grade and malignancy of HCC.[117,118] A study found that CCT8 attenuates the migration and invasion of HCC cells via glucose-regulated protein 94 (GRP94)/c-Jun/EMT signaling.[119] Likewise, cofilin 1 (CFL1) is increased in human HCC, and its high expression is associated with a low overall survival rate and poor progression in patients with HCC.[120] Both *in vivo* and *in vitro* experiments have revealed that silencing CFL1 remarkably suppresses the growth and metastasis of HCC cells.[121] This evidence indicates that CCT8 and CFL1 may serve as potential targets for the diagnosis and treatment of HCC. Interestingly, a recent study reported that CCT8 and CFL1 proteins in EVs isolated from HCC cells and serum had higher expression levels than in controls, and ROC analysis of the validation cohort showed that the AUC of serum EV-derived CCT8 protein (cutoff > 31 ng/mL) and CFL1 protein (cutoff > 1,040 pg/mL) were 0.698 and 0.677, respectively, to detect HCC, while the AUC of serum AFP (cutoff > 20 ng/mL) was 0.628.[122] However, the combination with AFP had the highest diagnostic performance, with an AUC of 0.838.[122] Hence, EV-derived CCT8 and CFL1 proteins represent promising novel biomarkers for HCC screening (Table 4).[95,97,99,104,105,122] Further research involving evaluation of their diagnostic efficiency in free serum will contribute to the selection of a better strategy.

EVs are rich in lipids, which not only protect their contents from degradation but also play a crucial role in the entire process of EV formation, including the creation of multivesicular bodies, endosomal sorting, secretion, and interactions with target cells.[123] The types and distribution of lipids in EV membranes vary depending on cell type. Additionally, studies have shown that different biological states of cells, including cancerous conditions, can significantly influence the composition and quantity of lipids in EVs.[124] Therefore, EV-derived lipids carry valuable information about the physiological state of the cells that produce them, making them potential biomarkers.[109] For instance, a recent study using ultra-high-resolution mass spectrometry identified 2,864 distinct EV-derived lipids across 52 classes that were differentially expressed in HCC patients compared with non-HCC

Table 4. The diagnostic efficacy of EV-derived mRNAs and proteins for HCC patients

Author	Country	Year	Sample size	Categories	Diagnostic efficacy	Reference controls
Cui *et al*.[95]	China	2020	100 NC 112 HCC	LDHC	0.945	GAPDH
Sasaki *et al*.[97]	Japan	2019	30 NC 17 CLD 20 HCC	HEPCIDIN	0.60	NA
Xu *et al*.[99]	China	2017	68 NC 135 CLD 88 HCC	hnRNPH1 AFP Combination	0.865 0.785 0.891	GAPDH
Zhu *et al*.[104]	China	2021	202 NC 243 HCC	MYL6B+THOC2	0.907	NA
Sun *et al*.[105]	China	2020	23 NC 51 CLD 46 HCC	AFP/GPC3/ALB/APOH /FABP1/FGB/FGG/AHSG /RBP4/TF AFP	0.93 0.65	NA
Cho *et al*.[122]	Korea	2021	34 NC 58 CLD 132 HCC	CCT8 CFL1 AFP Combination AFP+CCT8 AFP+CFL1 CCT8+CFL1	0.698 0.677 0.628 0.838 0.728 0.773 0.829	HMBS

NC, negative control; CLD, chronic liver disease; HCC, hepatocellular carcinoma; NA, not applicable; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDHC, lactate dehydrogenase C; AFP, alpha-fetoprotein; GPC3, glypican 3; ALB, albumin; APOH, apolipoprotein H; FABP1, fatty acid binding protein 1; FGB, fibrinogen beta chain; FGG, fibrinogen gamma chain; AHSG, alpha 2-HS glycoprotein; RBP4, retinol binding protein 4; TF, transferrin; CFL1, cofilin 1; CCT8, chaperonin-containing TCP1 subunit 8; HMBS, hydroxymethylbilane Synthase.

patients.[107] Certain lipid species, such as dilysocardiolipins, lysophosphatidylserines, cardiolipins, and sphingosines, show specific expression levels in early-stage HCC, indicating their diagnostic potential.[109] However, it is important to note that EV lipidomics is a relatively new area of research, and a limited number of studies have explored it. According to statistical data, the majority of current research on EV biology (over 95%) has focused on genomics and proteomics.[123] Nevertheless, advancements in technologies for isolating, purifying, and quantifying EVs have led to growing interest in the biological functions of EV-derived lipids. EV-derived lipids are being explored as new therapeutic and diagnostic markers for cancer.[124] Currently, there are limited reports on the detection capabilities of EV-derived lipids in HCC patients, but substantial clinical data are being gathered to identify potential EV-derived lipid biomarkers that could enhance the positive detection rate in HCC patients.

Critical analysis of key clinical trials

Strengths of current clinical trials

First, most clinical studies adopted well-defined case-control designs with strict inclusion criteria—enrolling treatment-naive HCC patients alongside cirrhosis, chronic hepatitis, and HCs—enabling direct comparison of EV biomarker levels across distinct liver disease stages. This design strength supports the clinical utility of EVs in discriminating early HCC from high-risk backgrounds, though it also introduces spectrum bias that may inflate apparent diagnostic performance. Second, several trials validated EV-derived multi-marker panels (e.g., multi-miRNA panels, lncRNA signatures, or combined protein assays) rather than relying on single markers, which markedly improved diagnostic specificity and reduced false-positive rates compared with AFP alone, demonstrating the added value of a panel-based strategy. Third, some studies specifically targeted clinically unmet needs—including AFP-negative early HCC, a notoriously difficult diagnostic scenario—achieving promising sensitivity and demonstrating the potential of EV-based biomarkers to complement and extend conventional screening strategies. Fourth, multiple trials confirmed the pre-analytical stability of EV cargoes (miRNAs, lncRNAs, circRNAs, and proteins) under common storage conditions, including repeated freeze-thaw cycles, a critical prerequisite for clinical laboratory feasibility.

Major limitations and methodological weaknesses

Despite the strengths noted above, critical methodological weaknesses substantially limit the clinical applicability of current findings. First, nearly all published trials are single-center, retrospective studies with small sample sizes (typically below 300 participants), resulting in insufficient statistical power and poor external generalizability. Large-scale, prospective, multicenter validation studies are universally lacking across all EV biomarker categories. Second, the overwhelming majority of studies recruited Asian patients with HBV-related HCC, leaving a critical evidence gap for NAFLD-related, HCV-related, or alcohol-related HCC. The extent to which current biomarker panels perform across different etiologies and ethnic populations remains largely unknown. Third, the absence of standardized protocols for EV isolation, quantification, and characterization is a fundamental limitation. Variations in ultracentrifugation speed, precipitation conditions, or size-exclusion chromatography parameters directly affect EV purity and biomarker yield, generating inconsistent and non-comparable results across studies. Fourth, few studies have included long-term clinical follow-up to assess the prognostic or predictive value of EV biomarkers beyond initial diagnostic performance, severely limiting understanding of their utility in disease monitoring. Fifth, and critically, none of the current trials have established clinically applicable diagnostic

cutoff values that have been independently validated in external cohorts, making real-world implementation currently unfeasible. Taken together, these methodological deficiencies collectively impede the translation of EV biomarkers from research findings into routine clinical practice.

The isolation strategies of EVs

High-quality EV isolation is a critical prerequisite for reliable diagnostic analysis. The challenges inherent in isolating and purifying EVs—primarily stemming from their wide range in size, compositional heterogeneity across cell types, and contamination by co-isolated impurities—remain significant obstacles, particularly for diagnostic applications requiring high purity and specificity.[125] Current methodologies for isolating EVs from cell culture media and biological fluids broadly exploit differences in density, particle size, solubility, surface molecular recognition, and microfluidic properties.[125,126] Ultracentrifugation remains the most widely employed approach, utilizing differential centrifugal forces or sucrose density gradients; however, it is equipment-intensive, time-consuming, and involves multistep procedures that may compromise EV integrity through repeated centrifugation.[127] Size-based isolation methods—including ultrafiltration, sequential filtration, and size-exclusion chromatography—have gained broad adoption in EV research.[128–130] These methods enrich EVs from complex biological samples using membranes with defined pore sizes (polyether sulfone, polycarbonate, or anodic aluminum oxide)[125] and preserve EV biological function[131,132]; however, they risk co-isolating other nanovesicles of similar size, potentially reducing specificity.[126] Polymer-based co-precipitation, widely used in commercial EV isolation kits, offers convenience and scalability; yet the purity of precipitated material is often compromised by co-isolated contaminants such as viruses, lipoproteins, and soluble proteins, which may interfere with downstream analyses.[133,134] More recently, advanced methods including microfluidic chips, molecularly imprinted polymers, and immunoaffinity capture have been developed, exploiting antibody binding to specific EV surface markers (TSG101, CD9, CD63, and HSP70) and employing nanowire- or nanopore-based microfluidic architectures to improve EV isolation specificity and purity.[135–137] Nevertheless, high fabrication costs, operational complexity, and limited sample throughput currently restrict their clinical scalability.[133] As diagnostic EV research constitutes nearly half of all registered EV-related clinical trials, the development of efficient, high-purity, high-throughput EV isolation platforms suitable for clinical implementation is an urgent priority.

The clinical translation challenges of EVs

The clinical translation of EVs from preclinical research to routine practice faces three interconnected challenges: cost, reproducibility, and the lack of standardized diagnostic thresholds. The workflow from EV isolation to downstream analysis entails considerable costs. For example, ultracentrifugation requires specialized equipment and trained personnel, while the cost per test of commercial kits is significantly higher than that of traditional AFP detection, which limits its application in large-scale screening in resource-limited settings.[133] Methodological heterogeneity severely compromises the consistency of results. This includes variations in product purity caused by different EV isolation methods, inconsistencies in the quantitative accuracy of detection platforms, and the impact of pre-analytical variables (e.g., sample processing and storage) on biomarker stability, making it difficult to directly compare data across different studies. In addition, there is a lack of widely validated and universally applicable diagnostic cut-off values. Current thresholds are mostly established based on small cohorts of specific populations and have not been verified through multicenter studies in populations with different etiologies (e.g., HBV- and NAFLD-related liver cancer), which restricts their clinical universality. Overcoming these challenges requires collaborative efforts from multiple parties, including the development of low-cost technologies and equipment, the formulation of internationally unified experimental guidelines, and large-scale validation in diverse populations.

Methodological considerations and challenges

Methodological heterogeneity in current EV research has become a critical bottleneck, limiting the comparability of findings and impeding clinical translation, with its core sources manifesting in three principal dimensions. First, variability in sample sources: existing studies employ both serum and plasma for EV isolation, and differences in sample preparation protocols generate substantial discrepancies in EV yield, purity, and cargo composition, directly influencing biomarker quantification and the establishment of diagnostic thresholds. Second, diversity of EV isolation and enrichment platforms: ultracentrifugation, polymer-based co-precipitation, size-exclusion chromatography, and immunoaffinity capture each carry inherent limitations that introduce systematic biases. Ultracentrifugation yields relatively pure EVs but is operationally cumbersome and may cause structural damage[127]; polymer-based co-precipitation is operationally convenient but results in low-purity preparations susceptible to lipoprotein and soluble protein contamination[134]; size-exclusion chromatography preserves EV biological activity but may co-isolate size-matched non-EV nanoparticles.[126] Third, variability of downstream biomolecular detection platforms: even for identical biomarkers, differences in analytical sensitivity, dynamic range, and specificity across platforms generate significant inter-study variation in reported diagnostic performance metrics (AUC, sensitivity, specificity), making cross-study comparisons inherently unreliable.

The aforementioned methodological heterogeneity not only hinders the integration and validation of results among different studies but also poses severe challenges to the clinical translation of EV-derived biomarkers. On the one hand, the lack of unified EV isolation and detection protocols leads to poor stability in the quantitative results of biomarkers, directly affecting the determination of diagnostic thresholds and the accuracy of clinical applications. On the other hand, inconsistent research results caused by heterogeneity reduce the level of evidence, making it difficult for most biomarkers to pass large-scale multicenter validation. In addition, some studies fail to conduct rigorous characterization of EVs (e.g., particle size distribution, surface marker detection, purity identification), which further exacerbates the unreliability of results. Therefore, establishing standardized operating procedures has become an urgent demand in the field of EV research. Strict adherence to the MISEV2023 guidelines issued by ISEV—covering EV isolation, characterization, cargo analysis, and data reporting—is essential to improving the comparability and reproducibility of research findings across studies. Future studies should clarify the selection of sample types (plasma is preferred to reduce coagulation-related interference), standardize EV isolation techniques (e.g., density gradient ultracentrifugation recommended by MISEV2023), unify detection platforms, and strengthen comprehensive characterization of EVs. Methodological standardization will lay a solid foundation for the clinical translation of EV-derived biomarkers.

The endogenous controls for EVs

Reference genes—commonly referred to as housekeeping genes—are stably expressed across diverse tissues and cell types and are routinely used as endogenous controls to normalize target gene expression and minimize experimental variability.[138] Selecting an appropriate endogenous control is particularly critical for accurate quantitative analysis of EV-derived nucleic acids, as the EV isolation process introduces additional sources of variability not encountered in conventional cell-based assays. Discrepancies in reference gene selection across studies contribute substantially to inter-study inconsistencies in HCC diagnostic accuracy and further impede meaningful cross-study comparisons. Researchers have advocated reference gene validation specific to cell type, HCC subtype, and sample source (including EVs).[139,140]

Currently, the snRNAs U6 and miR-16 are commonly used to normalize miRNAs in HCC EVs and plasma/serum.[141] However, recent findings indicate that U6 and miR-16 are highly unstable for normalizing EV-derived miRNAs, owing to significant expression variability within groups.[142] Tang *et al*. found that U6 was the least stable endogenous control when assessing miRNA levels in serum samples from patients with HCC, CLD, HBV, and healthy individuals.[143] Similarly, Jacobsen *et al*. noted a significant bias when using U6 and miR-211 between HBV-replicating cells and liver cells, highlighting their inadequacy as reference genes.[144] Additionally, some studies have raised concerns that U6 and miR-16 from EVs might be affected by contamination, particularly because U6 is primarily found in the nucleus, which contradicts its EV origin.[145] Consequently, new candidate reference genes have been proposed to enhance miRNA quantification accuracy, such as miR-106a[143] and miR-1280[146] for most HCC, miR-4644 for HBV/LC-HCC,[147] and miR-24-3p and miR-151a-3p for HBV-HCC.[144] Furthermore, cel-miR-39 has gained popularity as a normalization choice in many laboratories owing to its stable expression. Li *et al*. identified miR-221, miR-191, let-7a, miR-181a, and miR-26a as the optimal endogenous controls for EV-derived miRNAs in patients with HCC and HBV.[142] Notably, another independent study found that using miR-221, miR-181a, and miR-26a for normalization improved the quantitative accuracy of EV-derived miRNAs in HCC patients.[148]

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin (ACTB) are the housekeeping genes most commonly used in cancer research. GAPDH is a key enzyme in glycolysis, whereas ACTB is an important component of muscle filaments and cytoskeletal microfilaments.[149] Our analysis shows that these two genes are frequently used as internal reference genes in studies assessing the diagnostic value of EVs. However, only Bahri *et al*.[150] reported the precise standardization of suitable reference genes in EVs derived from cancer cells. Controversially, the three traditional genes ACTB, TBP, and HPRT1 were nearly absent in the EVs of liver cancer cells. This may be limited by the use of different algorithms (Delta Ct, NormFinder, RefFinder). However, subsequent validation confirmed the stability of the reference GAPDH. In subsequent research, YWHAZ and UBC could also be considered for data normalization in the study of liver cancer EVs. Some studies have indicated that the same reference genes may not be applicable to different cell types or culture environments. Therefore, it is recommended to validate the internal reference genes before evaluation, select appropriate reference genes, and achieve higher accuracy for data normalization.

Future perspectives

Extracellular vesicles have emerged as a transformative platform for early HCC liquid biopsy, offering superior biomarker stability, specificity, and sensitivity compared with traditional serum markers. Unlike most existing reviews that catalogue biomarkers in isolation, this work integrates mechanistic evidence, quantitative diagnostic performance data, methodological standardization considerations, and a critical appraisal of clinical trial quality to provide a clear, evidence-grounded translational roadmap for EV-based HCC diagnostics.

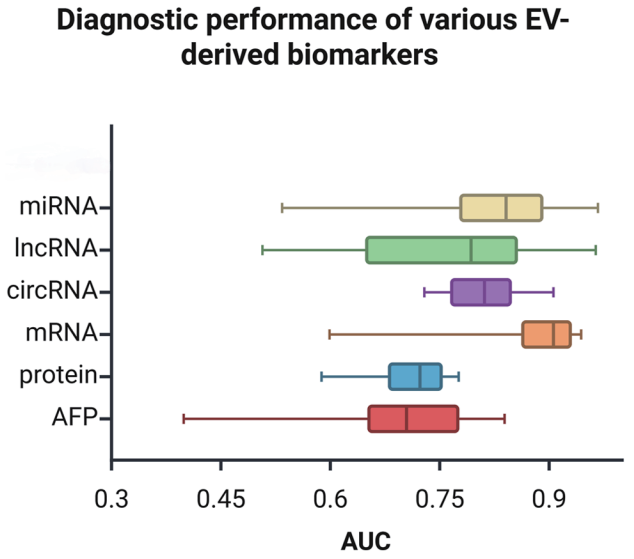

Fig. 3. The diagnostic performance of various EV-derived biomarkers. EV, extracellular vesicle; miRNA, microRNA; lncRNA, long noncoding RNA; circRNA, circular RNA; AFP, alpha-fetoprotein; AUC, area under the curve.

A central conclusion of this review is that EV-derived biomarkers often outperform AFP in many studies for detecting early-stage and AFP-negative HCC. Among the most clinically validated candidates, miRNA panels (particularly EV-miR-21, miR-10b-5p, miR-122, and miR-148a), lncRNA LINC00853, circRNA signatures (circ-0006602, circ-0070396), and combined EV protein markers (CCT8 and CFL1 paired with AFP) achieve AUC values consistently above 0.90 across independent cohorts (Fig. 3), approaching the performance expected of clinical-grade diagnostic tools. Despite these compelling results, widespread clinical implementation remains constrained by inconsistent EV isolation protocols, the absence of large-scale multicenter validation, and a lack of universally accepted diagnostic cutoff values. To translate EV-based liquid biopsy into clinical practice, a prioritized implementation strategy is proposed. In terms of target populations, EV-based screening should first be deployed in well-defined high-risk groups—specifically individuals with HBV or HCV infection, LC, advanced NAFLD-related fibrosis, or AFP-negative early HCC—where unmet diagnostic needs are greatest and the incremental benefit over the current standard of care is most pronounced. Regarding specific biomarker targets, priority should be assigned to EV-miRNA panels combined with AFP (achieving AUC ≥ 0.94), lncRNA LINC00853 (sensitivity 93.75%, specificity 89.77% for stage I HCC), and combined EV protein signatures, given their demonstrated accuracy, analytical feasibility, and clear biological relevance to HCC initiation and immune evasion. Concerning the translational pathway, adoption of MISEV2023-compliant standardized protocols for EV isolation and characterization is the first indispensable

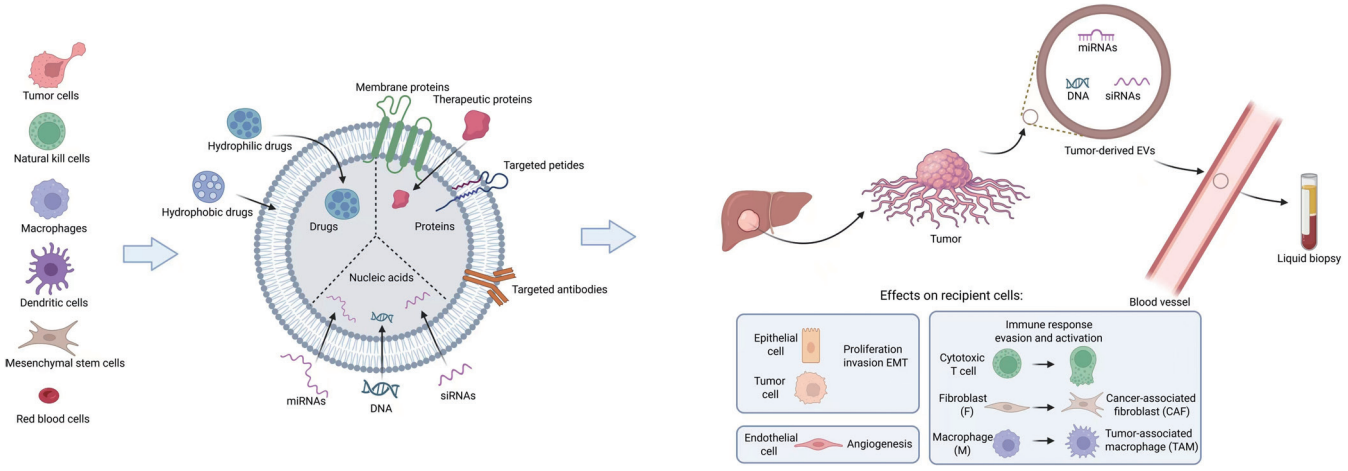

Fig. 4. Molecular mechanisms by which tumor-derived EVs regulate the HCC tumor microenvironment. Created with BioRender. EVs, extracellular vesicles; EMT, epithelial-mesenchymal transition; CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage.

step, followed by prospective multicenter validation studies enrolling diverse etiologies and ethnicities to establish robust, universally applicable diagnostic thresholds. From a therapeutic standpoint, engineered EVs also hold substantial promise as targeted drug delivery vehicles (Fig. 4), leveraging their natural hepatotropism, low immunogenicity, and superior membrane permeability; sorafenib- or doxorubicin-loaded EV formulations have already shown enhanced efficacy in preclinical HCC models.[20] Ultimately, integrating EV biomarkers with imaging data, conventional liquid biopsy panels, and clinical risk scores will generate clinically actionable precision models for HCC prevention, early diagnosis, and targeted treatment.

Conclusions

EVs have evolved from experimental biomarkers into a clinically actionable platform for HCC early detection, risk stratification, and targeted therapy. With the adoption of standardized isolation and characterization protocols—as defined by MISEV2023—and rigorous large-scale multicenter validation, EV-based liquid biopsy is poised to become an integral component of clinical guidelines for HCC management, transforming how this disease is detected and treated.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Writing-original draft (KL), conceptualization (KL, YG), writing-review and editing (ZZ, BZ, YG), visualization (KL, ZZ, XO, MY, YG), and validation (ZZ, YQ). All authors have approved the final version and publication of the manuscript.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71(3):209–249. doi:10.3322/caac.21660, PMID:33538338.
[2] Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, *et al*. Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7(1):6. doi:10.1038/s41572-020-00240-3, PMID:33479224.
[3] Franceschi S, Raza SA. Epidemiology and prevention of hepatocellular carcinoma. Cancer Lett 2009;286(1):5–8. doi:10.1016/j.canlet.2008.10.046, PMID:19070421.
[4] Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nat Rev Gastroenterol Hepatol 2010;7(8):448–458. doi:10.1038/nrgastro.2010.100, PMID:20628345.
[5] Gao Q, Zhu H, Dong L, Shi W, Chen R, Song Z, *et al*. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. Cell 2019;179(2):561–577.e22. doi:10.1016/j.cell.2019.08.052, PMID:31585088.
[6] Hu H, Zhao Y, He C, Qian L, Huang P. Ultrasonography of Hepatocellular Carcinoma: From Diagnosis to Prognosis. J Clin Transl Hepatol 2024;12(5):516–524. doi:10.14218/JCTH.2024.00018, PMID:38779517.
[7] Maluccio M, Covey A. Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma. CA Cancer J Clin 2012;62(6):394–399. doi:10.3322/caac.21161, PMID:23070690.
[8] Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med 2014;11(4):e1001624. doi:10.1371/journal.pmed.1001624, PMID:24691105.
[9] Ayuso C, Rimola J, Vilana R, Burrel M, Darnell A, García-Criado Á, *et al*. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. Eur J Radiol 2018;101:72–81. doi:10.1016/j.ejrad.2018.01.025, PMID:29571804.
[10] Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, *et al*. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology 2018;154(6):1706–1718.e1. doi:10.1053/j.gastro.2018.01.064, PMID:29425931.
[11] Di Martino M, De Filippis G, De Santis A, Geiger D, Del Monte M, Lombardo CV, *et al*. Hepatocellular carcinoma in cirrhotic patients: prospective comparison of US, CT and MR imaging. Eur Radiol 2013;23(4):887–896. doi:10.1007/s00330-012-2691-z, PMID:23179521.
[12] Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, *et al*. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles 2024;13(2):e12404. doi:10.1002/jev2.12404, PMID:38326288.
[13] Chen R, Xu X, Tao Y, Qian Z, Yu Y. Exosomes in hepatocellular carcinoma: a new horizon. Cell Commun Signal 2019;17(1):1. doi:10.1186/s12964-018-0315-1, PMID:30616541.
[14] Xue D, Han J, Liu Y, Tuo H, Peng Y. Current perspectives on exosomes in the diagnosis and treatment of hepatocellular carcinoma (review). Cancer Biol Ther 2021;22(4):279–290. doi:10.1080/15384047.2021.1898728, PMID:33847207.
[15] Zeng Y, Hu S, Luo Y, He K. Exosome Cargos as Biomarkers for Diagnosis and Prognosis of Hepatocellular Carcinoma. Pharmaceutics 2023;15(9):2365. doi:10.3390/pharmaceutics15092365, PMID:37765333.
[16] Yang Y, Mao F, Guo L, Shi J, Wu M, Cheng S, *et al*. Tumor cells derived-extracellular vesicles transfer miR-3129 to promote hepatocellular carcinoma metastasis by targeting TXNIP. Dig Liver Dis 2021;53(4):474–485. doi:10.1016/j.dld.2021.01.003, PMID:33563583.

[17] Wei JX, Lv LH, Wan YL, Cao Y, Li GL, Lin HM, *et al*. Vps4A functions as a tumor suppressor by regulating the secretion and uptake of exosomal microRNAs in human hepatoma cells. Hepatology 2015;61(4):1284–1294. doi:10.1002/hep.27660, PMID:25503676.
[18] Shi TT, Zhang RB, Wu Y, Zhang Y, Zhu L, Gao C, *et al*. Role of extracellular vesicles of different origins in the development and progression of hepatocellular carcinoma. J Clin Hepatol 2024;40(6):1264–1268. doi:10.12449/JCH240630.
[19] Wong SWK, Tey SK, Mao X, Fung HL, Xiao ZJ, Wong DKH, *et al*. Small Extracellular Vesicle-Derived vWF Induces a Positive Feedback Loop between Tumor and Endothelial Cells to Promote Angiogenesis and Metastasis in Hepatocellular Carcinoma. Adv Sci (Weinh) 2023;10(26):e2302677. doi:10.1002/advs.202302677, PMID:37387563.
[20] Zhang G, Huang X, Xiu H, Sun Y, Chen J, Cheng G, *et al*. Extracellular vesicles: Natural liver-accumulating drug delivery vehicles for the treatment of liver diseases. J Extracell Vesicles 2020;10(2):e12030. doi:10.1002/jev2.12030, PMID:33335695.
[21] Julich-Haertel H, Urban SK, Krawczyk M, Willms A, Jankowski K, Patkowski W, *et al*. Cancer-associated circulating large extracellular vesicles in cholangiocarcinoma and hepatocellular carcinoma. J Hepatol 2017;67(2):282–292. doi:10.1016/j.jhep.2017.02.024, PMID:28267620.
[22] Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Curr Genomics 2010;11(7):537–561. doi:10.2174/138920210793175895, PMID:21532838.
[23] Peng C, Ye Y, Wang Z, Guan L, Bao S, Li B, *et al*. Circulating microRNAs for the diagnosis of hepatocellular carcinoma. Dig Liver Dis 2019;51(5):621–631. doi:10.1016/j.dld.2018.12.011, PMID:30744930.
[24] Zhao L, Yu J, Wang J, Li H, Che J, Cao B. Isolation and Identification of miRNAs in exosomes derived from serum of colon cancer patients. J Cancer 2017;8(7):1145–1152. doi:10.7150/jca.18026, PMID:28607588.
[25] Zhu L, Zhao L, Wang Q, Zhong S, Guo X, Zhu Y, *et al*. Circulating exosomal miRNAs and cancer early diagnosis. Clin Transl Oncol 2022;24(3):393–406. doi:10.1007/s12094-021-02706-6, PMID:34524618.
[26] Wang S, Yang Y, Sun L, Qiao G, Song Y, Liu B. Exosomal MicroRNAs as Liquid Biopsy Biomarkers in Hepatocellular Carcinoma. Onco Targets Ther 2020;13:2021–2030. doi:10.2147/OTT.S232453, PMID:32210570.
[27] Ghosh S, Bhowmik S, Majumdar S, Goswami A, Chakraborty J, Gupta S, *et al*. The exosome encapsulated microRNAs as circulating diagnostic marker for hepatocellular carcinoma with low alpha-fetoprotein. Int J Cancer 2020;147(10):2934–2947. doi:10.1002/ijc.33111, PMID:32441313.
[28] Cho HJ, Eun JW, Baek GO, Seo CW, Ahn HR, Kim SS, *et al*. Serum Exosomal MicroRNA, miR-10b-5p, as a Potential Diagnostic Biomarker for Early-Stage Hepatocellular Carcinoma. J Clin Med 2020;9(1):281. doi:10.3390/jcm9010281, PMID:31968558.
[29] Zhang Y, Xi H, Nie X, Zhang P, Lan N, Lu Y, *et al*. Assessment of miR-212 and Other Biomarkers in the Diagnosis and Treatment of HBV-infection-related Liver Diseases. Curr Drug Metab 2019;20(10):785–798. doi:10.2174/1389200220666191011120434, PMID:31608838.
[30] Abd El Gwad A, Matboli M, El-Tawdi A, Habib EK, Shehata H, Ibrahim D, *et al*. Role of exosomal competing endogenous RNA in patients with hepatocellular carcinoma. J Cell Biochem 2018;119(10):8600–8610. doi:10.1002/jcb.27109, PMID:30015383.
[31] Xue X, Wang X, Zhao Y, Hu R, Qin L. Exosomal miR-93 promotes proliferation and invasion in hepatocellular carcinoma by directly inhibiting TIMP2/TP53INP1/CDKN1A. Biochem Biophys Res Commun 2018;502(4):515–521. doi:10.1016/j.bbrc.2018.05.208, PMID:29859935.
[32] Hao X, Xin R, Dong W. Decreased serum exosomal miR-320a expression is an unfavorable prognostic factor in patients with hepatocellular carcinoma. J Int Med Res 2020;48(4):300060519896144. doi:10.1177/0300060519896144, PMID:32339037.
[33] Wang Y, Zhang C, Zhang P, Guo G, Jiang T, Zhao X, *et al*. Serum exosomal microRNAs combined with alpha-fetoprotein as diagnostic markers of hepatocellular carcinoma. Cancer Med 2018;7(5):1670–1679. doi:10.1002/cam4.1390, PMID:29573235.
[34] Xue X, Zhao Y, Wang X, Qin L, Hu R. Development and validation of serum exosomal microRNAs as diagnostic and prognostic biomarkers for hepatocellular carcinoma. J Cell Biochem 2019;120(1):135–142. doi:10.1002/jcb.27165, PMID:30238497.
[35] Yang J, Dong W, Zhang H, Zhao H, Zeng Z, Zhang F, *et al*. Exosomal microRNA panel as a diagnostic biomarker in patients with hepatocellular carcinoma. Front Cell Dev Biol 2022;10:927251. doi:10.3389/fcell.2022.927251, PMID:36211468.
[36] Cui Y, Xu HF, Liu MY, Xu YJ, He JC, Zhou Y, *et al*. Mechanism of exosomal microRNA-224 in development of hepatocellular carcinoma and its diagnostic and prognostic value. World J Gastroenterol 2019;25(15):1890–1898. doi:10.3748/wjg.v25.i15.1890, PMID:31057302.
[37] Fründt T, Krause L, Hussey E, Steinbach B, Köhler D, von Felden J, *et al*. Diagnostic and Prognostic Value of miR-16, miR-146a, miR-192 and miR-221 in Exosomes of Hepatocellular Carcinoma and Liver Cirrhosis Patients. Cancers (Basel) 2021;13(10):2484. doi:10.3390/cancers13102484, PMID:34069692.
[38] Cho HJ, Baek GO, Seo CW, Ahn HR, Sung S, Son JA, *et al*. Exosomal microRNA-4661-5p-based serum panel as a potential diagnostic biomarker for early-stage hepatocellular carcinoma. Cancer Med 2020;9(15):5459–5472. doi:10.1002/cam4.3230, PMID:32537885.
[39] Hong Y, Ye M, Wang F, Fang J, Wang C, Luo J, *et al*. MiR-21-3p Promotes Hepatocellular Carcinoma Progression via SMAD7/YAP1 Regulation. Front Oncol 2021;11:642030. doi:10.3389/fonc.2021.642030, PMID:33763375.
[40] Cai M, Shao W, Yu H, Hong Y, Shi L. Paeonol Inhibits Cell Proliferation, Migration and Invasion and Induces Apoptosis in Hepatocellular Carcinoma by Regulating miR-21-5p/KLF6 Axis. Cancer Manag Res 2020;12:5931–5943. doi:10.2147/CMAR.S254485, PMID:32765094.
[41] Li ZB, Li ZZ, Li L, Chu HT, Jia M. Publisher Correction: MiR-21 and miR-183 can simultaneously target SOCS6 and modulate growth and invasion of hepatocellular carcinoma (HCC) cells. Eur Rev Med Pharmacol Sci 2024;28(9):3290. doi:10.26355/eurrev_202405_36196, PMID:38766781.
[42] Zhang H, Ding R, Chen D. Value of miR-21 levels as potential biomarkers in the early diagnosis of hepatocellular carcinoma:a meta-analysis. Biomarkers 2021;26(7):586–597. doi:10.1080/1354750X.2021.1955976, PMID:34266326.
[43] Wang H, Hou L, Li A, Duan Y, Gao H, Song X. Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. Biomed Res Int 2014;2014:864894. doi:10.1155/2014/864894, PMID:24963487.
[44] Lee JH, Kim JA, Jeong S, Rhee WJ. Simultaneous and multiplexed detection of exosome microRNAs using molecular beacons. Biosens Bioelectron 2016;86:202–210. doi:10.1016/j.bios.2016.06.058, PMID:27372573.
[45] Xu G, Bu S, Wang X, Ge H. MiR-122 radiosensitize hepatocellular carcinoma cells by suppressing cyclin G1. Int J Radiat Biol 2022;98(1):11–17. doi:10.1080/09553002.2021.1987561, PMID:34623217.
[46] Shi M, Jiang Y, Yang L, Yan S, Wang YG, Lu XJ. Decreased levels of serum exosomal miR-638 predict poor prognosis in hepatocellular carcinoma. J Cell Biochem 2018;119(6):4711–4716. doi:10.1002/jcb.26650, PMID:29278659.
[47] Nimitrungtawee N, Inmutto N, Chattipakorn SC, Chattipakorn N. Extracellular vesicles as a new hope for diagnosis and therapeutic intervention for hepatocellular carcinoma. Cancer Med 2021;10(23):8253–8271. doi:10.1002/cam4.4370, PMID:34708589.
[48] Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev 2009;23(13):1494–1504. doi:10.1101/gad.1800909, PMID:19571179.
[49] Wang D, Chen F, Zeng T, Tang Q, Chen B, Chen L, *et al*. Comprehensive biological function analysis of lncRNAs in hepatocellular carcinoma. Genes Dis 2021;8(2):157–167. doi:10.1016/j.gendis.2019.12.013, PMID:33997162.
[50] Shi T, Morishita A, Kobara H, Masaki T. The Role of Long Non-Coding RNA and microRNA Networks in Hepatocellular Carcinoma and Its Tumor Microenvironment. Int J Mol Sci 2021;22(19):10630. doi:10.3390/ijms221910630, PMID:34638971.
[51] Huang JL, Zheng L, Hu YW, Wang Q. Characteristics of long non-coding RNA and its relation to hepatocellular carcinoma. Carcinogenesis 2014;35(3):507–514. doi:10.1093/carcin/bgt405, PMID:24296588.
[52] Tang X, Ren H, Guo M, Qian J, Yang Y, Gu C. Review on circular RNAs and new insights into their roles in cancer. Comput Struct Biotechnol J 2021;19:910–928. doi:10.1016/j.csbj.2021.01.018, PMID:33598105.
[53] Wong LS, Wong CM. Decoding the Roles of Long Noncoding RNAs in Hepatocellular Carcinoma. Int J Mol Sci 2021;22(6):3137. doi:10.3390/ijms22063137, PMID:33808647.
[54] Huang X, Sun L, Wen S, Deng D, Wan F, He X, *et al*. RNA sequencing of plasma exosomes revealed novel functional long noncoding RNAs in hepatocellular carcinoma. Cancer Sci 2020;111(9):3338–3349. doi:10.1111/cas.14516, PMID:32506598.
[55] Yao Z, Jia C, Tai Y, Liang H, Zhong Z, Xiong Z, *et al*. Serum exosomal long noncoding RNAs lnc-FAM72D-3 and lnc-EPC1-4 as diagnostic biomarkers for hepatocellular carcinoma. Aging (Albany NY) 2020;12(12):11843–11863. doi:10.18632/aging.103355, PMID:32554864.
[56] Wang T, Zhu H, Xiao M, Zhou S. Serum exosomal long noncoding RNA CRNDE as a prognostic biomarker for hepatocellular carcinoma. J Clin Lab Anal 2021;35(11):e23959. doi:10.1002/jcla.23959, PMID:34612554.
[57] Tan C, Cao J, Chen L, Xi X, Wang S, Zhu Y, *et al*. Noncoding RNAs Serve as Diagnosis and Prognosis Biomarkers for Hepatocellular Carcinoma. Clin Chem 2019;65(7):905–915. doi:10.1373/clinchem.2018.301150, PMID:30996051.
[58] Kim SS, Baek GO, Ahn HR, Sung S, Seo CW, Cho HJ, *et al*. Serum small extracellular vesicle-derived LINC00853 as a novel diagnostic marker for early hepatocellular carcinoma. Mol Oncol 2020;14(10):2646–2659. doi:10.1002/1878-0261.12745, PMID:32525601.
[59] Sun L, Su Y, Liu X, Xu M, Chen X, Zhu Y, *et al*. Serum and exosome long non coding RNAs as potential biomarkers for hepatocellular carcinoma. J Cancer 2018;9(15):2631–2639. doi:10.7150/jca.24978, PMID:30087703.
[60] Yao J, Hua X, Shi J, Hu X, Lui K, He K, *et al*. LncRNA THEMIS2-211, a tumor-originated circulating exosomal biomarker, promotes the growth and metastasis of hepatocellular carcinoma by functioning as a competing endogenous RNA. FASEB J 2022;36(4):e22238. doi:10.1096/fj.202101564R, PMID:35224785.
[61] Xu H, Chen Y, Dong X, Wang X. Serum Exosomal Long Noncoding RNAs ENSG00000258332.1 and LINC00635 for the Diagnosis and Prognosis of Hepatocellular Carcinoma. Cancer Epidemiol Biomarkers Prev 2018;27(6):710–716. doi:10.1158/1055-9965.EPI-17-0770, PMID:29650788.
[62] Lu Y, Duan Y, Xu Q, Zhang L, Chen W, Qu Z, *et al*. Circulating exosome-derived bona fide long non-coding RNAs predicting the occurrence and metastasis of hepatocellular carcinoma. J Cell Mol Med 2020;24(2):1311–1318. doi:10.1111/jcmm.14783, PMID:31811749.
[63] Wang H, Ke J, Guo Q, Barnabo Nampoukime KP, Yang P, Ma K. Long non-coding RNA CRNDE promotes the proliferation, migration and invasion of hepatocellular carcinoma cells through miR-217/MAPK1 axis. J Cell Mol Med 2018;22(12):5862–5876. doi:10.1111/jcmm.13856, PMID:30246921.
[64] Lin C, Xiang Y, Sheng J, Liu S, Cui M, Zhang X. Long non-coding RNA CRNDE promotes malignant progression of hepatocellular carcinoma through the miR-33a-5p/CDK6 axis. J Physiol Biochem 2020;76(3):469–481. doi:10.1007/s13105-020-00754-0, PMID:32621257.
[65] Shiu TY, Lin HH, Shih YL, Feng AC, Huang HH, Huang TY, *et al*. CRNDE-h transcript/miR-136-5p axis regulates interleukin enhancer binding factor 2

expression to promote hepatocellular carcinoma cell proliferation. Life Sci 2021;284:119708. doi:10.1016/j.lfs.2021.119708, PMID:34153299.
[66] Abdelmohsen K, Panda AC, Kang MJ, Guo R, Kim J, Grammatikakis I, *et al*. 7SL RNA represses p53 translation by competing with HuR. Nucleic Acids Res 2014;42(15):10099–10111. doi:10.1093/nar/gku686, PMID:25123665.
[67] Xu LC, Chen QN, Liu XQ, Wang XM, Chang QM, Pan Q, *et al*. Up-regulation of LINC00161 correlates with tumor migration and invasion and poor prognosis of patients with hepatocellular carcinoma. Oncotarget 2017;8(34):56168–56173. doi:10.18632/oncotarget.17040, PMID:28915581.
[68] You LN, Tai QW, Xu L, Hao Y, Guo WJ, Zhang Q, *et al*. Exosomal LINC00161 promotes angiogenesis and metastasis via regulating miR-590-3p/ROCK axis in hepatocellular carcinoma. Cancer Gene Ther 2021;28(6):719–736. doi:10.1038/s41417-020-00269-2, PMID:33414518.
[69] Yang L, Peng X, Li Y, Zhang X, Ma Y, Wu C, *et al*. Long non-coding RNA HOTAIR promotes exosome secretion by regulating RAB35 and SNAP23 in hepatocellular carcinoma. Mol Cancer 2019;18(1):78. doi:10.1186/s12943-019-0990-6, PMID:30943982.
[70] Shen H, Liu B, Xu J, Zhang B, Wang Y, Shi L, *et al*. Circular RNAs: characteristics, biogenesis, mechanisms and functions in liver cancer. J Hematol Oncol 2021;14(1):134. doi:10.1186/s13045-021-01145-8, PMID:34461958.
[71] Wang P, Zhang Y, Deng L, Qu Z, Guo P, Liu L, *et al*. The function and regulation network mechanism of circRNA in liver diseases. Cancer Cell Int 2022;22(1):141. doi:10.1186/s12935-022-02559-1, PMID:35361205.
[72] Han TS, Hur K, Cho HS, Ban HS. Epigenetic Associations between lncRNA/circRNA and miRNA in Hepatocellular Carcinoma. Cancers (Basel) 2020;12(9):2622. doi:10.3390/cancers12092622, PMID:32937886.
[73] Xiong DD, Dang YW, Lin P, Wen DY, He RQ, Luo DZ, *et al*. A circRNA-miRNA-mRNA network identification for exploring underlying pathogenesis and therapy strategy of hepatocellular carcinoma. J Transl Med 2018;16(1):220. doi:10.1186/s12967-018-1593-5, PMID:30092792.
[74] Wang Y, Li Z, Xu S, Guo J. Novel potential tumor biomarkers: Circular RNAs and exosomal circular RNAs in gastrointestinal malignancies. J Clin Lab Anal 2020;34(7):e23359. doi:10.1002/jcla.23359, PMID:32419229.
[75] Lin Y, Zheng ZH, Wang JX, Zhao Z, Peng TY. Tumor Cell-Derived Exosomal Circ-0072088 Suppresses Migration and Invasion of Hepatic Carcinoma Cells Through Regulating MMP-16. Front Cell Dev Biol 2021;9:726323. doi:10.3389/fcell.2021.726323, PMID:34568335.
[76] Cao Y, Tao Q, Kao X, Zhu X. Hsa-circRNA-103809 Promotes Hepatocellular Carcinoma Development via MicroRNA-1270/PLAG1 Like Zinc Finger 2 Axis. Dig Dis Sci 2021;66(5):1524–1532. doi:10.1007/s10620-020-06416-x, PMID:32683589.
[77] Zhan W, Liao X, Chen Z, Li L, Tian T, Yu L, *et al*. Circular RNA hsa_circRNA_103809 promoted hepatocellular carcinoma development by regulating miR-377-3p/FGFR1/ERK axis. J Cell Physiol 2020;235(2):1733–1745. doi:10.1002/jcp.29092, PMID:31317555.
[78] Li X, Shen M. Circular RNA hsa_circ_103809 suppresses hepatocellular carcinoma proliferation and invasion by sponging miR-620. Eur Rev Med Pharmacol Sci 2019;23(2):555–566. doi:10.26355/eurrev_201902_16868, PMID:30720163.
[79] Huang C, Yu W, Wang Q, Huang T, Ding Y. CircANTXR1 Contributes to the Malignant Progression of Hepatocellular Carcinoma by Promoting Proliferation and Metastasis. J Hepatocell Carcinoma 2021;8:1339–1353. doi:10.2147/JHC.S317256, PMID:34786378.
[80] Chen W, Quan Y, Fan S, Wang H, Liang J, Huang L, *et al*. Exosome-transmitted circular RNA hsa_circ_0051443 suppresses hepatocellular carcinoma progression. Cancer Lett 2020;475:119–128. doi:10.1016/j.canlet.2020.01.022, PMID:32014458.
[81] Zhu C, Su Y, Liu L, Wang S, Liu Y, Wu J. Circular RNA hsa_circ_0004277 Stimulates Malignant Phenotype of Hepatocellular Carcinoma and Epithelial-Mesenchymal Transition of Peripheral Cells. Front Cell Dev Biol 2020;8:585565. doi:10.3389/fcell.2020.585565, PMID:33511111.
[82] Sun XH, Wang YT, Li GF, Zhang N, Fan L. Serum-derived three-circRNA signature as a diagnostic biomarker for hepatocellular carcinoma. Cancer Cell Int 2020;20:226. doi:10.1186/s12935-020-01302-y, PMID:32536814.
[83] Lyu L, Yang W, Yao J, Wang H, Zhu J, Jin A, *et al*. The diagnostic value of plasma exosomal hsa_circ_0070396 for hepatocellular carcinoma. Biomark Med 2021;15(5):359–371. doi:10.2217/bmm-2020-0476, PMID:33666515.
[84] Guo S, Hu C, Zhai X, Sun D. Circular RNA 0006602 in plasma exosomes: a new potential diagnostic biomarker for hepatocellular carcinoma. Am J Transl Res 2021;13(6):6001–6015. PMID:34306340.
[85] Wang Y, Pei L, Yue Z, Jia M, Wang H, Cao LL. The Potential of Serum Exosomal hsa_circ_0028861 as the Novel Diagnostic Biomarker of HBV-Derived Hepatocellular Cancer. Front Genet 2021;12:703205. doi:10.3389/fgene.2021.703205, PMID:34367259.
[86] Wang Y, Gao R, Li J, Tang S, Li S, Tong Q, *et al*. Downregulation of hsa_circ_0074854 Suppresses the Migration and Invasion in Hepatocellular Carcinoma via Interacting with HuR and via Suppressing Exosomes-Mediated Macrophage M2 Polarization. Int J Nanomedicine 2021;16:2803–2818. doi:10.2147/IJN.S284560, PMID:33880025.
[87] Luo Y, Liu F, Gui R. High expression of circulating exosomal circAKT3 is associated with higher recurrence in HCC patients undergoing surgical treatment. Surg Oncol 2020;33:276–281. doi:10.1016/j.suronc.2020.04.021, PMID:32561093.
[88] Lai Z, Wei T, Li Q, Wang X, Zhang Y, Zhang S. Exosomal circFBLIM1 Promotes Hepatocellular Carcinoma Progression and Glycolysis by Regulating the miR-338/LRP6 Axis. Cancer Biother Radiopharm 2023;38(10):674–683. doi:10.1089/cbr.2020.3564, PMID:32907351.
[89] Sasaki R, Kanda T, Yokosuka O, Kato N, Matsuoka S, Moriyama M. Exosomes and Hepatocellular Carcinoma: From Bench to Bedside. Int J Mol Sci 2019;20(6):1406. doi:10.3390/ijms20061406, PMID:30897788.
[90] Wu ZH, Li C, Zhang YJ, Lin R. Bioinformatics Study Revealed Significance of Exosome Transcriptome in Hepatocellular Carcinoma Diagnosis. Front Cell Dev Biol 2022;10:813701. doi:10.3389/fcell.2022.813701, PMID:35573701.
[91] Goldberg E, Eddy EM, Duan C, Odet F. LDHC: the ultimate testis-specific gene. J Androl 2010;31(1):86–94. doi:10.2164/jandrol.109.008367, PMID:19875487.
[92] Odet F, Duan C, Willis WD, Goulding EH, Kung A, Eddy EM, *et al*. Expression of the gene for mouse lactate dehydrogenase C (Ldhc) is required for male fertility. Biol Reprod 2008;79(1):26–34. doi:10.1095/biolreprod.108.068353, PMID:18367675.
[93] Chen L, Wu Q, Xu X, Yang C, You J, Chen F, *et al*. Cancer/testis antigen LDHC promotes proliferation and metastasis by activating the PI3K/Akt/GSK-3β-signaling pathway and the in lung adenocarcinoma. Exp Cell Res 2021;398(2):112414. doi:10.1016/j.yexcr.2020.112414, PMID:33301764.
[94] Cui Z, Chen Y, Hu M, Lin Y, Zhang S, Kong L, *et al*. Diagnostic and prognostic value of the cancer-testis antigen lactate dehydrogenase C4 in breast cancer. Clin Chim Acta 2020;503:203–209. doi:10.1016/j.cca.2019.11.032, PMID:31794764.
[95] Cui Z, Li Y, Gao Y, Kong L, Lin Y, Chen Y. Cancer-testis antigen lactate dehydrogenase C4 in hepatocellular carcinoma: a promising biomarker for early diagnosis, efficacy evaluation and prognosis prediction. Aging (Albany NY) 2020;12(19):19455–19467. doi:10.18632/aging.103879, PMID:33035196.
[96] Pan F, Lin X, Hao L, Wang T, Song H, Wang R. The Critical Role of Ferroptosis in Hepatocellular Carcinoma. Front Cell Dev Biol 2022;10:882571. doi:10.3389/fcell.2022.882571, PMID:35800895.
[97] Sasaki K, Kohgo Y, Ohtake T. Splicing variant of hepcidin mRNA. Vitam Horm 2019;110:131–141. doi:10.1016/bs.vh.2019.01.006, PMID:30798808.
[98] Liu M, Yang L, Liu X, Nie Z, Zhang X, Lu Y, *et al*. HNRNPH1 Is a Novel Regulator Of Cellular Proliferation and Disease Progression in Chronic Myeloid Leukemia. Front Oncol 2021;11:682859. doi:10.3389/fonc.2021.682859, PMID:34295818.
[99] Xu H, Dong X, Chen Y, Wang X. Serum exosomal hnRNPH1 mRNA as a novel marker for hepatocellular carcinoma. Clin Chem Lab Med 2018;56(3):479–484. doi:10.1515/cclm-2017-0327, PMID:29252188.
[100] Maliga Z, Junqueira M, Toyoda Y, Ettinger A, Mora-Bermúdez F, Klemm RW, *et al*. A genomic toolkit to investigate kinesin and myosin motor function in cells. Nat Cell Biol 2013;15(3):325–334. doi:10.1038/ncb2689, PMID:23417121.
[101] Xie X, Wang X, Liao W, Fei R, Wu N, Cong X, *et al*. MYL6B, a myosin light chain, promotes MDM2-mediated p53 degradation and drives HCC development. J Exp Clin Cancer Res 2018;37(1):28. doi:10.1186/s13046-018-0693-7, PMID:29439719.
[102] Tamhankar V, Tamhankar P, Chaubal R, Chaubal J, Chaubal N. Novel Consensus Splice Site Pathogenic Variation in THOC2 Gene Leads to Recurrent Arthrogryposis Multiplex Congenita Phenotype: A Case Report. Cureus 2021;13(11):e19682. doi:10.7759/cureus.19682, PMID:34976470.
[103] Kumar R, Gardner A, Homan CC, Douglas E, Mefford H, Wieczorek D, *et al*. Severe neurocognitive and growth disorders due to variation in THOC2, an essential component of nuclear mRNA export machinery. Hum Mutat 2018;39(8):1126–1138. doi:10.1002/humu.23557, PMID:29851191.
[104] Zhu J, Tang B, Gao Y, Xu S, Tu J, Wang Y, *et al*. Predictive Models for HCC Prognosis, Recurrence Risk, and Immune Infiltration Based on Two Exosomal Genes: MYL6B and THOC2. J Inflamm Res 2021;14:4089–4109. doi:10.2147/JIR.S315957, PMID:34466015.
[105] Sun N, Lee YT, Zhang RY, Kao R, Teng PC, Yang Y, *et al*. Purification of HCC-specific extracellular vesicles on nanosubstrates for early HCC detection by digital scoring. Nat Commun 2020;11(1):4489. doi:10.1038/s41467-020-18311-0, PMID:32895384.
[106] Qi F, Zhou A, Yan L, Yuan X, Wang D, Chang R, *et al*. The diagnostic value of PIVKA-II, AFP, AFP-L3, CEA, and their combinations in primary and metastatic hepatocellular carcinoma. J Clin Lab Anal 2020;34(5):e23158. doi:10.1002/jcla.23158, PMID:31821607.
[107] Sanchez JI, Jiao J, Kwan SY, Veillon L, Warmoes MO, Tan L, *et al*. Lipidomic Profiles of Plasma Exosomes Identify Candidate Biomarkers for Early Detection of Hepatocellular Carcinoma in Patients with Cirrhosis. Cancer Prev Res (Phila) 2021;14(10):955–962. doi:10.1158/1940-6207.CAPR-20-0612, PMID:34253566.
[108] Li W, Li C, Zhou T, Liu X, Liu X, Li X, *et al*. Role of exosomal proteins in cancer diagnosis. Mol Cancer 2017;16(1):145. doi:10.1186/s12943-017-0706-8, PMID:28851367.
[109] Hsu MT, Wang YK, Tseng YJ. Exosomal Proteins and Lipids as Potential Biomarkers for Lung Cancer Diagnosis, Prognosis, and Treatment. Cancers (Basel) 2022;14(3):732. doi:10.3390/cancers14030732, PMID:35158999.
[110] Baran J, Baj-Krzyworzeka M, Weglarczyk K, Szatanek R, Zembala M, Barbasz J, *et al*. Circulating tumour-derived microvesicles in plasma of gastric cancer patients. Cancer Immunol Immunother 2010;59(6):841–850. doi:10.1007/s00262-009-0808-2, PMID:20043223.
[111] Zhao L, Shi J, Chang L, Wang Y, Liu S, Li Y, *et al*. Serum-Derived Exosomal Proteins as Potential Candidate Biomarkers for Hepatocellular Carcinoma. ACS Omega 2021;6(1):827–835. doi:10.1021/acsomega.0c05408, PMID:33458533.
[112] Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, *et al*. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 2015;523(7559):177–182. doi:10.1038/nature14581, PMID:26106858.
[113] Lim JW, Mathias RA, Kapp EA, Layton MJ, Faux MC, Burgess AW, *et al*. Restoration of full-length APC protein in SW480 colon cancer cells induces exosome-mediated secretion of DKK-4. Electrophoresis 2012;33(12):1873–

1880. doi:10.1002/elps.201100687, PMID:22740476.
[114] Wang R, Yin Y, Zhu ZJ. Advancing untargeted metabolomics using data-independent acquisition mass spectrometry technology. Anal Bioanal Chem 2019;411(19):4349–4357. doi:10.1007/s00216-019-01709-1, PMID:30847570.
[115] Sajic T, Liu Y, Aebersold R. Using data-independent, high-resolution mass spectrometry in protein biomarker research: perspectives and clinical applications. Proteomics Clin Appl 2015;9(3-4):307–321. doi:10.1002/prca.201400117, PMID:25504613.
[116] Zhu J, Warner E, Parikh ND, Lubman DM. Glycoproteomic markers of hepatocellular carcinoma-mass spectrometry based approaches. Mass Spectrom Rev 2019;38(3):265–290. doi:10.1002/mas.21583, PMID:30472795.
[117] Li W, Liu J, Zhao H. Prognostic Power of a Chaperonin Containing TCP-1 Subunit Genes Panel for Hepatocellular Carcinoma. Front Genet 2021;12:668871. doi:10.3389/fgene.2021.668871, PMID:33897772.
[118] Huang X, Wang X, Cheng C, Cai J, He S, Wang H, *et al*. Chaperonin containing TCP1, subunit 8 (CCT8) is upregulated in hepatocellular carcinoma and promotes HCC proliferation. APMIS 2014;122(11):1070–1079. doi:10.1111/apm.12258, PMID:24862099.
[119] Wei PL, Huang CY, Tai CJ, Batzorig U, Cheng WL, Hunag MT, *et al*. Glucose-regulated protein 94 mediates metastasis by CCT8 and the JNK pathway in hepatocellular carcinoma. Tumour Biol 2016;37(6):8219–8227. doi:10.1007/s13277-015-4669-3, PMID:26718209.
[120] Zhang L, Chai Z, Kong S, Feng J, Wu M, Tan J, *et al*. Nujiangexanthone A Inhibits Hepatocellular Carcinoma Metastasis via Down Regulation of Cofilin 1. Front Cell Dev Biol 2021;9:644716. doi:10.3389/fcell.2021.644716, PMID:33791303.
[121] Yao B, Li Y, Chen T, Niu Y, Wang Y, Yang Y, *et al*. Hypoxia-induced cofilin 1 promotes hepatocellular carcinoma progression by regulating the PLD1/AKT pathway. Clin Transl Med 2021;11(3):e366. doi:10.1002/ctm2.366, PMID:33784016.
[122] Cho HJ, Baek GO, Yoon MG, Ahn HR, Son JA, Kim SS, *et al*. Overexpressed Proteins in HCC Cell-Derived Exosomes, CCT8, and Cofilin-1 Are Potential Biomarkers for Patients with HCC. Diagnostics (Basel) 2021;11(7):1221. doi:10.3390/diagnostics11071221, PMID:34359304.
[123] Donoso-Quezada J, Ayala-Mar S, González-Valdez J. The role of lipids in exosome biology and intercellular communication: Function, analytics and applications. Traffic 2021;22(7):204–220. doi:10.1111/tra.12803, PMID:34053166.
[124] Skotland T, Ekroos K, Kauhanen D, Simolin H, Seierstad T, Berge V, *et al*. Molecular lipid species in urinary exosomes as potential prostate cancer biomarkers. Eur J Cancer 2017;70:122–132. doi:10.1016/j.ejca.2016.10.011, PMID:27914242.
[125] Wang J, Ma P, Kim DH, Liu BF, Demirci U. Towards Microfluidic-Based Exosome Isolation and Detection for Tumor Therapy. Nano Today 2021;37:101066. doi:10.1016/j.nantod.2020.101066, PMID:33777166.
[126] Xu K, Jin Y, Li Y, Huang Y, Zhao R. Recent Progress of Exosome Isolation and Peptide Recognition-Guided Strategies for Exosome Research. Front Chem 2022;10:844124. doi:10.3389/fchem.2022.844124, PMID:35281563.
[127] Kim J, Tan Z, Lubman DM. Exosome enrichment of human serum using multiple cycles of centrifugation. Electrophoresis 2015;36(17):2017–2026. doi:10.1002/elps.201500131, PMID:26010067.
[128] Cheruvanky A, Zhou H, Pisitkun T, Kopp JB, Knepper MA, Yuen PS, *et al*. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. Am J Physiol Renal Physiol 2007;292(5):F1657–F1661. doi:10.1152/ajprenal.00434.2006, PMID:17229675.
[129] Zheng H, Guan S, Wang X, Zhao J, Gao M, Zhang X. Deconstruction of Heterogeneity of Size-Dependent Exosome Subpopulations from Human Urine by Profiling N-Glycoproteomics and Phosphoproteomics Simultaneously. Anal Chem 2020;92(13):9239–9246. doi:10.1021/acs.analchem.0c01572, PMID:32495629.
[130] Guo J, Wu C, Lin X, Zhou J, Zhang J, Zheng W, *et al*. Establishment of a simplified dichotomic size-exclusion chromatography for isolating extracellular vesicles toward clinical applications. J Extracell Vesicles 2021;10(11):e12145. doi:10.1002/jev2.12145, PMID:34514732.
[131] Liu F, Vermesh O, Mani V, Ge TJ, Madsen SJ, Sabour A, *et al*. The Exosome Total Isolation Chip. ACS Nano 2017;11(11):10712–10723. doi:10.1021/acsnano.7b04878, PMID:29090896.
[132] Gao M, Cai J, Zitkovsky HS, Chen B, Guo L. Comparison of Yield, Purity, and Functional Properties of Large-Volume Exosome Isolation Using Ultrafiltration and Polymer-Based Precipitation. Plast Reconstr Surg 2022;149(3):638–649. doi:10.1097/PRS.0000000000008830, PMID:35196679.
[133] Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, *et al*. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics 2020;10(8):3684–3707. doi:10.7150/thno.41580, PMID:32206116.
[134] Shao H, Im H, Castro CM, Breakefield X, Weissleder R, Lee H. New Technologies for Analysis of Extracellular Vesicles. Chem Rev 2018;118(4):1917–1950. doi:10.1021/acs.chemrev.7b00534, PMID:29384376.
[135] Lin B, Tian T, Lu Y, Liu D, Huang M, Zhu L, *et al*. Tracing Tumor-Derived Exosomal PD-L1 by Dual-Aptamer Activated Proximity-Induced Droplet Digital PCR. Angew Chem Int Ed Engl 2021;60(14):7582–7586. doi:10.1002/anie.202015628, PMID:33382182.
[136] Liu C, Su C. Design strategies and application progress of therapeutic exosomes. Theranostics 2019;9(4):1015–1028. doi:10.7150/thno.30853, PMID:30867813.
[137] Liang LG, Kong MQ, Zhou S, Sheng YF, Wang P, Yu T, *et al*. An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer. Sci Rep 2017;7:46224. doi:10.1038/srep46224, PMID:28436447.
[138] Sharan RN, Vaiphei ST, Nongrum S, Keppen J, Ksoo M. Consensus reference gene(s) for gene expression studies in human cancers: end of the tunnel visible? Cell Oncol (Dordr) 2015;38(6):419–431. doi:10.1007/s13402-015-0244-6, PMID:26384826.
[139] Wang H, Lv Y, Wang C, Leng D, Yan Y, Blessing Fasae M, *et al*. Systematic Analysis of Intestinal MicroRNAs Expression in HCC: Identification of Suitable Reference Genes in Fecal Samples. Front Genet 2019;10:687. doi:10.3389/fgene.2019.00687, PMID:31456816.
[140] Gao Q, Wang XY, Fan J, Qiu SJ, Zhou J, Shi YH, *et al*. Selection of reference genes for real-time PCR in human hepatocellular carcinoma tissues. J Cancer Res Clin Oncol 2008;134(9):979–986. doi:10.1007/s00432-008-0369-3, PMID:18317805.
[141] Jiang Y, He J, Li Y, Guo Y, Tao H. The Diagnostic Value of MicroRNAs as a Biomarker for Hepatocellular Carcinoma: A Meta-Analysis. Biomed Res Int 2019;2019:5179048. doi:10.1155/2019/5179048, PMID:31871941.
[142] Li Y, Zhang L, Liu F, Xiang G, Jiang D, Pu X. Identification of endogenous controls for analyzing serum exosomal miRNA in patients with hepatitis B or hepatocellular carcinoma. Dis Markers 2015;2015:893594. doi:10.1155/2015/893594, PMID:25814782.
[143] Tang G, Shen X, Lv K, Wu Y, Bi J, Shen Q. Different normalization strategies might cause inconsistent variation in circulating microRNAs in patients with hepatocellular carcinoma. Med Sci Monit 2015;21:617–624. doi:10.12659/MSM.891028, PMID:25719241.
[144] Jacobsen KS, Nielsen KO, Winther TN, Glebe D, Pociot F, Hogh B. Identification of valid reference genes for microRNA expression studies in a hepatitis B virus replicating liver cell line. BMC Res Notes 2016;9:38. doi:10.1186/s13104-016-1848-2, PMID:26801621.
[145] Occhipinti G, Giulietti M, Principato G, Piva F. The choice of endogenous controls in exosomal microRNA assessments from biofluids. Tumour Biol 2016;37(9):11657–11665. doi:10.1007/s13277-016-5164-1, PMID:27438704.
[146] Pratama MY, Cavalletto L, Tiribelli C, Chemello L, Pascut D. Selection and validation of miR-1280 as a suitable endogenous normalizer for qRT-PCR Analysis of serum microRNA expression in Hepatocellular Carcinoma. Sci Rep 2020;10(1):3128. doi:10.1038/s41598-020-59682-0, PMID:32081930.
[147] Zhao J, Zhu XC, Wu XS, Wang L, Zhu CC, Yang K, *et al*. Identification of miR-4644 as a suitable endogenous normalizer for circulating miRNA quantification in hepatocellular carcinoma. J Cancer 2020;11(23):7032–7044. doi:10.7150/jca.48903, PMID:33123293.
[148] Li Y, Xiang GM, Liu LL, Liu C, Liu F, Jiang DN, *et al*. Assessment of endogenous reference gene suitability for serum exosomal microRNA expression analysis in liver carcinoma resection studies. Mol Med Rep 2015;12(3):4683–4691. doi:10.3892/mmr.2015.3919, PMID:26082194.
[149] Dundas J, Ling M. Reference genes for measuring mRNA expression. Theory Biosci 2012;131(4):215–223. doi:10.1007/s12064-012-0152-5, PMID:22588998.
[150] Gorji-Bahri G, Moradtabrizi N, Vakhshiteh F, Hashemi A. Validation of common reference genes stability in exosomal mRNA-isolated from liver and breast cancer cell lines. Cell Biol Int 2021;45(5):1098–1110. doi:10.1002/cbin.11556, PMID:33501690.