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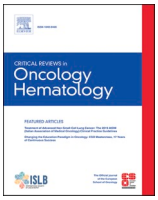

Emerging role of circulating cell-free RNA as a non-invasive biomarker for hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is a severe neoplastic disease associated with high morbidity and mortality rates. HCC is often detected at advanced stages leading to ineffective curative treatments. Recently, liquid biopsy has emerged as a non-invasive method to identify highly specific HCC biomarkers in bodily fluids such as blood, serum, urine, and saliva. Circulating cell-free nucleic acids (cfNAs), particularly cell-free DNA (cfDNA) and cell-free RNA (cfRNA), have become promising candidates for biomarkers in liquid biopsy applications. While cfDNA presented significant challenges, researchers have turned their attention to cfRNA, which can be efficiently identified through various methods and is considered a potential biomarker for cancer diagnosis and prognosis. This review primarily focuses on studies related to detecting various cfRNA in body fluids as biomarkers. The aim is to provide a summary of available information to assist researchers in their investigations and the development of new diagnostic and prognostic tools.

1. Introduction

According to data from the International Agency for Research on Cancer 2020, liver cancer held the unenviable position of being the sixth most prevalent cancer and the third leading cause of cancer-related death across the globe (Sung et al., 2021). The projections for the future are equally disheartening, with an estimated one million lives expected to be claimed by liver cancer in 2030 (Villanueva, 2019). Medical guidelines identified several risk factors closely associated with the development of hepatocellular carcinoma (HCC). Among these, major contributors include chronic infections of hepatitis B or C viruses (HBV and HCV), excessive alcohol consumption, and non-alcoholic steatohepatitis (NASH) a serious form of non-alcoholic fatty liver disease (NAFLD) is a frequent risk factor observed in the western population, leading to cirrhosis and ultimately progress to HCC (Llovet et al., 2021).

In healthcare, individuals with a high risk of developing HCC undergo regular surveillance.

Liver cancer diagnosis typically involves imaging techniques such as ultrasonography, computed tomography (CT), and magnetic resonance tomography (MRT). The medical professionals further confirm the diagnosed result by histological assessment of tissue biopsies and alpha-fetoprotein (AFP) levels in the blood (A. Tang et al., 2018). Currently, AFP remains a relevant non-invasive biomarker in the clinical management of patients with HCC. Nevertheless, it possesses certain limitations in terms of its sensitivity and specificity. (Galle et al., 2019; Sherman, 2010). With an AFP threshold value of 20 ng/ul, the sensitivity and specificity for detecting HCC are 41%-65% and 80%-84% respectively. Lowering the AFP cutoff increases sensitivity but raises false positives, whereas raising the cutoff to 50 ng/ml enhances specificity to 96% and reduces sensitivity to 47% (Hanif et al., 2022). Detecting early-stage HCC is crucial for clinical outcomes, as it

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significantly improves the prognosis, with 5-year survival rates exceeding 60–80% following curative interventions (J. D. Yang, 2019). This emphasizes the critical need for identifying novel, non-invasive biomarkers that are both highly accurate and feasible for the early diagnosis of HCC.

In oncology, liquid biopsy stands as a growing and pivotal field, offering a non-invasive method for the identification of highly specific cancer biomarkers through an array of bodily fluids, including blood, serum, urine, and saliva. This provides vast information about the intricacies of tumor characteristics and their dynamic evolution, which are usually obtained by tissue biopsy. The focal point of liquid biopsy revolves around circulating tumor cells (CTCs), circulating nucleic acids, circulating extracellular vesicles (EVs), tumor-educated platelets (TEPs), proteins, and metabolites (Alix-Panabières & Pantel, 2013; Schwarzenbach et al., 2011; van de Stolpe et al., 2011). Specifically circulating cell-free nucleic acids (cfNAs), such as cell-free DNA (cfDNA) and cell-free RNA (cfRNA), have emerged as compelling candidates for biomarkers in the context of liquid biopsy applications. However, due to the low DNA content in bodily fluids, cfDNA analysis has experienced considerable challenges in liquid biopsy (Song et al., 2022). To overcome this limitation, researchers have focused on cfRNA which can be detected through various methods and can be considered as a potential biomarker for cancer diagnosis and prognosis. This review article mainly focuses on the emerging role of cfRNA as a non-invasive biomarker for HCC. An extensive discussion on the recent progress of different cfRNA in liquid biopsy developments, and prospects for the future of liquid biopsy, particularly focusing on clinical application in HCC, is conducted based on the currently available literature.

2. Mechanobiology of cfRNA in circulation

During the past decade, increasing attention has been paid to cfRNA that are present at high concentrations in the blood of cancer patients . Despite elevated RNases in the bloodstream, circulating RNAs remain remarkably stable, suggesting that their protection from degradation may be attributed to encapsulation within exosomes, including microparticles, microvesicles, or multi-vesicles (Schwarzenbach et al., 2011). cfRNAs are released into body fluids through various mechanisms, primarily involving passive leakage from dead cells or active secretion from membrane-bound vesicles or vesicle-free RNAs binding proteins (e.g., with HDL, AGO2, NPM1 proteins) ((R et al., 2017); Zhao et al., 2019) (Fig. 1). In passive leakage, cells shrink and disintegrate into minute structures known as granules that are subsequently packaged into apoptotic bodies, releasing cfRNAs into the bloodstream (Green & Llambi, 2015). Active secretion via membranes involves larger vesicles compared to exosomes. D'Souza et al. referred to this as tumor-derived microvesicles (TMVs), generated through a process that includes outward budding and subsequent fission from the surface of tumor cells (D'Souza-Schorey, and Clancy). Active secretion by protein-RNA complexes occurs in conjunction with molecules such as high-density lipoproteins and RNA-binding proteins (RBPs) such as Argonaute 2 Protein (AGO2) (Zhao et al., 2019). RBPs play a dual role by facilitating the import and serving as carriers of extracellular RNA (exRNA) in circulation (Borniego & Innes, 2023).

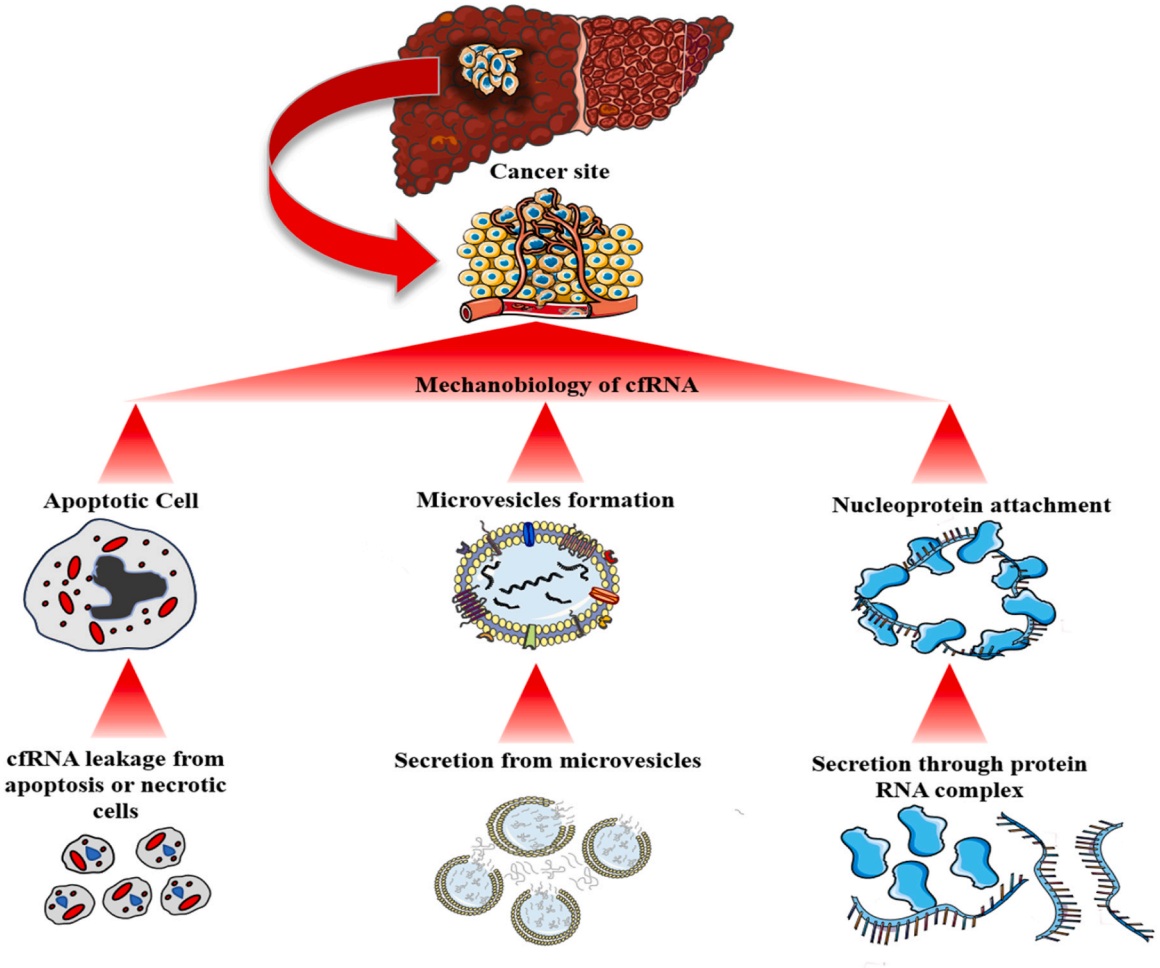

Fig. 1. Mechanobiology of cfRNA in circulation.

2.1. Detection and authentication of cfRNA in liquid biopsy

The isolation of cfRNA from body fluids is followed by its quantification. There are several methods to quantify the initial amount of cfRNA, including the Qubit Fluorometer (Technologies) or spectrophotometer-based technologies such as Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), Tecan Infinite® 200 PRO Nanoquant Spectrophotometer (Tecan, Maennedorf, Switzerland) or Nanodrop Spectrophotometers (Thermo Scientific, Waltham, MA, (USA). The major disadvantage of these quantification methods is their inability to distinguish between different types of RNAs, therefore producing false cfRNA yield. However, the spectrophotometry method-based Qubit system, developed by Thermo Scientific in Waltham, MA, USA, employs specialized fluorescent dyes that are specific for small RNA or miRNA over other RNA variants (Martinez-Dominguez et al., 2021). This establishes the Qubit system as a notable method for detecting cfRNA in body fluids. A concise overview of the various methods used for the detection of cfRNA is depicted in **(**Fig. 2**),** including the advantages and disadvantages of each, follows the initial quantification steps in (Table 1)

2.2. Representative circulatory RNA Biomarkers

cfRNA comprises coding RNA, specifically messenger RNA (mRNA) that encodes protein, and non-coding RNA (ncRNA), which doesn't encode a protein but plays a crucial part in regulating various cellular functions (Y. Li, 2023). Primarily, ncRNAs are divided based on their size: long non-coding RNAs (lncRNAs) >200 bp and short non-coding RNAs (sncRNAs) ~28 bp long (P. Zhang et al., 2019). These RNA molecules exhibit dysregulation in tumors and are released into body fluids, providing crucial information about a patient's health status and various medical conditions (Bradley & Anczukow, 2023). Currently, the majority of studies are carried out in circulatory RNA-based liquid biopsy biomarkers focusing on mRNA, microRNA (miRNA), lncRNA, circular RNA (circRNA), small nucleolar RNA (snoRNA), and transpose RNA (tRNA).

2.3. Protein Coding RNA

2.3.1. Messenger RNA

mRNA is an RNA molecule that plays a pivotal role in the central dogma of molecular biology. It translates the genetic information from DNA to proteins, making it a crucial component in the complex and tightly regulated process of gene expression. Dysregulation of genes in tumors often leads to the production of various proteins through abnormal control of alternative splicing. Oncogenic variations produced by alternative splicing, such as variants a and b of osteopontin (OPN) (Chae et al., 2009) CD44 variants 5 and 6 (T. Zhang et al., 2010), and variant J of transcription factor 7-like 2 (TCF7L2) (Tomimaru et al., 2013) which contribute to HCC. Researcher has alos focoused on the expression profile and presence of the VEGF mRNA isoforms, VEGF165 and VEGF121, in the peripheral blood of HCC patients who underwent curative surgery. It has been observed that the VEGF165 isoforms significantly contributes to predicting postoperative recurrence of HCC, which has important implication for diagnostic and prognostic purposes. (Jeng et al., 2004).

2.4. Small non-coding RNA

2.4.1. Micro RNA

miRNAs are short, ncRNA molecules (18–25 nucleotides) that regulate gene expression by binding to target mRNAs, leading to suppression or degradation. miRNAs have a crucial role to play in cell growth, maturation, apoptosis, and proliferation. Therefore, when dysregulated, they can act on oncogenes based on their downstream targets and drive the process of tumor development and tumor suppression (Annese et al., 2020). Several studies have shown that miRNA sequencing in body fluids helps in monitoring the status of cancer, treatment response, and predicting the prognosis (Bagheri et al., 2023; Cortez et al., 2011). Huang et al. identified a plasma miRNA panel comprising miR-122, miR-21, miR-223, and miR-801, which exhibits high diagnostic specificity for HCC, enabling differentiation from healthy controls, individuals with chronic hepatitis B (CHB), and those with liver cirrhosis (LC) (A. Huang et al., 2021). The presence of miRNAs in non-blood fluids such as saliva, seminal fluid, breast milk, cerebrospinal fluid, tear gland secretions aqueous and vitreous humor of the eye also holds promising an non-invasive-biomarker in HCC patients (Fernandez-Mercado et al., 2015).

2.4.2. Small nucleolar RNA

snoRNAs are typically around 60–300 nucleotides in length and assist in chemical modifications such as methylation and conversion of uridine residues to pseudouridine. Recent research on SNHG8 demonstrates its role in promoting liver cancer development via miR-149

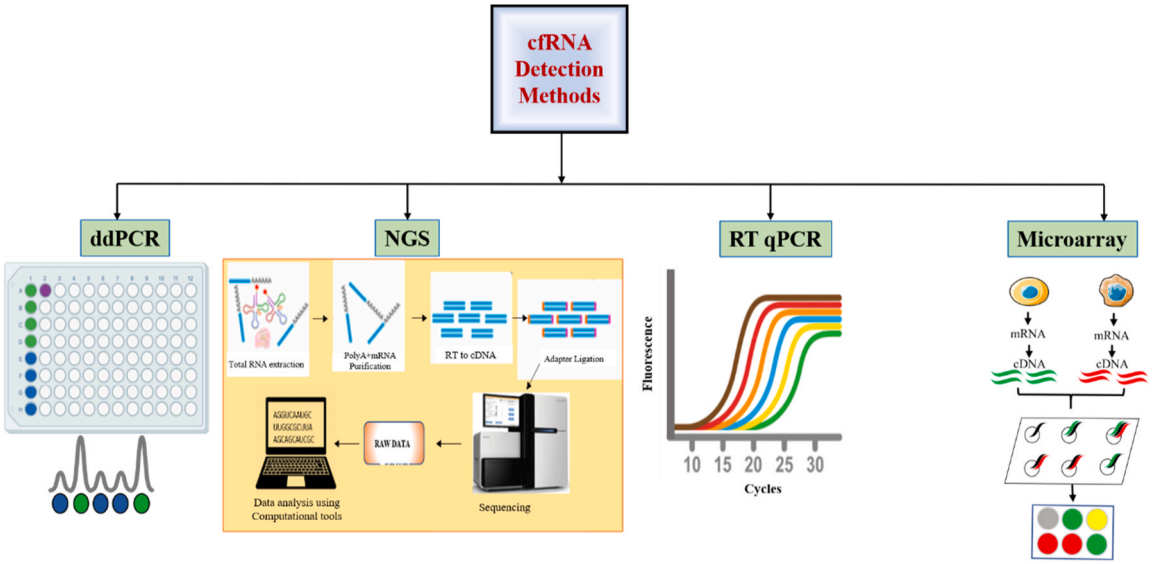

Fig. 2. Detection methods of cfRNAs.

Table 1
Approaches for Detecting cfRNAs and its respective advantages and disadvantages.

Methods	Description	Price and duration	Performance	Advantage	Disadvantage
RT-qPCR	Amplifies the genes in real-time	Less than 100 dollars 4–7 hr preparation time	• Sensitive (Madlener & Gojo, 2020) • 1% Detection limit	• Established method • Use of Taqman or FRET probes to Specificity (Androvic et al., 2019; Saliminejad et al., 2019) Multiplexing • Use of Taqman or Molecular Beacon (Duan et al., 2013; Wu et al., 2012)	• Standard curve requirement. • Limited multiplexing (Stewart & Tsui, 2018)
ddPCR	Water–oil emulsion droplet technology-based PCR	Hundred Dollars or less (Baker, 2010) 4–5 hr complete workflow	• Ultrasensitive (Saliminejad et al., 2019) • Detecting specific RNA sequence, • Detection limit 0.01–0.001% (Baker, 2010)	• Multiplexing • Ultraprecision	• Researchers can influence the distribution of the drops, and therefore the outcome (Saliminejad et al., 2019). • Requires previous optimization. • Difficult multiplexing.
Microarray	large number of specific RNA detections at the same time	Hundreds of Dollars (D. Li et al., 2020) 2–4 days preparation	• Specific • Genome-wide RNA (Jaksik et al., 2015; Saliminejad et al., 2019)	• Open array—Thermo Fisher Scientific • Can analyzing hundreds of probes.	• Short sequence specialized equipment (Y.-K. Kim et al., 2012).
NGS-RNA Seq	Analysis of whole RNA sequencing	Hundreds of dollars (Baker, 2010) 3–5 days of preparation time (Baker, 2010)	• Precise • Maximum of 0.1% detection (Mohanty et al., 2021) • To increase the sensitivity with no background noise.	• Massive parallel Genome-wide RNA • Multiplexing • New variants identification • CleanPlex® is a highly scalable and ultrasensitive NGS amplicon sequencing technology (Mohanty et al., 2021)	• Sensitivity • Lower than ddPCR sensitivity. • Complex bioinformatics and computational resources (Stewart & Tsui, 2018)

sponging, thereby marking it as an independent predictor of HCC recurrence. This, in turn nominates it as a promising biomarker of HCC (Dong et al., 2018). Moreover, several cancerous malignancies are linked to the expression of SNORA71A, whose downregulation in HCC serves as a prognostic marker (Ding et al., 2020).

2.5. Long non-coding RNA

lncRNAs play a significant role in a variety of fundamental biological processes, including transcription regulation, cell differentiation, and chromatin modification (Mallela et al., 2024). Notably, in the advanced stages of HCC, elevated levels of MALAT1 and lncSPRY4–1T1 have been detected in plasma samples. Meanwhile, the plasma levels of LINC00152, XLOC014172, and RP11–160H22.5 have shown effective differentiation between HCC and conditions such as chronic hepatitis, cirrhosis, or normal liver status, showcasing their potential as a biomarker (Jing et al., 2016; Yuan et al., 2017).

2.6. Circular RNA

circRNA is a type of RNA molecule that forms a covalently closed loop structure, making it distinct from the more common linear RNA molecules. Unlike linear RNAs which have a 5' end and a 3' end, circRNAs lack free ends and instead form a continuous circle. This unique circular structure is generated through a process called back-splicing (Niu & Wang, 2022; W. Wang et al., 2020). Due to these features, circRNAs play a variety of biological roles, such as regulating encoding proteins and peptides, binding RBPs, acting as sponges for miRNAs, and regulating the transcription of parental genes (Peng et al., 2020). circRNAs have been shown to play important regulatory roles in various cellular processes and have the potential to be stable and detectable in body fluids, making them promising candidates for diagnostic and prognostic markers for HCC

3. cfRNA as diagnostic biomarkers

Diagnosing early-stage HCC presents significant challenges, frequently leading to detection the cancer in later stages. This delayed identification often results in more advanced local or distant spread, requiring complex treatments that result in a less favorable prognosis. Traditionally, diagnosing early liver cancer relied on examining AFP levels in the serum. However, elevated AFP levels are not specific to HCC and appear in individuals with chronic HBV and HCV infections (Harris et al., 2019). This lack of specificity has sparked debates about AFP's effectiveness as an early diagnostic tool for HCC (Xie et al., 2020). The growing number of studies providing evidence of identifying cfRNA in body fluids could significantly improve the early detection of HCC by enhancing both sensitivity and specificity. In Table 2, we have summarized cfRNAs candidates previously demonstrated to show high sensitivity, specificity, and significant AUC for diagnosing HCC.

3.1. MicroRNA

In the pursuit of enhancing the early diagnostic methods for HCC, the focus has significantly shifted toward exploring the potential of circulating miRNAs. The differential expression of miRNAs in plasma samples from HCC and control groups serves as a major factor for considering the miRNAs as a diagnostic markers. A study containing a group of 23 HCC patients and 17 chronic hepatitis C (CHC) individuals reported higher miR-21 levels cut of value 3.8 relative quantity (RQ)) and lower miR-199a levels (cut-off value 0.68 (RQ)) in the plasma of HCC patients compared to the CHC. This low expression of miR-199a accurately diagnoses HCC, showing a sensitivity of 54.5%, specificity of 100%, and AUC of 0.85. Furthermore, the study highlighted miR-21 as a promising diagnostic factor, with an impressive AUC of 0.94, 100% sensitivity, and 81.2% specificity. The accuracy of diagnosis HCC was way higher than the AFP with AUC of 0.61 (Amr et al., 2016; Tomimaru et al., 2012). In another study, the researcher observed a similar pattern to the above study considering two different miRNA levels namely miR-122 and miR-214. They found that miR-122 was significantly decreased (cut of value <0.67 (RQ)), while miR-214 was significantly increased (cut of value >1.2 (RQ)) in 40 HCC cases related to HCV compared to CHC and control groups. With a sensitivity of 87.5%, specificity of 95%, and an AUC of 0.98, miR-122 shows a promising indicator for HCC prognosis,

Table 2
Studies on cfRNAs as diagnostic biomarkers of HCC.

cfRNA	Expression	Sample number	Body Fluids	AUC	Sensitivity	Specificity	AUC of AFP	Reference
mRNA								
GPX3 mRNA	Down	HBV-HCC n=132 CHB n= 78 Healthy n=33	PBMCs	0.76	78%	60.3%	0.6	(Han et al., 2023)
RhoA mRNA	Up	HCC n=20 cirrhosis patients n=20 Healthy n=10	Plasma EVs	0.75	N/A	N/A	N/A	(Waqar et al., 2021)
CPE mRNA	Up	HCC n=22 Healthy n=30	Plasma EVs	0.87	N/A	N/A	N/A	(Hareendran et al., 2022)
hTERTmRNA	Up	HCC n=303, CH n= 89, LC n= 45 Healthy n=201	Serum	N/A	90.2%	85.4%	N/A	(Miura et al., 2010)
Circular RNA								
circRNA_104075	Up	HCC n=60, Healthy n=60	Serum	0.97	96.0%	98.3%	0.7	(X. Zhang et al., 2018)
circ-0051443	Down	HCC n=60, Healthy n=60	Plasma Exosome	0.80	N/A	N/A	N/A	(W. Chen et al., 2020a)
circ-ADD3	Down	HCC n= 31 Healthy n= 19	Plasma	0.88	N/A	N/A	N/A	(Sun et al., 2019)
circ-LRIG3 (hsa_circ_0027345)	Up	HCC n=36 Healthy n=36	Plasma	0.86	N/A	N/A	N/A	(Sun et al., 2020)
hsa_circ_0003998	Up	HCC n=100, HB n=50, Healthy n=50	Serum	0.89	84%	80%	N/A	(Qiao et al., 2019)
circ-FOXP1	Up	HCC n= 30 Healthy n=16	Serum	0.93	N/A	N/A	N/A	(W. Wang et al., 2020)
hsa_circ_0000976 hsa_circ_0007750 hsa_circ_0139897	Up	HCC n= 600 CHB n=186 LC n=180 Healthy n=179	Plasma	0.84	87.5%	82.2%	0.7	(J. Yu et al., 2020)
hsa_circ_0027089	Up	HBV-HCC n= 98 Healthy n= 72	Plasma	0.78	57.8%	84.8%	0.86	(K. Zhu et al., 2020)
circ_0000437	Up	HCC n=3, Healthy n=3	Serum	0.92	N/A	N/A	N/A	(W. Chen et al., 2020b)
Circ-0072088	Up	HCC n=50, Healthy n=50	Serum	0.89	N/A	N/A	N/A	(Lin et al., 2021)
micorRNA								
miR-92–3p, miR-107, miR-3126–5p	Up Up Down	HCC n=115 Healthy n=40	Serum	0.705 0.730 0.881	N/A	N/A	0.73	(Y. Zhang et al., 2017)
miR-21 miR-96 miR-122,	Up Up Down	HCC n=50 Hepatic cirrhosis n=50	Exosome	0.91 0.8 0.85	82% (combination)	92%	N/A	(Wang et al., 2020)
miR-375	Up	HBV HCC n=55 Healthy n=50	Serum	0.96	96%	100%	N/A	(L.-M. Li et al., 2010)
miR-155 miR-665	Up Up	HCV HCC n=80 Healthy n=40	Serum	0.74 0.93	80% 92%	62% 86%	0.6	(Mohamed et al., 2020)
miR-21	Up	HCC n=126 Healthy n=50	Plasma	0.95	87%	92%	0.88	(Tomimaru et al., 2012)
miR-199-a	Down	HCC n= 23 Chronic Hepatatis n=17	Serum	0.85	54%	100%	0.83	(Amr et al., 2016)
miR-122 miR-224	Up Down	HCV-HCC n=40, CHC n=40, Healthy n=20	Plasma	0.98 0.93	87% 87%	95% 97%	0.61	(Amr et al., 2017)
microRNA-574–3p	UP	HCC n=70 CHC n= 40, Healthy n=45	Serum	0.837	77%	82%	N/A	(Shen et al., 2018)
miR-34a	Down	HCC n=60 Healthy n=60	Serun	0.85(combination with AFP)	68.3%	98.3%	0.82	(S. Chen et al., 2022)
long noncoding RNAs								
EV-MALAT1 EV-SNHG1	Up	HCC n=95, Healthy n= 434	Serum	0.95 0.94	92.0% 80.7%	81.5% 85.2%	0.64	(S. S. Kim et al., 2021)
LINC01793	Up	HCC n=52, CHB n= 30, LC n= 30 Healthy n=30	Whole blood	0.82 (combination with AFP	67.3%	100%	0.774	(Mo et al., 2022)
Linc00152 UCA1	Up	HCC n=129, CHB n= 27	Serum	0.89 0.85	78.3% 81.4%	89.2% 75.3%	0.862	(J. Huang et al., 2020)

(*continued on next page*)

Table 2 (*continued*)

cfRNA	Expression	Sample number	Body Fluids	AUC	Sensitivity	Specificity	AUC of AFP	Reference
		LC n=49 Healthy n=93						
RP11–160H22.5, XLOC_014172 and LOC149086	Up	HCC n=217 Healthy n=250 CH n=100	Plasma	0.89	82%	73%	N/A	(J. Tang et al., 2015)
SHNG1	Up	HCC n=72 Healthy n=50	Blood	0.92	87.3	86	0.85	(S. Gao et al., 2018)
LncRNA-UCA1, lncRNA-WRAP53	Up	HCC n= 82 chronic CHC n= 34 Healthy n=44	Serum	0.76 0.87	N/A	N/A	N/A	(Kamel et al., 2016)
LINC00978	UP	HCC n=58 Healthy n=45	Serum	0.91	0.76%	0.98%	N/A	(X. Xu et al., 2019)
SPRYA-IT1	UP	HCC n= 87, Healthy n=87	Plasma	0.93	N/A	N/A	N/A	(Jing et al., 2016)
EV-LINC00853	UP	HCC n=10 Healthy n=10	Serum	0.93	93%	89%	0.71	(S. S. Kim et al., 2020)
uc001ncr AX800134	UP	HCC n=61 HBV n= 60, Healthy n=60	Serum	0.94 0.94	N/A	N/A	0.93	(K. Wang et al., 2015)

Abbreviations: AUC, area under the curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; N/A, not available data; UP, High expression of cfRNA; Down, Low expression of cfRNA; EVs, Extracellular vesicles; n, number of patients; CHB, Chronic Hepatitis B; CHC, Chronic HCV infection; LC, Liver Cirrhosis; AFP, Alpha-fetoprotein.

while miR-224 exhibits a potential diagnostic marker, with an AUC of 0.93, sensitivity of 87.5%, and specificity of 97%. Meanwhile, the accuracy of AFP for diagnosing HCC is lower, with an AUC of 0.619 indicating that miR-122 and miR-224 are potential non-invasive diagnostic biomarkers for HCC (Amr et al., 2017).

The combination of various biomarkers has the potential to overcome single biomarker limitations. For this reason, researchers have examined miRNAs, both alone and in combination with AFP, to enhance the diagnosis performance. Zhang et al. utilized a strategy that combined two or more miRNAs as a powerful diagnostic tool for HCC, especially in early tumor screening and patients with low-level AFP in early HCC stage. The microarray analysis of plasma smaples from 115 individuals with HCC identified a panel of three miRNAs (miR92–3p, miR-107, and miR-3126–5p) as a valuable diagnostic marker, particularly in early-stage patients (AUC = 0.975) and those with low-level AFP (AUC = 0.971). Additionally, combining this 3-miRNA panel with AFP significantly improved the effectiveness of discriminating between early-stage HCC patients (AUC = 0.988) and those with low-level AFP (AUC = 0.989) compared to controls, highlighting the robust diagnostic potential of these miRNAs (Y. Zhang et al., 2017). In a study comparing serum levels of miRNA-574–3p among 70 individuals with primary HCC, 40 with cirrhosis, and 45 healthy participants, researchers detected a significant elevation in HCC patients (2.3 RQ). Compared with the healthy control group, the AUC of serum pmicroRNA-574–3p in HCC group was 0.83, with a diagnostic accuracy of 79.1%, surpassing that of AFP (76.5%) and AFU (62.6%). Combining miRNA-574–3p with AFP increased sensitivity to 94.5%, outperforming the combination with AFU (85.7%). However, the highest accuracy in HCC detection was achieved by combining all three markers, resulting in an exceptional sensitivity of 97.1%. (Shen et al., 2018).

Circulating exosomal miRNAs are considered potential non-invasive biomarkers due to their high specificity to cancer. In the study conducted on 60 HCC patients before and after surgery and 60 health samples, results showed a significant decrease in miR-34a post-surgery exosome. The AUC level for serum exosome miR-34a was 0.664, lower than the AUC level of 0.82 for AFP. However, the combined AUC level for exosome miR-34a and AFP increased to 0.855, with a sensitivity of 68.3% and a specificity of 98.3%. These findings indicate that combining serum exosome miR-34a with AFP significantly improves the AUC, sensitivity, and specificity, making it an effective tool for distinguishing between HCC patients and the healthy group and underscoring its potential as a biomarker for the early clinical diagnosis of HCC (S. Chen et al., 2022).

3.2. Long non coding RNAs

Several types of cfRNA, apart from miRNA, have emerged as promising diagnostic biomarkers in HCC. Among these lncRNAs play a critical role in regulating genes through epigenetic modifications within the nucleus. Wang et al. elucidated the function of LINC001225, and demonstrated its ability to bind to the Epidermal Growth Factor Receptor (EGFR), thereby elevating EGFR protein levels and triggering the activation of the mitogen-activated protein kinase pathway (MAPK). They also observed high expression of LINC001225 in 60 HCC patients compared to 60 control, with a sensitivity of 76.1% and a specificity of 44.3% which can act as a novel biomarker in predicting the diagnosis of HCC. (X. Wang et al., 2016). Xu et al. observed upregulation of LINC00978 in serum from 58 HCC patients compared to 49 patients with benign liver diseases (BLD), and 45 healthy controls. Furthermore, LINC00978 promotes the progression of HCC by inhibiting the expression of p21 and E-cadherin through EZH2-mediated epigenetic silencing. This study elucidates the oncogenic role of LINC00978 in HCC progression and offers a diagnostic marker along with a therapeutic target(X. Xu et al., 2019).

In a combination biomarker study involving 87 HCC patients and healthy individuals, the expression level of lncSPRY4-IT1 combined with AFP (the cut-off value of AFP was at 200 ng/ml) resulted in improved AUC scores of 0.8, representing effective diagnostic markers (Jing et al., 2016). Kim et al. emphasized that extracellular vesicle-linked LINC00853 (EV-LINC00853) in serum vesicles could detect HCC more efficiently than AFP. Their study revealed that EV-LINC00853 exhibited a sensitivity of 93.75% and a specificity of 89.77% in diagnosing early-stage HCC (mUICC stage I), significantly outperforming AFP, which demonstrated only a sensitivity of 9.38% and a specificity of 72.73%. Additionally, EV-LINC00853 tested positive in 97% of early HCC cases that were AFP-negative and 67% of those that were AFP-positive (S. S. Kim et al., 2020). The analysis of various lncRNAs, including uc001ncr and AX800134, effectively diagnosed HBV-positive HCC, achieving AUC of 0.9494 and 0.9491 respectively. These lncRNAs also detected HCC effectively in cases where AFP levels were lower than 400 ng/ml, with AUC of 0.9371 and 0.9527, and in early-stage HCC, where they reached AUC of 0.9450 and 0.9564 (K. Wang et al., 2015). lncRNAs hold great promise in advancing HCC diagnosis. Continued research and validation of these discoveries may

pave the way for more precise diagnostic tools and strategies to effectively manage HCC.

3.3. Circular RNAs

Various circRNAs have emerged as potential non-invasive biomarkers for HCC. Notably, circ104075 exhibited significantly elevated levels in serum and tissue samples obtained from 101 HCC patients compared to a control group of 60 healthy individuals, thus highlighting its potential diagnostic relevance for HCC. Its diagnostic performance was characterized by an AUC of 0.97 with a sensitivity of 96.0% and a specificity of 98.3% which was more significant than AFP, which had an AUC of 0.7 (X. Zhang et al., 2018). Researchers detected high expression of circ0072088 in serum of 50 HCC patients compared to 50 healthy individuals. This expression, particularly prominent within serum exosomes, demonstrated promising diagnostic and prognostic utility in HCC cases, supported by an AUC of 0.89. Functional investigations revealed its involvement in exosome-mediated secretion, leading to the degradation of miR-375 and subsequent upregulation of MMP-16 (Lin et al., 2021). Another functional study delineates the role of circ-0051443 which suppresses the malignant biological behaviors by promoting cell apoptosis and arresting the cell cycle. Plasma exosomal circ-0051443 levels were significantly lower in the plasma of 60 HCC patients compared to 60 healthy individuals, with an AUC of 0.8. This suggests that circ-0051443 act as a diagnostic biomarker for HCC (W. Chen et al., 2020a). These collective findings underscore the potential feasibility of circular cfRNA as a promising diagnostic biomarker in HCC cases, particularly in scenarios where conventional biomarkers like AFP may exhibit limitations in effectiveness. A list of recently studied cfRNA and its significance as a diagnostic biomarker is collected in (Table 2).

4. Prognostic values of cfRNA

Plasma/serum cell-free cfRNA levels have been explored as a vital indicator for determining tumor stage and prognosis in HCC patients. Many studies have shown the association between miRNA levels with pathological characteristics with prognosis. A study conducted on 76 patients with HCC, 62 with benign liver diseases, and 55 healthy volunteers, shed light on the association of low levels of miR-148a with tumor size and tumor node metastasis (TNM) stage. In contrast, low miR-152 levels were only associated with TNM stage. Decreased serum levels of miR-148a and miR-152 were closely correlated with shortened overall survival (OS), with miR-148a being a potent prognostic marker in HCC patients (F. Wang et al., 2016). A recent study measured average serum levels of miR-125a-5p at 1.44 (RQ) in healthy individuals, 3.66 (RQ) in those with liver fibrosis, and 0.68 (RQ) in HCC patients. Patients with low expression levels of miR-125a-5p exhibited a lower survival rate, with a median survival of 30.66 months, compared to 39.04 months in those with high expression levels (J. Zheng et al., 2015). A retrospective study considering 122 HCC subgroup analysis by gender showed that high serum miR-122 level was independently associated with better OS in male patients, compared to female patients (Y. Xu et al., 2015). The 120 patients with HBV-related HCC who underwent radiofrequency ablation (RFA n = 57) had high levels of miR-122 in plasma and were considered one of the risk factors for poor OS. Additionally, combining the expression level of miR-122 with tumor stage predicted one year OS (Cho et al., 2015). These findings supported the potential utility of circulating miRNAs in evaluating the prognosis of HCC.

Recent research has shown a link between the levels of miRNAs in serum exosomes and the prognostic factors in HCC patients. A prospective study involving 76 HCC patients with elevated levels of miRNA-21 ($\geq$0.09 vs. $<$0.09) in serum exosomes displayed a significant association with TNM stage and other prognostic factors, such as T stage and portal vein thrombosis. The patients with higher miRNA-21 levels had significantly lower OS and progression-free survival (PFS) indicating miRNA-21 as a novel prognostic marker (Lee et al., 2019). The lower levels of exosomal miR-125b in HCC patients (n=158) compared to those in CHB (n=30) and LC (n=30) were associated with shorter OS and time to recurrence (TTR), with miR-125b acting as an independent predictor for both outcomes. Exosomal miR-125b levels were predictive of recurrence and survival in HCC patients, showing an AUC of 0.739 with 83.0% sensitivity and 67.9% specificity (W. Liu et al., 2017). Another study indicated that exosomal miRNA-21 served as a standalone indicator for predicting disease progression in 76 HCC patients. During the study, 44 patients showed disease progression after about 3.4 months on average, and 34 patients passed away around the same time. It was observed that the level of AFP in the serum was notably higher in patients who died compared to those who survived. The authors also reported an increased level of exosomal miRNA-21($\geq$0.09 RQ) in the bloodstream was associated with poorer OS and a shorter PFS suggesting that miRNA-21 novel prognostic markers and therapeutic targets for HCC (Lee et al., 2019). Recent studies have explored the prognostic value of mRNA expression changes in exosomes for HCC patients. One study utilized mRNA data derived form RNA-seq data obtained from the TCGA and exoRBase 2.0 databases to establish a robust prognostic scoring model for HCC patients based on exosomal mRNA expression. Identifying six genes (CLEC3B, CYP2C9, GNA14, NQO1, NT5DC2, and S100A9 mRNA) linked to liver cancer and high expression of these mRNA is linked to higher OS (L. Zhu et al., 2023).

Currently, expression profiles of lncRNAs in plasma are extensively studied, reflecting the characteristics of a tumor, and their role in prognosis. An elevated level of lncRNA-ATB ($\geq$0.0016 RQ) was observed in serum exosomes of 79 HCC patients and served as an independent predictor of mortality and disease progression, along with larger tumor size. However, OS and PFS were significantly lower in patients with higher circulating levels of lncRNA-ATB (Lee et al., 2019). In another study conducted on HCC (n = 60), LC (n = 85), CHB (n = 96), and healthy subjects (n = 60), levels of ENSG00000258332.1 (1.85 RQ), LINC00635 (2.1 RQ), LRB1 (54 ng/ml), were higher in HCC than other groups. The elevated levels of ENSG00000258332.1 and LINC00635 could distinguish HCC patients from CHB patients with AUC of 0.71 and 0.75 respectively. Serum AFP at a cutoff value of 20 mg/L yielded an AUC of 0.666. Higher levels of ENSG00000258332.1, and LINC00635 were associated with, lymph node metastasis, TNM stage, and lower OS. Moreover, higher levels of LRB1 were positively associated with AFP expression, large tumor sizes, tumor stage (TNM or Barcelona Clinic Liver Cancer stage), and venous invasion, and were negatively associated with OS. These findings underscore the potential of lncRNAs as a biomarker for prognosis in HCC (Z.-F. Wang et al., 2018; H. Xu et al., 2018). Many studies have highlighted the potential of cfRNA as a predictive marker in liver cancer. However, it's crucial to recognize that current research primarily centers on miRNA, neglecting the possible impacts of other types of cfRNA. This knowledge gap encourages further exploration for a more comprehensive and non-invasive biomarker candidate in cancer diagnostics. Some of the recently studied cfRNAs expression and its importance as a prognostic biomarker in blood for the HCC population are discussed in (Table 3).

5. Monitoring the effect of therapy

Therapeutic advancements have led to the discovery of several drugs for HCC, showing promising results in improving the survival and prognosis. However, the complex nature of cancer cells poses challenges, particularly in terms of drug resistance, which arises from multifactorial cancer mechanisms. Currently, well-defined non-invasive biomarkers for assessing drug resistance and predicting outcomes are lacking. Following therapy, the presence of cfRNA in bodily fluids may reflect specific tumor characteristics and treatment responses (M. Cui et al., 2019). This discovery has prompted numerous studies dedicated to detecting miRNA, aiming to serve as a non-invasive biological indicator for assessing treatment efficacy.

Table 3
Studies on cfRNAs as prognostic biomarkers of HCC.

cfRNA	Expression	Sample number	Body Fluids	Survival Outcome	Reference
mRNA					
ST2 mRNA	Up	HCC n=565 Healthy n=561	Peripheral blood cells	OS rates lower	(Pan et al., 2023)
GNA14, NQO1, NT5DC2, and S100A9 mRNA	Up	HCC n=468 Healthy n=168	Plasma EVs	OS rates lower	(L. Zhu et al., 2023)
CLEC3B, CYP2C9	Up	HCC n=468 Healthy n=168	Plasma EVs	OS rate Higher	
Circular RNA					
hsa_circ_0003998	Up	HCC n=100 HB n=50 Health n=50	Plasma	OS rate Lower	(Qiao et al., 2019)
microRNA					
miR-122	Up	HCC n= 122	Serum	OS rate Higher	(Y. Xu et al., 2015)
miR-125a-5p	Down	HCC n=120 CHB n= 91 Healthy n= 164	Serum	OS rate Higher	(J. Zheng et al., 2015)
miR-21	UP	HCC n=79	Serum Exosome	OS and PFS rate Lower	(Lee et al., 2019)
miR-150	Up	HCC n=120, Healthy n=120 CHB n=110	Serum	OS rate Lower	(F. Yu et al., 2015)
miR-125b	Down	HCC n= 158 CHB n= 30 LC n=30	Serum Exosome	OS and TTR rate Lower	(W. Liu et al., 2017)
miR-200a	Up	HCC n=136	Serum	OS rate Lower	(M. Liu et al., 2014)
miR122	Up	HCC n=120 (Hepatic resection n= 63, RFA n=57)	Plasma	OS rate lower in RFA treatment	(Cho et al., 2015)
miR-139	Down	HCC n=31, Chronic HBV n=31	Plasma	-	(T. Li et al., 2014)
miR-182 miR-331–3p	Up	HCC n=103, Benign liver n= 95, Healthy n=40	Serum	Associated with postoperative survival	(L. Chen et al., 2015)
miR-1 miR-122	Up	HCC n=195, Liver cirrhosis n=54	Serum	-	(Köberle et al., 2013)
miR-148a	Down	HCC n= 76 Liver Benign Disease n= 62 Healthy n= 55	Serum	OS rate Lower	(F. Wang et al., 2016)
miR-1247–3p	Up	HCC n=90 Healthy n= 25	Serum exosomal	OS and DFS rate Lower	(Fang et al., 2018)
miR-143	Down	HCC n= 131 Healthy n= 121	Serum	-	(J. Zhang et al., 2017)
miR-192–5p miR-29a-3p	Up Up	HCC n=174, Healthy n=130 Cirrhosis n=43	Serum	OS and PFS rate lower	(Zhu et al., 2016)
miR-218	Down	HCC n=156 Healthy n=64 BLD n= 98	Serum	OS rate lower	(L. Yang et al., 2016)
miR-221	Up	HCC n=46 Healthy n=20	Serum	OS rate lower	(J. Li et al., 2011)
miR-224	Up	HCC n=182	Serum	OS rate lower	(Zhuang & Meng, 2015)
miR24–3p	Up	HCC n=84 Healthy n= 46 CLD n=31	Serum	OS, DFS rate lower	(Meng et al., 2014)
miR-638	Down	HCC n=126	Serum Exosomes	OS rate lower	(Shi et al., 2018)
miR-96	Up	HCC n= 104 Healthy n= 120 LC n=190 CHB n=100	Serum	OS rate lower	(Chen et al., 2015)
Long noncoding RNA					
lncRNA-ATB	Up	HCC n=79	Serum Exosome	OS, DFS rate Lower	(Lee et al., 2019)
DANCR	Up	HCC n= 52 HVs n= 43 CHB n=29 Cirrhosis n=22	Plasma	-	(X. Ma et al., 2016)
ENSG00000258332.1 + LINC00635	Up	HCC n=60 Healthy n=60 LC n= 85 CHB n=96	Serum	OS rate Lower	(H. Xu et al., 2018)
JPX XIST	Down	HCC n=42 Healthy n=68	Plasma	OS rate Lower	(W. Ma et al., 2017)
LRB1	Up	HCC n=326, Healthy n= 73	Serum	OS rate Lower	(Z.-F. Wang et al., 2018)
UCA1	Up	HCC n=105 Healthy n=105 BLD n=105	Serum	OS rate Lower	(Z.-K. Zheng et al., 2018)

Abbreviations: AUC, Area under the receiver-operating characteristic curve; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; N/A, not available data; UP, High expression of cfRNA; Down, Low expression of cfRNA; EVs, Extracellular vesical; BLD, Benign liver diseases; CHB, Chronic hepataitis B; LC, Liver cirrhosis; TTR, Time to recurrence; PFS, Progression free survival: CLD, Chronic liver disease.

Recent studies have shed light on the relationship between plasma cfRNA and the outcomes of Transarterial Chemoembolization (TACE) treatment, liver transplantation, resection, and sorafenib treatment, showcasing a growing interest in non-invasive methods to track various treatment responses in HCC. The recommended first-line treatment for intermediate-stage HCC (BCLC B) typically involves non-curative therapies like TACE, which exhibit varying response rates (15–55%) (European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu & European Association for the Study of the Liver, 2018; Kong et al., 2018). One study of 177 HCC patients treated with TACE revealed an association between higher levels of plasma miR-122 (> 100) and inadequate responses to TACE (Hazardous ratio (HR, 2.77; 95%), Confidence interval (CI), 1.12–6.86;). Moreover, univariate analyses showed that high miR-122 expression tends to be associated with poor liver transplantation-free survival (HR, 1.42; 95% CI, 0.95–2.11;)(S. S. Kim et al., 2017).

Conversely, a multivariate Cox proportional hazards regression analysis of clinical parameters in 75 HCC patients and LC treated with TACE revealed a notable reduction in exosomal miR-122 levels. This reduction is associated with disease-specific survival, suggesting that exosomal miR-122 levels may serve as a predictive biomarker in HCC patients with TACE treatment. (Suehiro et al., 2018). In another study of 125 HCC patients treated with TACE, the mean serum miR-335 level was slightly elevated at 1.12 (RQ), yet it remained significantly lower than in hepatitis patients 1.88 (RQ) and healthy controls 1.98 (RQ). Higher response rate was observed in patients with high miR-335, whereas lower levels were linked to shorter OS.

Moreover, reduced miR-200 expression in plasma samples from 136 HCC patients has been associated with a more favorable prognosis, yielding an ROC curve area of 88.19% with 93.62% specificity and 68.54% sensitivity (L. Cui et al., 2015; M. Liu et al., 2014). Furthermore, drastic changes in the expression levels of miR-133b and miR-26a were observed

compared to baseline after TACE therapy for 51 HCC patients. Particularly elevated levels of miR-133b and miR-26a in plasma distinguish between complete response (CR), partial response (PR), and no response (NR) to treatment. The combination of miR-133b and miR-26a showed excellent ability to differentiate responders from non-responders with high accuracy AUC=1.0 when CR compared with NR, AUC=0.997 when PR compared with NR, AUC=0.919 when CR was compared with PR, and AUC=0.998 when responders to TACE (CR&PR) compared with NR (Ali et al., 2019). The level of miR-26a reaches its peak (≥ 2.5) 24 hours after TACE treatment, and combining the expression level of miR-26a with that of other miRNAs serves as a predictive indicator for the efficacy of TACE treatment (S. S. Kim et al., 2018).

Sorafenib is often the first-line systemic therapy for advanced-stage HCC (BCLC C). Researchers have found that evaluating circular miRNAs could aid in anticipating the response to sorafenib. 93 Patients who responded to sorafenib treatment exhibited an increase serum level of miR-221, while lowerpre-treatmnet of levels of miR-221 were associated with a better response to the drug (Fornari et al., 2017). During sorafenib treatment, analysis of miRNA in 53 patients revealed significant differences in serum levels of miR-181a-5p and miR-3395p among those with PR, stable disease (SD), and progressive disease (PD), patients with PR exhibited with highest levels, and those with PD, the lowest. However, serum miR-181a-5p was the only independent factor associated with achieving disease control (DC) ($p = 0.0092$, odds ratio 0.139, and 95% confidence interval 0.011–0.658). Additionally, the level of miR-181a-5p independently influenced OS ($p = 0.0194$, hazard ratio (HR) 0.267, and 95% confidence interval 0.070–0.818) (Nishida et al., 2017). A pilot study involving 83 HCC patients demonstrated that miRNAs correlated with treatment response and also predicted prognostic value. Specifically, the miR-222–5p and miR-512–3p levels increased after one month of sorafenib treatment, which was associated with a poor prognosis (de la Cruz-Ojeda et al., 2022).

Radiofrequency treatment typically applies to early-stage HCC patients (J. Gao et al., 2015). Patients undergoing radiotherapy showed increased levels of circulating miR-122. Furthermore, high (>100) plasma miRNA-122 expression (HR = 2.67; 95% confidence interval [CI]= 1.12–6.35;) and advanced tumor stage (HR = 2.27; 95% CI = 1.23–4.18;) are independent risk factors for poor OS in patients with HBV-related HCC who underwent RFA (Cho et al., 2015). Beyond miRNAs, circRNAs efficiently serves as a potential prognostic biomarker. For instance, plasma samples from 124 HCC patients and 100 healthy individual revealed upregulated exosomal circAKT3. Approximately 63% of patients with high circulating exosomal circAKT3 experience higher tumor recurrence rates (HR= 3.14) and increased mortality (HR= 1.89). The data also show that these patients have lower OS rates and (RFS) rates compared to patients with low exosomal circAKT3, indicating the potential of exosomal circAKT3 as a prognostic marker after surgical treatment(Luo et al., 2020).

In the analysis of serum miRNA levels in 76 HCC patients, as well as 62 controls with benign liver diseases and 55 healthy individuals, levels of miR-148/152 were significantly lower in HCC patients than those in benign and healthy individuals. Notably, serum levels of the miR-148/152 family drastically decreased in cases of recurrent or metastatic HCC compared to postoperative groups. Kaplan-Meier analyses reveal that HCC patients with low levels of serum miR-148a and miR-152 experience shorter OS compared to those with low levels of serum miR-148b. Additionally, Cox multivariate analysis suggests that serum miR-148a, as opposed to serum miR-148b and miR-152, could serve as an independent prognostic factor for HCC patients (F. Wang et al., 2016;.). This evidence suggests an association between differential levels of cfRNAs in serum and treatment assessment in HCC. However, most studies focus on analyzing miRNAs, with insufficient investigation into other cfRNAs for treatment management. Exploring these could help identify appropriate non-invasive biomarkers for treatment assessment. The roles of different cfRNA as biomarkers for monitoring and predicting responses in the treatment of HCC are emphasized in the following table (Table 4).

6. Technical and clinical challenges

The field of liquid biopsy faces some challenges, primarily stemming from the variability in technical approaches adopted by research groups worldwide (Cabús et al., 2022). This inconsistency can be attributed to the complex nature of cfRNA, where factors like RNA type and specialized secondary structures make extraction difficult. (Buschmann et al., 2016; Kroh et al., 2010).

Analyzing cfRNA poses challenges because the input sample typically contains a blend of both small and large RNAs, and there are no specialized methods for separating them (Androvic et al., 2019). Various pre-analytical variables significantly impact the detection of cfRNA in body fluids. Notably, the inflammatory state leads to an increase in white blood cell count, which can affect cfRNA expressions. This can potentially lead to the identification of false-positive biomarkers that are attributed to the secondary effects of leukocytes rather than cancer cells themselves. Additionally, hemolysis and nutritional status of patients dramatically affect miRNA profiles due to increased lipoprotein concentrations, which cfRNAs can bind to (Marzi et al., 2016; Vickers et al., 2011). Researchers encounter environmental stimuli as a technical challenge that affects the RNA expression, leading to differences between samples, even with no symptom. These challenges underscore the

Table 4
Studies on cfRNAs as monitoring the therapeutic effect.

cfRNA	Expression	Sample number	Body Fluids	Clinical Setting	Treatment	Reference
Circular RNA						
circAKT3	UP	HCC n= 124	Serum	Recurrence	Resection	(Luo et al., 2020)
microRNA						
miR-200	Up	HCC n= 136	Serum	Response prediction	TACE	(M. Liu et al., 2014)
miR133b, miR-26a	UP	HCC n=51	Serum	Responsive and non-responsive	TACE	(Ali et al., 2019)
miR-122	Up	HBV-HCC n= 57	Plasma	Response Prediction	RFA	(Cho et al., 2015)
miR-182 miR-331–3 P	Up	HCC n=18	Serum	Monitoring and Responsive and non-responsive	TACE	(L. Chen et al., 2015)
miR-148/ miR-152	Down	HCC n= 76	Serum	Responsive and non-responsive	Resection post	(F. Wang et al., 2016)
miR-122	Up	HCC N=177	Plasma	Response Prediction	TACE	(S. S. Kim et al., 2017)
miR-122	Down	HCC n=75	Exosome	Responsive vs. non responsive	TACE	(Suehiro et al., 2018)
miR-335	Down	HCC n=125	Serum	Responsive vs. non-responsive	TACE	(L. Cui et al., 2015)
miR-221	Down	HCC n=28 Responder n = 12 Nonresponse n= 16	Serum	Responsive vs. non-responsive	Sorafenib	(Fornari et al., 2017)
miR-21	Up	HCC n-97	Serum	Response Prediction	Resection	(Wang et al., n.d)
miR-1246 miR-148a	Up Up	HCC pre-LT n=33, HCC LT-1Day n= 36 HCC LT- 1week n= 38	Plasma	Responsive vs non responsive	LT	(Ng et al., 2016)
miR-181a-5p miR-339–5p	Down	HCC n=16	Serum	Response Prediction	Sorafenib	(Nishida et al., 2017)
miR-26a miR-29a	Down	HBV HCC n=120	Plasma	Response prediction	Resection or RFA	(Cho et al., 2017)
miR-34a	Down	HCC n=10	Serum	Response Prediction	Resection	(Xiang et al., 2016)
miR-718	Down	HCC n=59	Serum Exosomes	Response Prediction	LT	(Sugimachi et al., 2015)
miR-423–5p	Down	HCC n=39	Serum	Responsive vs. non responsive	Sorafenib	(Stiuso et al., 2015)
miRNA-21, miRNA-26a, miRNA-29a-3p	Up Up Down	HCC n=198	Plasma	TACE refractoriness	TACE	(S. S. Kim et al., 2018)
miR-222–5p miR-512–3p	Up Up	HCC n=36	Plasma	Responsive vs. non responsive	Sorafenib	(de la Cruz-Ojeda et al., 2022)

Abbreviations: AUC, area under the receiver-operating characteristic curve; HCC, hepatocellular carcinoma; N/A, not available data; UP, High expression of cfRNA; Down, Low expression of cfRNA; LT, Liver Transplant; TACE, Transarterial Chemoembolization; RFA, Radiofrequency Ablation

need for standardization and the development of specific isolation and analysis methods to improve the reliability and accuracy of liquid biopsy techniques. Currently, cfRNA, such as U6 snRNA, RNU44, RNU6B, miR-16, miR-191, and cel-miR-39 are used as internal control, although the appropriate choice of reference cfRNA varies depending on the specific experimental setup and type of samples being studied (Faraldi et al., 2018). Despite all the technical challenges encountered in processing the cfRNA, the clinical utility of cfRNA as a non-invasive biomarker holds immense promise, yet its full potential remains largely unexplored.

As of July 6, 2023, a larger number of human clinical trials have been registered on clinicaltrials.gov, and numerous ongoing and forthcoming clinical trials are employing cfRNA as a biomarker (Hanna et al., 2019). Recent advancements and cost reductions in sequencing technology have convinced the medical field to adopt the cfRNA profiling of body fluids from cancer patients using techniques like PCR, and next-generation sequencing (NGS) (Shegekar et al., n.d). However, it is noticed predominantly that clinicians encounter significant hurdles when incorporating liquid biopsy as a screening tool, primarily encountering issues related to data tests, specificity, technical reproducibility, and the variability inherent in cfRNA detection methods. These challenges are ultimately rooted in the absence of a gold standard for isolation, storage, and detection techniques, and the presence of high interpersonal variability (Geeurickx & Hendrix, 2020; H. Wang et al., 2018).

Recently, the FDA has granted the approval of cfDNA-based tests such as Guardant360 CDx and Foundation One Liquid CDx in 2020 which is NGS-based liquid biopsy using cfDNA for diagnosis (FDA Approves Blood Tests, 2020). This approval allows the test to serve as a companion diagnostic device for numerous biomarkers identified in cell-free DNA obtained from plasma samples. Till now several cfRNA-based studies have undertaken clinical trials and utilized advanced cloud computing platforms demonstrating promising strides in developing dependable cfRNA-based biomarkers (Cabús et al., 2022; University of Southern California, 2021). Despite this progress, no cfRNA-based kit has secured FDA approval for clinical application. Nevertheless, there is considerable optimism regarding their potential in the future.

7. Conclusion

HCC is known for its stealthy nature, often remaining undetected until later stages. By the time it's detected, the tumor may have already spread to other areas through the portal vein and cancerous clots. Presently, diagnosing HCC involves a combination of imaging and the AFP serum marker. However, early lesions may evade detection through imaging, and the accuracy of AFP is limited due to its low specificity and sensitivity. In the realm of liquid biopsy, the last few years have seen a significant emphasis on investigating cfRNA majorly focusing on miRNA as a prominent non-invasive biomarker. However, in the past five years, the attention has been shifting from miRNAs to various cfRNAs, revealing the previously undiscovered disease-associated RNAs. Studies have shown significant changes in the levels of ncRNA in body fluids before and after surgery. Furthermore, during TACE and radiofrequency treatments, specific circulating miRNAs have been recognized as important biomarkers for assessing treatment effectiveness and predicting the recurrence of the condition after surgery. cfRNAs show promising results for the diagnostic and prognostic markers in HCC

patients, but it's crucial to confirm and validate these markers across diverse patient groups. This includes individuals with different causes of HCC, varied cancer cell characteristics, and various stages of the disease. In summary, initial research on cfRNA indicates potential in addressing different stages of liver cancer, including its early development. To effectively diagnose HCC, extensive studies involving large groups of patients are necessary.

Statement of ethics

Not applicable

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Credit authorship contribution statement

Dattatrya Shetti: Conceptualization, Writing – original draft, Visualization, Writing – review & editing, making Images and tables. **Venkata Ramana Mallela:** Visualization, Writing – review & editing, making Images and tables. **Wenjing Ye:** Writing – review & editing. **Mahyar Sharif:** Writing – review & editing. **Filip Ambrozkiewicz:** Feedback & critical review. **Andriy Trailin:** Feedback & critical review. **Václav Liška:** Writing – review & editing, Funding acquisition. **Kari Hemminki:** Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

No potential of conflict of interest was reported regarding to this article.

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