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REVIEW

# Circulating Non-Coding RNAs as Potential Diagnostic Biomarkers in Hepatocellular Carcinoma

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**Abstract:** Hepatocellular carcinoma (HCC) is currently the second leading cause of cancer-related deaths worldwide, with high morbidity and mortality. The clinical diagnosis of HCC mainly depends on imaging technology, such as ultrasound and computed tomography, and serum biomarkers, such as alpha-fetoprotein (AFP). However, HCC is still hard to diagnose at an early stage due to the low sensitivity of the above mentioned traditional methods. Typically, HCC is diagnosed at an advanced stage when limited treatment options are available. It is urgent to identify effective biomarkers for the early diagnosis of HCC. Increasing evidence uncovered ncRNAs, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), could be used in HCC diagnosis. The aim of this review is to summarize our understanding of circulating miRNAs, lncRNAs and circRNAs as fluid-based non-invasive biomarkers, and aiming at providing new insights into the diagnosis of HCC.

**Keywords:** hepatocellular carcinoma, microRNAs, long non-coding RNAs, circular RNAs, diagnosis

## Introduction

Hepatocellular carcinoma (HCC), one of the most common cancers worldwide, lists as a primary cause of cancer-related deaths globally.[1] Currently, most patients with HCC grow into advanced stages after diagnosis, at which point the efficacy of radiotherapy and chemotherapy is limited and the time of surgical treatment was missed.[2] Traditional imaging techniques including computed tomography (CT), positron emission computed tomography (PET) and magnetic resonance imaging (MRI) are the most widely used screening tools for HCC;[3] however, they have limited sensitivity and are less efficient for detecting early HCC.[4] Moreover, as the most commonly used clinical serum biomarker for detection of HCC, alpha-fetoprotein (AFP) also lacks the desired sensitivity and specificity for early diagnosis of HCC.[5] It has been reported that the serum levels of AFP remain normal in up to 40% of the patients with HCC, particularly during the early stage.[6] Therefore, there is an urgent need to develop clinical biomarkers for the detection of early-stage HCC.

Recently, there is extensive evidence suggesting that several circulating ncRNAs including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) in body fluids, such as urine, plasma, serum or saliva, could be potential non-invasive biomarkers for the diagnosis of cancers.[7,8] These cell-free ncRNAs in body fluids are proposed to be leaked from cells following cell injury and death or be secreted by extracellular vesicles, such as exosomes.[9,10] Due to the fact that ncRNAs have highly specific expression among different tissues or diseases, circulating ncRNAs are considered to be tumor-specific.[11,12]

A growing body of research has illuminated that ncRNAs play essential roles in the occurrence and development of HCC.[13] Abnormal expression of circulating ncRNAs in HCC may provide potential diagnostic biomarkers.[14] In this review, we will summarize the available knowledge on the diagnostic potential of circulating miRNAs, lncRNAs and circRNAs in HCC, aiming at providing new insights into the diagnosis of HCC.

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## Origins of Circulating ncRNAs

1. It has been demonstrated that circulating ncRNAs are packaged into various membrane-bound vesicles including exosomes and microvesicles, and apoptotic bodies.[15]
2. Lipoproteins complexes such as high-density lipoprotein and RNA-binding proteins such argonaute 2 are known to be another sources of circulating ncRNAs.[16]

## Advantages of ncRNAs as Potential Biomarkers

1. ncRNAs exhibit high stability in the circulation. Circulating ncRNAs are stably maintained in diverse biological fluids including cerebrospinal fluid and peripheral serum/plasma due to their protection in exosomes, microvesicles, apoptotic bodies, and protein complexes.[17] In terms of circRNAs, they have excellent stability owning to the covalently closed RNA circle without 5′ end caps or 3′ poly tails.[18]
2. Circulating ncRNAs can be quantified, even at low amounts, by quantitative reverse transcription polymerase chain reaction (qRT-PCR) with high sensitivity, specificity, and high dynamic range, a technique readily available in clinical laboratories.[19] Additionally, the global profiles of ncRNAs can be obtained in a single experiment using qRT-PCR panels, next-generation sequencing or microarrays.
3. Circulating ncRNAs are known to have tissue-specific expression patterns and profound relationships to development and diseases.[20]

## Detection Methods of Circulating ncRNAs

It has been reported that different sources of liquid biopsy including whole blood, plasma, and serum can be used to quantify circulating ncRNAs. Different approaches including qRT-PCR, RNA-sequencing, and microarray, have been developed to study ncRNA expression.

1. The most commonly used method to detect the expression of specific ncRNAs is qRT-PCR. This method allows evaluation of the expression of a few specific molecules but do not permit the discovery of new ncRNAs or provide an overview of all ncRNAs.[21]
2. RNA-sequencing is a high-throughput sequencing method which can be used to sequence the entire transcriptome, leading to the identification of new ncRNAs.[22]
3. Microarray-based technique is another powerful high-throughput method extensively used for ncRNA profiling, because of their ability to screen large number of ncRNAs simultaneously in large variety of samples. However, microarrays use already known sequences, thus making them unsuitable for the discovery of novel ncRNAs.[23,24]

## MiRNA Biomarkers

MiRNAs, a class of small non-coding RNAs with a length of 18–25 nucleotides, are endogenous and function as negative regulators of gene expression at the post-transcriptional level by promoting the degradation of messenger RNA (mRNA) or repressing its translation.[25] It has been well demonstrated that miRNAs are secreted by cells through exosomes and extracellular vesicles, and secreted miRNAs remain stable in bodily fluids.[26] Abnormal expression of miRNAs has been identified in various kinds of malignancies, which can function as oncogenes or tumor suppressors.[27,28]

Over the past several years, numerous studies have focused on exploring the possibility of circulating miRNAs as potential biomarkers for HCC diagnosis (Table 1). For example, it has been shown that the plasma miRNA-21 levels in the 126 patients with HCC were significantly increased than in patients with chronic hepatitis and healthy volunteers, respectively.[29] ROC analysis showed that the area under the curve (AUC) of miRNA-21 in plasma was 0.773 with 61.1% sensitivity and 83.3% specificity in distinguishing patients with HCC from patients with chronic hepatitis; the AUC of miRNA-21 in plasma was 0.953 with 87.3% sensitivity and 92.0% specificity in distinguishing patients with HCC from patients with healthy volunteers.[29] Recently, another study evaluated the diagnostic value of circulating levels of miRNA-9-3p in hepatitis C virus infection (HCV)-related HCC patients. The results showed that at a cutoff point of 1.01, miRNA-9-3p can discriminate between HCC patients and healthy volunteers with a sensitivity of 91.43% and

**Table 1** Circulating miRNAs Serve as Potential Diagnostic Biomarkers for HCC

| miRNAs | Source | Cohort Size | Ethnic Population | Detection Method of ncRNAs | AUC | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|---|---|
| miR-107 | Serum | 115 HCC vs 40 HC | Chinese | Microarray | 0.730 | | | [33] |
| miR-122 | Plasma | 40 HCC vs 20 HC | Egyptian | qRT-PCR | 0.96 | 87.5 | 95 | [35] |
| miR-122+AFP | Plasma | 40 HCC vs 40 CHC | Egyptian | qRT-PCR | 1 | 97.5 | 100 | [35] |
| miR-199a | Serum | 23 HCC vs.17 CH | Egyptian | qRT-PCR | 0.856 | 54.5 | 100 | [36] |
| miR-21 | Plasma | 126 HCC vs 30 CH | Japanese | qRT-PCR | 0.773 | 61.1 | 83.3 | [29] |
| miR-21 | Plasma | 126 HCC vs 50 HC | Japanese | qRT-PCR | 0.953 | 87.3 | 92.0 | [29] |
| miR-21 | Serum | 23 HCC vs.17 CH | Egyptian | qRT-PCR | 0.943 | 100.0 | 81.2 | [36] |
| miR-21+AFP | Plasma | 126 HCC vs 30 CH | Japanese | qRT-PCR | 0.823 | 81.0 | 76.7 | [29] |
| miR-21+AFP | Plasma | 126 HCC vs 50 HC | Japanese | qRT-PCR | 0.971 | 92.9 | 90 | [29] |
| miR-224 | Plasma | 40 HCC vs 20 HC | Egyptian | qRT-PCR | 0.94 | 92.5 | 90 | [35] |
| miR-224 | Plasma | 40 HCC vs 40 CHC | Egyptian | qRT-PCR | 0.93 | 87.5 | 97 | [35] |
| miR-224+AFP | Plasma | 40 HCC vs 40 CHC | Egyptian | qRT-PCR | 0.93 | 90 | 100 | [35] |
| miR-3126-5p | Serum | 115 HCC vs 40 HC | Chinese | Microarray | 0.881 | | | [33] |
| miR-34a | Serum exosome | 60 HCC vs 60 HC | Chinese | qRT-PCR | 0.664 | 78.3 | 51.7 | [37] |
| miR-34a+AFP | Serum exosome | 60 HCC vs 60 HC | Chinese | qRT-PCR | 0.855 | 68.3 | 93.3 | [37] |
| miR-424 | Serum | 123 HCC vs 76 HC | Chinese | qRT-PCR | 0.768 | 75.0 | 72.4 | [38] |
| miR-665 | Serum | 80 HCC vs 80 LC | Egyptian | qRT-PCR | 0.930 | 92.5 | 86.3 | [39] |
| miR-9-3p | Serum | 35 HCC vs 33 HCV | Egyptian | qRT-PCR | | 91.43 | 87.88 | [30] |
| miR-9-3p | Serum | 35 HCC vs 32 HC | Egyptian | qRT-PCR | | 91.43 | 87.50 | [30] |
| miR-92a-3p | Serum | 115 HCC vs 40 HC | Chinese | Microarray | 0.705 | | | [33] |
| miR-122 + miR-192 + miR-21 + miR-223 + miR-26a + miR-27a + miR-801 | Plasma | 204 HCC vs 60 LC + 75 CHB + 68 HC | Chinese | Microarray and qRT-PCR | 0.864 | 68.6 | 90.1 | [32] |
| miR-122 + miR-192 + miR-21 + miR-223 + miR-26a + miR-27a + miR-801 | Plasma | 196 HCC vs 56 LC + 72 CHB + 66 HC | Chinese | Microarray and qRT-PCR | 0.888 | 81.8 | 83.5 | [32] |
| miR-29a + miR-29c + miR-133a + miR143 + miR-145 + miR-192 + miR-505 | Serum | 108 HCC vs 47 LC + 51 CHB + 51 HC | Chinese | qRT-PCR | 0.826 | 80.6 | 84.6 | [34] |
| miR-29a + miR-29c + miR-133a + miR143 + miR-145 + miR-192 + miR-505 | Serum | 153 HCC vs 71 LC + 68 CHB + 60 HC | Chinese | qRT-PCR | 0.817 | 74.5 | 88.9 | [34] |
| miR-29a + miR-29c + miR-133a + miR143 + miR-145 + miR-192 + miR-505 | Serum | 49 HCC vs 42 IHC + 48 HC | Chinese | qRT-PCR | 0.884 | 85.7 | 91.1 | [34] |
| miR-29a + miR-29c + miR-133a + miR143 + miR-145 + miR-192 + miR-505 | Serum | Nested cohort: 568 samples from 135 CHB/LC vs 100 samples from 27 HCC | Chinese | qRT-PCR | 0.670 | 55.6 | 78.5 | [34] |

**Abbreviations**: AFP, alpha-fetoprotein; AUC, area under the curve; CH, chronic hepatitis; CHB, chronic hepatitis B infection; CHC, chronic hepatitis C infection; HC, healthy control; HCC, hepatocellular carcinoma; HCV, hepatitis C virus infection; IHC, inactive HBsAg carrier; LC, liver cirrhosis.

a specificity of 87.50%.[30] The performance of serum miRNA-224 as diagnostic biomarker for HCC at early stage has been assessed.[31] The results of ROC analysis demonstrated that the AUC was 0.880 with 86.5% sensitivity and 76.7% specificity for serum miR-224 in discriminating early-stage HCC from all three controls (liver cirrhosis (LC), chronic hepatitis B (CHB) and healthy subjects), higher than that for AFP.[31]

Combining detection of different biomarkers has been reported to be a useful strategy due to the increased sensitivity and specificity of each biomarker. Consistently, several studies have evaluated the clinical value of miRNA panel for the early diagnosis of HCC. It has been shown that a miRNA panel (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801) can differentiate HCC from healthy (AUC 0.941), chronic hepatitis B (AUC 0.842), and cirrhosis (AUC 0.884), respectively.[32] Similarly, another study has demonstrated that the unique 3-miRNA signature (miR-92a-3p, miR-107, and miR-3126-5p) combined with AFP can serve as a sensitive, specific, and noninvasive biomarker for the diagnosis of HCC, especially in the patients at early stages or with low AFP level.[33] A large-scale, multicentre, retrospective longitudinal study has been conducted to assess the performance of serum miRNA for HCC detection.[34] The results revealed that a seven-miRNA classifier (miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505) had significantly higher sensitivity than AFP to distinguish patients with HCC from healthy controls, inactive HBsAg carriers, chronic hepatitis B patients, and patients with hepatitis B-induced liver cirrhosis.[34]

## LncRNA Biomarkers

LncRNAs, a diverse class of ncRNAs, are defined as untranslated RNA molecules greater than 200 nucleotides in length with low protein-coding potential.[40] Mounting evidence have suggested that lncRNAs play important roles in regulating gene expression at transcriptional, posttranscriptional and epigenetic levels in the development and progression of tumors.[41,42] More importantly, aberrant signatures of lncRNAs are developed as the potential diagnostic biomarkers and therapeutic targets of cancer due to their specific expression pattern in carcinomas.[43,44]

Recent studies have evaluated the possibility of circulating lncRNAs as diagnostic biomarkers for HCC (Table 2). Ma et al recruited a cohort of 146 participants including healthy volunteers (HVs) and patients with chronic hepatitis B (CHB), cirrhosis and HCC, and investigated the potential use of circulating lncRNA differentiation antagonizing non-protein coding RNA (DANCR) in plasma as a diagnostic biomarker for HCC. The results showed that plasma DANCR exhibited significantly increased discriminatory power for differentiating patients with HCC from HVs and non-HCC patients compared to AFP.[45] Similarly, another study showed that LINC00978 was upregulated in tissues and serum of HCC patients.[46] The results of ROC analysis indicated that the upregulation of LINC00978 had a high diagnostic value to distinguish HCC patients from patients with hepatitis and liver cirrhosis and healthy controls.[46] In addition, Zeng et al investigated the clinical value and functional role of lncRNA DQ786243 (lncDQ) in the pathogenesis of HCC.[47] It has been found that serum lncDQ level could differentiate HCC patients from healthy controls, with an AUC of 0.804.

In terms of the combination of lncRNA and AFP, it has been reported that combination of lncRNA SPRY4 intronic transcript 1 (SPRY4-IT1) and AFP possessed a moderate ability for discrimination between HCC patients and controls.[48] The AUC of the combined indicators (0.800) is higher than that of SPRY4-IT1 alone (0.702).[48] More recently, it has been indicated that a combination of lncRNA ubiquitin conjugating enzyme E2 C pseudogene 3 (UBE2CP3) and AFP yielded an AUC of 0.933, which was improved as compared to lncRNA UBE2CP3 (0.839) or AFP (0.912) alone.[49] Similarly, combination of lncRNA JPX and AFP possessed a promoted ability for discrimination between HCC patients and controls (AUC 0.905, 97.1% sensitivity, 72.2% specificity) compared to JPX alone (AUC 0.814, 100% sensitivity, 52.4% specificity).[50]

In terms of the panel of lncRNAs, Kamel et al demonstrated that the panel of serum lncRNA urothelial carcinoma associated-1 (lncRNA UCA1) and WD repeat containing, antisense to TP53 (WRAP53) is superior to the AFP in specificity for the diagnosis of patients with HCC.[51] Moreover, a panel based on the expression of lncRNAs uc001ncr and AX800134 accurately diagnosed HBV-positive HCC from control.[52] It is worth noting that the diagnostic performance of the panel remained high in patients with AFP $\leq$ 400 ng/mL or in patients with early HCC.[52] Furthermore, a plasma panel of lncRNAs HULC and 00152 possessed a moderate ability to discrimination between HCC and control with an area under ROC value of 0.87.[53]

**Table 2** Circulating lncRNAs Serve as Potential Diagnostic Biomarkers for HCC

| lncRNAs | Source | Cohort Size | Ethnic Population | Detection Method of ncRNAs | AUC | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|---|---|
| lnc00152 | Plasma | 66 HCC vs 53 HC | Chinese | qRT-PCR | 0.850 | | | [53] |
| lnc00152 + HULC | Plasma | 66 HCC vs 53 HC | Chinese | qRT-PCR | 0.870 | | | [53] |
| lnc00152 + HULC + AFP | Plasma | 66 HCC vs 53 HC | Chinese | qRT-PCR | 0.890 | | | [53] |
| lnc00853 | Serum exosome | 90 HCC vs 35 LC + 28 CH + 29 HC | Korean | qRT-PCR | 0.935 | 83.3 | 89.8 | [54] |
| lnc00853 + AFP | Serum exosome | 90 HCC vs 35 LC + 28 CH + 29 HC | Korean | qRT-PCR | 0.969 | 93.8 | 89.8 | [54] |
| lnc00974 | Plasma | Operation: 150 preoperative vs postoperative | Chinese | qRT-PCR | 0.733 | 51.1 | 95.6 | [55] |
| lnc00974 | Plasma | Tumor size (5 cm): 78 large vs 72 small | Chinese | qRT-PCR | 0.755 | 67.5 | 83.5 | [55] |
| lnc00974 | Plasma | Metastasis: 62 positive vs 88 negative | Chinese | qRT-PCR | 0.791 | 68.5 | 89.6 | [55] |
| lnc00978 | Serum | 58 HCC vs 49 CH/LC and 45 HC | Chinese | qRT-PCR | 0.910 | 76.0 | 96.0 | [46] |
| lnc01225 | Serum | 66 HCC vs 70 HC | Chinese | qRT-PCR | | 76.1 | 44.3 | [56] |
| AF085935 | Serum | 137 HCC vs 104 CHB | Chinese | qRT-PCR | 0.860 | | | [57] |
| AF085935 | Serum | 137 HCC vs 138 HC | Chinese | qRT-PCR | 0.960 | | | [57] |
| DANCR | Plasma | 52 HCC vs 22 LC + 29 CHB + 43 HC | Chinese | qRT-PCR | 0.868 | 83.8 | 72.7 | [45] |
| DANCR | Plasma | 52 HCC vs 22 LC + 29 CHB | Chinese | qRT-PCR | 0.864 | 80.8 | 84.3 | [45] |
| DGCR5 | Serum | 60 HCC vs HC | Chinese | qRT-PCR | 0.782 | 63.3 | 83.3 | [58] |
| LncDQ | Serum | 50 HCC vs 30 HC | Chinese | qRT-PCR | 0.804 | 72.0 | 80.0 | [47] |
| lnc-GPR89B-15 | Serum exosome | 45 HCC vs 45 HC | Chinese | qRT-PCR | 0.717 | | | [59] |
| HULC | Plasma | 66 HCC vs 53 HC | Chinese | qRT-PCR | 0.780 | | | [53] |
| JPX | Plasma | 42 HCC vs 68 HC | Chinese | qRT-PCR | 0.814 | | | [50] |
| JPX + AFP | Plasma | 42 HCC vs 68 HC | Chinese | qRT-PCR | 0.905 | 97.1 | 72.2 | [50] |
| MALAT1 | Plasma | 88 HCC vs 28 CH | Japanese | qRT-PCR | 0.660 | 51.1 | 89.3 | [60] |
| MALAT1 + AFP + PIVKA II | Plasma | 88 HCC vs 28 CH | Japanese | qRT-PCR | | 88.6 | 75.0 | [60] |
| PVT1 + uc002mbe.2 | Serum | 40 HCC vs 33 HC | Chinese | qRT-PCR | 0.764 | 60.6 | 90.6 | [61] |
| SPRY4-IT1 | Plasma | 60 HCC vs HC | Chinese | qRT-PCR | 0.702 | 87.3 | 50.0 | [48] |
| SPRY4-IT1 + AFP | Plasma | 60 HCC vs HC | Chinese | qRT-PCR | 0.800 | 87.3 | 65.0 | [48] |
| SPRY4-IT1 | Plasma | 60 HCC vs 85 CHB/LC | Chinese | qRT-PCR | 0.611 | 43.5 | 86.7 | [48] |

(*Continued*)

**Table 2** (Continued).

| lncRNAs | Source | Cohort Size | Ethnic Population | Detection Method of ncRNAs | AUC | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|---|---|
| SPRY4-IT1 | Plasma | 60 preoperative vs postoperative | Chinese | qRT-PCR | 0.624 | 83.3 | 49.0 | [48] |
| UBE2CP3 | Serum | 80 HCC vs 75 HC | Chinese | Microarray and qRT-PCR | 0.839 | | | [49] |
| UBE2CP3 + AFP | Serum | 80 HCC vs 75 HC | Chinese | Microarray and qRT-PCR | 0.933 | | | [49] |
| uc001ncr + AX800134 | Serum | 121 HCC vs 95 CHB + 137 HC | Chinese | Microarray and qRT-PCR | 0.949 | 95.0 | 88.1 | [42] |
| uc001ncr + AX800134 | Serum | 61 HCC vs 60 CHB + 60 HC | Chinese | Microarray and qRT-PCR | 0.949 | 78.7 | 90.9 | [42] |
| uc003wbd | Serum | 137 HCC vs 104 CHB | Chinese | qRT-PCR | 0.700 | | | [47] |
| uc003wbd | Serum | 137 HCC vs 138 HC | Chinese | qRT-PCR | 0.860 | | | [47] |
| UCA1 | Serum | 82 HCC vs 34 CHC + 44 HC | Egyptian | qRT-PCR | 0.861 | 92.7 | 82.1 | [41] |
| WRAP53 | Serum | 82 HCC vs 34 CHC + 44 HC | Egyptian | qRT-PCR | 0.896 | 85.4 | 82.1 | [41] |
| UCA1 + WRAP53 | Serum | 82 HCC vs 34 CHC + 44 HC | Egyptian | qRT-PCR | | 95.1 | 82.1 | [41] |
| UCA1 + WRAP53 + AFP | Serum | 82 HCC vs 34 CHC + 44 HC | Egyptian | qRT-PCR | 0.727 | 100.0 | 62.8 | [41] |
| lnc-ZEB2-19 | Serum exosome | 45 HCC vs 45 HC | Chinese | qRT-PCR | 0.852 | | | [49] |

**Abbreviations**: AFP, alpha-fetoprotein; AUC, area under the curve; CH, chronic hepatitis; CHB, chronic hepatitis B infection; CHC, chronic hepatitis C infection; HC, healthy control; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

## CircRNA Biomarkers

As a novel class of endogenous ncRNAs, circRNAs form covalently closed continuous loops without 3' end poly (A) tails and 5' end caps.[62] Owing to the outstanding characteristics including high stability, abundant expression, tissue-specific expression pattern, and wide distribution in various body fluids, circRNAs have great potential as suitable biomarkers for disease diagnosis.[63] Recently, several reports have shown the potential of circulating circRNAs as a clinical biomarker in HCC diagnosis (Table 3). It has been reported that the AUC for circ_104075 was 0.973 with a sensitivity of 96.0% and a specificity of 98.3% in distinguishing patients with HCC from patients with healthy controls.[64] Moreover, plasma circSMARCA5 showed a high accuracy (AUC = 0.938, 0.853, 0.711) for diagnosing HCC from healthy controls, hepatitis and cirrhosis.[65] It is worth noting that plasma circSMARCA5 presented a high accuracy (AUC = 0.847, 0.706) for detecting HCC with serum AFP below 200 ng/mL from those hepatitis and cirrhosis with AFP below 200 ng/mL.[65] These results suggested that circSMARCA5 may serve as a potential diagnostic biomarker for HCC, especially in HCC patients with AFP blow the cutoff value.

Exosomes, a specific subtype of extracellular vesicles of 30–150 nm, secreted by almost all types of cells and widely distributed in body fluids, including blood.[66] As an important carrier of cell-to-cell signal transmission, exosomes carry a variety of important signal molecules such as proteins, nucleic acids and lipids, and are closely related to the development and occurrence of many disease processes, especially in cancer.[67] Recent studies have indicated that circRNAs are enriched and stable in exosomes and exosomal circRNAs show great potential as biomarkers in human diseases.[68] ROC curve analysis revealed that serum exosomal circWHSC1,[69] circ_0072088,[70] and circANTXR1[71] exhibited a potential diagnostic value for discriminating patients with HCC from healthy controls, respectively.

**Table 3** Circulating circRNAs Serve as Potential Diagnostic Biomarkers for HCC

| circRNAs | Source | Cohort Size | Ethnic Population | Detection Method of ncRNAs | AUC | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|---|---|
| circ_0004277 | Plasma exosome | 60 HCC vs 60 HC | Chinese | qRT-PCR | 0.816 | 58.3 | 96.7 | [76] |
| circ_0028861 | Serum exosome | 56 HCC vs 57 HBV | Chinese | qRT-PCR | 0.83 | 76.79 | 78.95 | [72] |
| circ_0028861 | Serum exosome | HCC vs CIRR | Chinese | qRT-PCR | 0.75 | 67.86 | 76.60 | [72] |
| circ_0028861 | Serum exosome | HCC vs HBV + CIRR | Chinese | qRT-PCR | 0.79 | 67.86 | 82.69 | [72] |
| circ_0051443 | Plasma exosome | 60 HCC vs 60 HC | Chinese | qRT-PCR | 0.809 | | | [77] |
| circ_0070396 | Plasma exosome | 111 HCC vs 54 HC | Chinese | qRT-PCR | 0.857 | 62.16 | 98.15 | [73] |
| circ_0070396 | Plasma exosome | 111 HCC vs 50 CHB | Chinese | qRT-PCR | 0.774 | 76.58 | 68 | [73] |
| circ_0070396 | Plasma exosome | 111 HCC vs 58 cirrhosis | Chinese | qRT-PCR | 0.661 | 46.85 | 81.03 | [73] |
| circ_0072088 | Serum exosome | 50 HCC vs 50 HC | Chinese | qRT-PCR | 0.899 | | | [70] |
| circ_104075 | Serum | 101 HCC vs 60 HC | Chinese | qRT-PCR | 0.973 | 96.0 | 98.3 | [64] |
| circANTXR1 | Serum exosome | 70 HCC vs 50 HC | Chinese | qRT-PCR | 0.760 | | | [71] |
| circSMARCA5 | Plasma | 135 HCC vs HC | Chinese | qRT-PCR | 0.938 | 86.7 | 89.3 | [65] |
| circSMARCA5 | Plasma | 135 HCC vs 117 CH | Chinese | qRT-PCR | 0.853 | 74.8 | 88.9 | [65] |
| circSMARCA5 | Plasma | 135 HCC vs 143 LC | Chinese | qRT-PCR | 0.711 | 77.0 | 63.6 | [65] |
| circSMARCA5 + AFP | Plasma | 135 HCC vs HC | Chinese | qRT-PCR | 0.992 | 100.0 | 100.0 | [65] |
| circSMARCA5 + AFP | Plasma | 135 HCC vs 117 CH | Chinese | qRT-PCR | 0.903 | 77.8 | 89.7 | [65] |
| circSMARCA5 + AFP | Plasma | 135 HCC vs 143 LC | Chinese | qRT-PCR | 0.858 | 71.9 | 83.2 | [65] |
| circPTGR1 | Serum | 82 HCC vs 47 HC | Chinese | qRT-PCR | | | | [78] |
| circWHSC1 | Serum exosome | 50 HCC vs 35 HC | Chinese | qRT-PCR | 0.869 | | | [69] |
| circ_0000976 + circ_0007750 + circ_0139897 | Plasma | 158 HCC vs 50 LC + 52 CHB + 53 HC | Chinese | qRT-PCR | 0.863 | 81.6 | 91.0 | [74] |

(*Continued*)

**Table 3** (Continued).

| circRNAs | Source | Cohort Size | Ethnic Population | Detection Method of ncRNAs | AUC | Sensitivity (%) | Specificity (%) | Reference |
|---|---|---|---|---|---|---|---|---|
| circ_0000976 + circ_0007750 + circ_0139897 | Plasma | 152 HCC vs 50 LC + 54 CHB + 50 HC | Chinese | qRT-PCR | 0.843 | 87.5 | 81.2 | [74] |
| circ_0000976 + circ_0007750 + circ_0139897 | Plasma | 290 HCC vs 80 LC + 82 CHB + 76 HC | Chinese | qRT-PCR | 0.864 | 85.5 | 87.3 | [74] |
| circ_0000976 + circ_0007750 + circ_0139897 + AFP | Plasma | 158 HCC vs 50 LC + 52 CHB + 53 HC | Chinese | qRT-PCR | 0.878 | 91.8 | 83.9 | [74] |
| circ_0000976 + circ_0007750 + circ_0139897 + AFP | Plasma | 152 HCC vs 50 LC + 54 CHB + 50 HC | Chinese | qRT-PCR | 0.863 | 92.8 | 79.9 | [74] |
| circ_0000976 + circ_0007750 + circ_0139897 + AFP | Plasma | 290 HCC vs 80 LC + 82 CHB + 76 HC | Chinese | qRT-PCR | 0.874 | 91.7 | 83.1 | [74] |
| circ_0004001 | Serum exosome | 21 HCC vs 32 HC | Chinese | qRT-PCR | 0.79 | 76.19 | 81.25 | [75] |
| circ_0004123 | Serum exosome | 21 HCC vs 32 HC | Chinese | qRT-PCR | 0.73 | 66.67 | 84.38 | [75] |
| circ_0075792 | Serum exosome | 21 HCC vs 32 HC | Chinese | qRT-PCR | 0.76 | 80.95 | 68.75 | [75] |
| circ_0004001+ circ_0004123+ circ_0075792 | Serum exosome | 21 HCC vs 32 HC | Chinese | qRT-PCR | 0.89 | 90.5 | 78.1 | [75] |

**Abbreviations**: AFP, alpha-fetoprotein; AUC, area under the curve; CH, chronic hepatitis; CHB, chronic hepatitis B infection; CIRR, cirrhosis; HBV, hepatitis B virus; HC, healthy control; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

Moreover, several studies have focused on distinguishing patients with HCC from patients with benign liver diseases by exosomal circRNAs. It has been demonstrated that the AUC for serum exosomal circ_0028861 was 0.79 for discriminating HCC from chronic HBV and cirrhosis individuals.[72] Similarly, plasma exosomal circ_0070396 could discriminate HCC individuals from patients with chronic hepatitis B and liver cirrhosis.[73]

The ability of a combined panel of circRNAs to discriminate HCC patients has also been evaluated. It has been identified that a plasma circRNA panel (circPanel) containing three circRNAs (circ_0000976, circ_0007750 and circ_0139897) could detect HCC patients in three sets from three hospitals in China.[74] Specifically, circPanel showed a higher accuracy than AFP to distinguish individuals with HCC from controls with AUCs of 0.843–0.864.[74] Notably, circPanel also performed well in detecting small-HCC (solitary, ≤3 cm), AFP-negative HCC and AFP-negative Small-HCC.[74] Another study has investigated the differentially expressed circRNAs in human blood exosomes from patients with HCC and investigated their diagnostic value.[75] The results showed that the AUCs of serum exosomal circ_0004001, circ_0004123, and circ_0075792 were 0.79, 0.73, and 0.76 with adequate sensitivity and specificity in discriminating HCC patients from the healthy controls.[75] Combination of these circRNAs considerably improved the AUC value (0.89), sensitivity and specificity, suggesting that the three circRNA signatures could be utilized as a diagnostic biomarker in HCC.[75]

## Future Directions

1. Although aberrant expression profiles of circulating ncRNAs in patients with HCC have been well demonstrated in numerous studies, the sample size is relative small and further confirmation with large-sample, multicentre, prospective clinical trial is needed in the future.
2. Current studies on the abnormal expressions of circulating ncRNAs in patients with HCC are all cross-sectional, the dynamic change of circulating ncRNAs levels during the progression of the disease has not been reported.
3. Although accumulating evidence has detailed the close relationship between HCC and ncRNAs, little is known about the causal relationship between them.
4. There are no universally accepted methods and assays for measuring the levels of circulating ncRNAs, which might result in the lack of consistency in various studies. Therefore, the methodology of the detection of circulating ncRNAs levels needs to be standardized for future clinical use.

## Conclusions

With the development of high-throughput technologies, a large number of biologically functional ncRNAs have now been identified. Accumulating evidence has indicated that ncRNAs can be used as novel biomarkers for the diagnosis and screening of diseases due to the molecular characteristics. Although the above described circulating ncRNAs have adequate sensitivity and specificity in discriminating patients with HCC, it is necessary to cross-check and validate these markers with various cohorts of patients representing high variabilities of HCCs, including patients with different etiologies, HCC cellular profiles, and stages of cancer.

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## Disclosure

The author declares that there are no conflicts of interest in this work.

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