Chapter 5

# The Diagnostic and Prognostic Potential of MicroRNAs for Hepatocellular Carcinoma

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**Abstract**

Hepatocellular carcinoma (also termed hepatocarcinoma) is the third cancer-related cause of death worldwide. To our knowledge, markers such as $\alpha$-fetoprotein display poor performance in the early diagnosis and prognosis prediction of hepatocarcinoma. MicroRNAs are an evolutionarily conserved class of small noncoding single-stranded RNA typically consisting of 18–24 nucleotides. They have been reported to act as tumor suppressors or oncogenes via reversely regulating gene expression. Recent evidence has revealed that microRNAs, especially in body fluids such as the blood and urine, display important diagnostic and prognostic potential for hepatocarcinoma. Here, we reviewed currently available data on microRNAs and hepatocarcinoma, with emphasis on the biogenesis and function of microRNAs and their potential diagnostic and prognostic value for hepatocarcinoma. We also discussed the clinical utility perspectives of microRNAs in hepatocarcinoma and possible challenges.

**Keywords:** hepatocarcinoma, microRNA, diagnosis, prognosis

## 1. Introduction

Hepatocarcinoma, also termed as hepatocellular carcinoma (HCC) or liver carcinoma, is the most common primary liver malignant disease in adults [1]. One of the most striking features of this malignant tumor is the wide variation in its incidence in different parts of the world. In areas of high incidence, such as China, hepatocarcinoma is among the leading cause of cancer-correlated deaths in recent years, with an annual incidence of approximately 40 per 100,000 [2, 3]. However, the countries in the low incidence, such as the USA, have only 2.3% of cancer-related deaths in

past decades. Globally, hepatocarcinoma is the fifth most common cancer among males and the eighth most common among females [1]. Furthermore, the incidence of this tumor generally increases with age, although there are geographic and gender differences. The precise reasons for this difference is not known, but growing evidence has exhibited that multiple factors including chronic viral hepatitis B (HBV) and C (HCV), aflatoxin (such as aflatoxin B1) exposure, hepatic cirrhosis, obesity, diabetes, and vitamin D deficiency play an important role [4–6]. Although the molecular mechanism of hepatocarcinoma has been unclear, these hepatocarcinoma patients with early diagnosis often have good prognosis with more than 50% of five overall survival rate [6]. This is mainly because they benefit from the curative treatment such as curative resection and orthotropic liver transplantation [6]. However, if patients are lately diagnosed, the cumulative 5-year survival rate remarkably reduces to less than 10%, and tumor recurrence risk noticeably increases (about 70–80% of 5-year recurrence rate). Thus, it is very urgent to identify specific and sensitive markers for early diagnosing hepatocarcinoma at a curative stage, monitoring recurrence of tumor, and predicting prognosis of tumor [6].

Currently, the early diagnosis of hepatocarcinoma is based on the following two classes of methods: imaging examination which mainly consists of ultrasonography, magnetic resonance imaging, and computed tomography and serological tests such as serous $\alpha$-fetoprotein (AFP) [4, 7]. Although advances in imaging technologies have significantly improved the early screening of hepatocarcinoma, these methods are so costly and unsatisfactory in early diagnosis that is not suitable for daily clinical practice [4, 7]. About serological methods, AFP is the most widely utilized marker for the diagnosis and prognosis prediction of hepatocarcinoma. However, this biomarker is limited because of its modest accuracy (with sensitivity of 40–65% and specificity of 87–96%) and about 30–40% of the false-negative rate for patients with early-stage hepatocarcinoma [8]. Additionally, serum AFP levels of some benign hepatic lesions, such as liver nodular hyperplasia, inflammation lesions of liver, and liver fibrotic cirrhosis, may give false-positive results [8]. Therefore, the reliability of this biomarker to determine hepatocarcinoma is inadequate because of its low sensitivity and specificity.

Emerging evidence has exhibited a correlation between dysregulation of microRNAs and development of hepatocarcinoma. Particularly, microRNAs are characterized by high stability in body fluids (including the blood and urine) and tissue specific in expression patterns, indicative of microRNAs in body fluids acting as potentially novel and ideal biomarkers for hepatocarcinoma diagnosis and prognosis prediction [8–16].

This review attempts to briefly review currently available data on microRNAs and hepatocarcinoma, with emphasis on (1) the biogenesis and function of microRNA, (2) potential diagnostic and prognostic value for hepatocarcinoma, and (3) the different value for hepatocarcinoma induced by different causes. Additionally, we summarized the clinical applicative perspectives and potential challenges of microRNAs in hepatocarcinoma.

## 2. MicroRNA biogenesis and function

Previous several reports have thoroughly reviewed biogenesis and function of microRNAs [8, 11, 17–29]. In brief, microRNAs are an evolutionarily conserved class of small noncoding

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single-stranded RNA typically consisting of 18–24 nucleotides. Originally, they are first transcribed by the RNA polymerase enzyme II into a kind of primary production named as primary microRNA that is characterized by long nucleotide sequences, 5′-cap structure, and 3′-poly-A tail, resembling protein-coding mRNAs. Then, primary microRNAs form a hairpin-shaped stem-loop structure and are processed into microRNA precursors (usually containing 60–70 nucleotides) by the microprocessor complex (consisting of DGCR8/Pasha and Drosha). After that, their precursors are transported to the cytoplasm and treated into a short double-strand duplex structure by another RNase endonuclease III (also called Dicer). Finally, the duplex structure (also called microRNA-microRNA*) is unwound into mature microRNAs by helicases. To date, it has been identified that there are more than 1800 microRNAs in the mammalian genome (miRDatabase) (**Figure 1**) [30]. Functionally, microRNAs are involved in regulating the expression of their

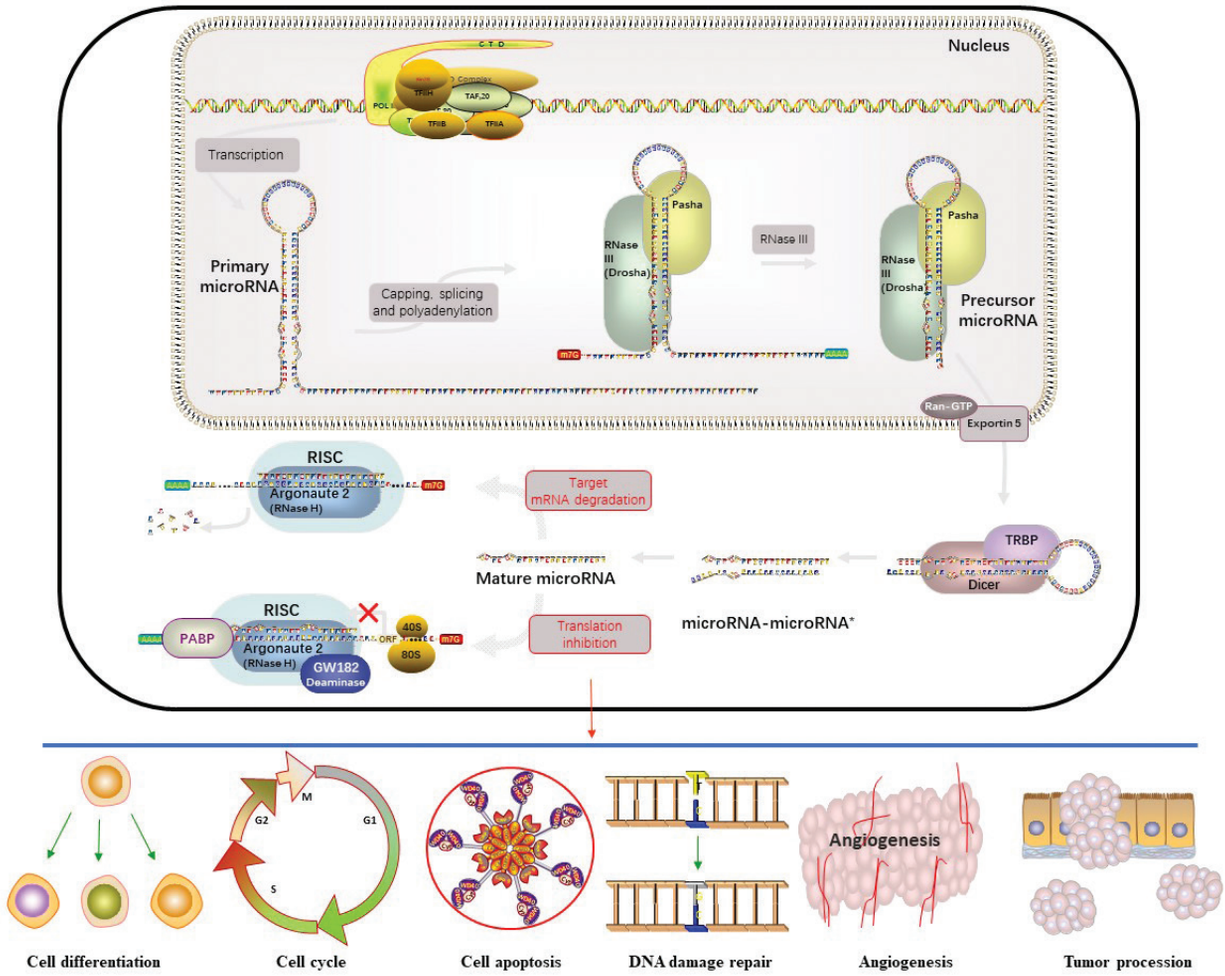


**Figure 1.** Biosynthesis and functions of microRNA. In the nucleus, the microRNA genes are transcribed into primary microRNAs by RNA polymerase II (Pol II). The primary microRNAs are then cleaved by Drosha and DGCR8 and produce their precursor molecules (also named as precursor microRNA). After that, the precursor molecules are transported to the cytoplasm by Exportin-5 and Ran-GTP and undergo final processing step including the cleavage by Dicer and the formation of stem-loop duplex molecule structure which contains the single-stranded mature microRNA molecule and a microRNA* fragment. Finally, the duplex molecule structure is incorporated into the RNA-induced silencing complex (RISC), the microRNA* fragments are degraded, and mature microRNA molecules are formed. The mature microRNAs can display genic regulation role via recognizing and binding to the 3′-untranslated region of their target genes' mRNAs. *Note*: This figure is plotted according to ScienceSlides (version#2016).

targeting genes via recognizing and integrating into the 3′-untranslated region of these genes' mRNAs. On the basis of perfect or imperfect base-base complementarity of microRNAs-their targeting mRNA binding, one microRNA specifically regulates the expression of multiple mRNAs, and at the same time, one mRNA might be inhibited by multiple microRNAs. This indicates the specificity and diversity of microRNAs regulating gene expression. In the past decades, microRNAs are emerged as important players in a very wide range of physiological processes including cell differentiation, cell proliferation and apoptosis, cycle regulation, survival, detoxification, physiological timing, metabolism, angiogenesis, hormone secretion, and DNA damage repair (**Figure 1**). Furthermore, growing evidence has shown that microRNAs can also display a role in the etiology and pathogenesis of various cancers by targeting many oncogenes or tumor inhibitive genes (**Figure 1**) [24, 27, 29–32]. Recent several reports have exhibited that some microRNAs involve in the tumorigenesis and procession of hepatocarcinoma and may become new potential markers for hepatocarcinoma diagnosis and prognosis [24, 27, 29, 31, 32].

## 3. MicroRNAs as novel biomarkers for hepatocarcinoma diagnosis

### 3.1. Diagnostic potential of single microRNA for hepatocarcinoma

With increasing incidence and death rate of hepatocarcinoma, it is very expected to identify one or several diagnostic biomarkers (with both high sensitivity and specificity) such as microRNAs for this malignancy. Growing evidence has shown that the expression change of all microRNAs in the peripheral blood may have a unique advantage because they exhibit tissue specificity and relative stability and can also provide some specific cues for early and small hepatocarcinoma [8–14]. Until now, more than 30 circulating microRNAs have been identified to have diagnostic potential for hepatocarcinoma (**Table 1**). For example, microRNA-122 has been reported as a hepatic-specific microRNA, accounting for 70% of the total microRNAs in hepatic tissues. This microRNA, a high conservative microRNA between vertebrate species, is indicative of a regulator of fatty acid metabolism and playing a critical role in liver homeostasis and tumorigenesis [19, 33, 34]. Increasing evidence has shown that elevated serum amount of microRNA-122 is positively associated with the severity of hepatic diseases including hepatitis, fatty- and alcohol-related liver damage, and drug-induced hepatotoxicity [35–39]. Interestingly, this increasing serum expression of microRNA-122 is noticeable and indicated that it could serve as a potential biomarker for the detection of patients with hepatocarcinoma from healthy controls with about 85% of the area under the receiver operating characteristic curve (AUC), 80% of sensitivity, and 80% of specificity [40, 41]. These results indicate that the dysregulated miR-122 in the peripheral blood may be used as a potential marker for hepatocarcinoma diagnosis. Results from retrospective studies have suggested that the microRNA-200 family (consisting of microRNA-200a and microRNA -200b) is also a promising biochemical biomarker for hepatocarcinoma diagnosis because of its deregulation during the development of both hepatic fibrosis and hepatocarcinoma [42, 43]. The elevated plasma levels of microRNA-21 can distinguish patients with hepatocarcinoma from cases with chronic hepatitis (with 61.1% of sensitivity and 83.3% of specificity) or healthy controls (the corresponding sensitivity and specificity are 87.3 and 92.0%, respectively) [44]. This suggests that this biomarker may have higher diagnostic potential than AFP. Some

| MicroRNAs | Source | Diagnostic relevance | Expression level | AUC (95% CI) | Sen (%) | Spe (%) | Refs |
|---|---|---|---|---|---|---|---|
| miR-12 | Serum | HCCs (n = 101) vs. HCs (n = 89) | Upregulated | 0.87 (0.81–0.93) | 84.0 | 75.3 | [41] |
| miR-122 | Serum | HCCs (n = 101) vs. HCs (n = 89) | Upregulated | 0.79 (0.71–0.86) | 70.7 | 69.1 | [41] |
| miR-223 | Serum | HCCs (n = 101) vs. CHCs (n = 89) | Upregulated | 0.86 (0.80–0.92) | 80.0 | 76.5 | [41] |
| miR-12 | Serum | HCCs (n = 101) vs. HCs (n = 48) | Upregulated | 0.91 (0.84–0.97) | 80.0 | 95.6 | [41] |
| miR-122 | Serum | HCCs (n = 101) vs. HCs (n = 48) | Upregulated | 0.93 (0.88–0.98) | 80.0 | 91.2 | [41] |
| miR-223 | Serum | HCCs (n = 101) vs. HCs (n = 48) | Upregulated | 0.88 (0.81–0.94) | 80.0 | 75.0 | [41] |
| miR-122 | Serum | HCCs (n = 70) vs. HCs (n = 34) | Upregulated | 0.87 (0.79–0.95) | 81.6 | 83.3 | [40] |
| miR-122 | Serum | HCCs (n = 70) vs. CHCs (n = 45) | Upregulated | 0.63 (0.52–0.74) | 77.6 | 57.8 | [40] |
| miR-21 | Plasma | HCCs (n = 126) vs. HCs (n = 50) | Upregulated | 0.77 | 61.1 | 83.3 | [44] |
| miR-21 | Plasma | HCCs (n = 126) vs. CHCs (n = 30) | Upregulated | 0.95 | 87.3 | 92.0 | [44] |
| miR-143 | Serum | HCCs (n = 95) vs. CTLs (n = 245) | Upregulated | 0.80 (0.68–0.92) | 73.0 | 83.0 | [46] |
| miR-215 | Serum | HCCs (n = 95) vs. CTLs (n = 245) | Upregulated | 0.82 (0.72–0.97) | 80.0 | 91.0 | [46] |
| miR-10b | Serum | HCCs (n = 27) vs. HCs (n = 50) | Upregulated | 0.85 (0.76–0.94) | / | / | [51] |
| miR-10b | Serum | HCCs (n = 27) vs. CLDs (n = 31) | Upregulated | 0.73 (0.60–0.86) | / | / | [51] |
| miR-106b | Serum | HCCs (n = 27) vs. HCs (n = 50) | Upregulated | 0.82 (0.72–0.91) | / | / | [51] |
| miR-106b | Serum | HCCs (n = 27) vs. CLDs (n = 31) | Upregulated | 0.71 (0.57–0.84) | / | / | [51] |
| miR-181a | Serum | HCCs (n = 27) vs. HCs (n = 50) | Upregulated | 0.89 (0.81–0.97) | / | / | [51] |
| miR-181a | Serum | HCCs (n = 27) vs. CLDs (n = 31) | Upregulated | 0.81 (0.70–0.92) | / | / | [51] |
| miR-206 | Serum | HCCs (n = 261) vs. HCs (n = 173) | Upregulated | 0.62 (0.55–0.68) | 48.1 | 78.8 | [52] |
| miR-143-3p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Upregulated | 0.76 (0.70–0.80) | 68.1 | 83.3 | [52] |
| miR-433-3p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Upregulated | 0.74 (0.67–0.80) | 79.3 | 64.4 | [52] |
| miR-1228-5p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Upregulated | 0.55 (0.44–0.60) | 79.3 | 27.8 | [52] |
| miR-199a-5p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Downregulated | 0.64 (0.57–0.71) | 59.3 | 66.7 | [52] |

| MicroRNAs | Source | Diagnostic relevance | Expression level | AUC (95% CI) | Sen (%) | Spe (%) | Refs |
|---|---|---|---|---|---|---|---|
| miR-122-5p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Downregulated | 0.70 (0.63–0.77) | 48.9 | 82.2 | [52] |
| miR-192-5p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Downregulated | 0.70 (0.62–0.77) | 71.9 | 75.6 | [52] |
| miR-26a-5p | Serum | HCCs (n = 261) vs. HCs (n = 173) | Downregulated | 0.76 (0.70–0.82) | 68.9 | 74.4 | [52] |
| miR-206 | Serum | HCCs (n = 261) vs. CCs (n = 233) | Upregulated | 0.69 (0.62–0.77) | 77.8 | 68.9 | [52] |
| miR-143-3p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Upregulated | 0.66 (0.60–0.73) | 60.7 | 72.7 | [52] |
| miR-433-3p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Upregulated | 0.64 (0.58–0.71) | 56.4 | 67.4 | [52] |
| miR-1228-5p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Upregulated | 0.54 (0.47–0.61) | 66.7 | 47 | [52] |
| miR-199a-5p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Downregulated | 0.59 (0.52–0.66) | 59.3 | 57.6 | [52] |
| miR-122-5p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Downregulated | 0.75 (0.69–0.81) | 48.9 | 90.2 | [52] |
| miR-192-5p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Downregulated | 0.69 (0.62–0.75) | 54.8 | 83.3 | [52] |
| miR-26a-5p | Serum | HCCs (n = 261) vs. CCs (n = 233) | Downregulated | 0.74 (0.68–0.81) | 60.7 | 90.9 | [52] |
| miR-16 | Serum | HCCs (n = 105) vs. CTLs (n = 188) | Downregulated | / | 72.1 | 88.8 | [14] |
| miR-199 | Serum | HCCs (n = 105) vs. CTLs (n = 188) | Downregulated | / | 62.9 | 93.5 | [14] |
| miR-199a | Serum | HCCs (n = 105) vs. CTLs (n = 188) | Downregulated | / | 78.1 | 64.5 | [14] |
| miR-375 | Serum | HCCs (n = 78) vs. HCs (n = 156) | Downregulated | 0.64 (0.56–0.74) | / | / | [53] |
| miR-199a-3p | Serum | HCCs (n = 78) vs. HCs (n = 156) | Downregulated | 0.88 (0.83–0.94 | / | / | [53] |
| miR-30c-5p | Plasma | HCCs (n = 8) vs. CTLs (n = 86) | Upregulated | / | / | / | [54] |
| miR-223-3p | Plasma | HCCs (n = 8) vs. CTLs (n = 86) | Downregulated | / | / | / | [54] |
| miR-202c-3p | Plasma | HCCs (n = 8) vs. CTLs (n = 86) | Upregulated | / | / | / | [54] |
| miR-17-57 | Plasma | HCCs (n = 8) vs. CTLs (n = 86) | Upregulated | / | / | / | [54] |
| miR-4651 | Serum | AHCCs (n = 279) vs. HCs (n = 338) | Upregulated | 0.89 (0.86–0.92) | 78.1 | 99.1 | [55] |
| miR-4651 | Serum | AHCCs (n = 279) vs. AHCs (n = 292) | Upregulated | 0.82 (0.78–0.85) | 78.1 | 85.3 | [55] |
| miR-4651 | Serum | AHCCs (n = 279) vs. ALCs (n = 32) | Upregulated | 0.80 (0.71–0.88) | 78.1 | 81.2 | [55] |

| MicroRNAs | Source | Diagnostic relevance | Expression level | AUC (95% CI) | Sen (%) | Spe (%) | Refs |
|---|---|---|---|---|---|---|---|
| miR-4651 | Serum | AHCCs (n = 279) vs. CTLs (n = 662) | Upregulated | 0.85 (0.82–0.88) | 78.1 | 92.1 | [55] |
| miR-143 | Serum | HCCs (n = 131) vs. HCs (n = 122) | Downregulated | 0.83 | 80.3 | 82.4 | [56] |
| miR-125b | Plasma | HCCs (n = 64) vs. HCs (n = 56) | Downregulated | 0.89 | 90.0 | 80.0 | [57] |
| miR-125b | Plasma | HCCs (n = 64) vs. CHBs (n = 63) | Downregulated | 0.96 | 90.0 | 90.0 | [57] |
| miR-125b | Plasma | HCCs (n = 64) vs. CCs (n = 59) | Downregulated | 0.96 | 90.0 | 90.0 | [57] |
| miR-150 | Serum | HCCs (n = 120) vs. CHBs (n = 110) | Downregulated | 0.88 (0.84–0.93) | 79.1 | 76.5 | [58] |
| miR-150 | Serum | HCCs (n = 120) vs. HCs (n = 120) | Downregulated | 0.93 (0.90–0.96) | 82.5 | 83.7 | [58] |
| miR-106b | Plasma | HCCs (n = 47) vs. CTLs (n = 61) | Upregulated | 0.81 | 0.7 | 0.8 | [59] |
| miR-200a | Serum | HCCs (n = 22) vs. HCs (n = 15) | Downregulated | 0.82 (0.69–0.97) | / | / | [60] |
| miR-200a | Serum | HCCs (n = 22) vs. CCs (n = 22) | Downregulated | 0.73 (0.56–0.89) | / | / | [60] |
| miR-143 | Serum | HCCs (n = 95) vs. CHCs (n = 118) | Upregulated | 0.62 (0.51–0.76) | 78.0 | 64.0 | [46] |
| miR-215 | Serum | HCCs (n = 95) vs. CHCs (n = 118) | Upregulated | 0.80 (0.67–0.95) | 78.0 | 89.0 | [46] |
| miR-143 | Serum | HCCs (n = 95) vs. HCs (n = 127) | Upregulated | 0.80 (0.68–0.92) | 78.0 | 89.0 | [46] |
| miR-215 | Serum | HCCs (n = 95) vs. HCs (n = 127) | Upregulated | 0.82 (0.72–0.97) | 80.0 | 91.0 | [46] |
| miR-101 | Serum | HCCs (n = 67) vs. HCs (n = 30) | Downregulated | 0.79 (0.69–0.87) | 76.1 | 70.0 | [61] |
| miR-483-5p | Serum | HCCs (n = 49) vs. HCs (n = 49) | Upregulated | 0.91 | 75.5 | 89.8 | [62] |
| miR-122a | Plasma | HCCs (n = 85) vs. HCs (n = 85) | Downregulated | 0.71 | 70.6 | 67.1 | [63] |
| miR-618 | Urine | HCCs (n = 32) vs. CTLs (n = 74) | Upregulated | 0.66 | 64.0 | 68.0 | [47] |
| miR-650 | Urine | HCCs (n = 32) vs. CTLs (n = 74) | Downregulated | 0.65 | 72.0 | 58.0 | [47] |
| miR-126 | tumor tissue | HCCs vs. CAs | Upregulated | / | / | / | [48] |

Abbreviation: miR, microRNA; HCCs, cases with hepatocarcinoma; CTLs, non-HCC controls (including healthy control and other nontumor controls); HCs, healthy controls; CCs, controls with liver cirrhosis; CHBs, patients with chronic hepatitis B; CLDs, cases with nontumor chronic liver diseases; Sen, sensitivity; Spe, specificity; AUC, the area under the receiver operating characteristic curve; CI, confidence interval; Refs, references.

**Table 1.** The microRNAs as diagnostic biomarkers for hepatocarcinoma.

other serum microRNAs, such as microRNA-15b, microRNA-130b, miR-143, and miR-215, are additional potential biomarkers that are significantly dysregulated in hepatocarcinoma [45, 46]. Noticeably, these biomarkers also exhibit their diagnostic potential for patients with early-stage hepatocarcinoma and/or negative-status AFP [45, 46].

Recently, some evidence has also exhibited that microRNAs in urine samples and liver tissues have screening potential for hepatocarcinoma (**Table 1**). Actually, the detection of five deregulated microRNAs, including microR-618, microRNA-625, microRNA-650, microRNA-532, and miR-516-5P, in the urine samples has already been used for screening patients with the early and small hepatocarcinoma from these with risk factors such as chronic virus hepatitis, liver cirrhosis, and dysplasia [47]. Barshack et al. [48] investigated differential diagnosis potential of microRNAs for discriminating hepatocarcinoma from metastatic tumors in the liver using custom microarray expression technique. In their study, they tested the distributed features of microRNAs among 144 tumor samples with or without metastatic adenocarcinoma and similar hepatocarcinoma in the morphology and immune types and found that microR-141 and microR-200c can promote non-hepatic epithelial phenotypes while microRNA-126 displays hepatic epithelial phenotypes. Higher expression of microRNA-126 is further shown in these tissue samples with hepatocarcinoma. Therefore, the change profiles of microRNAs in body fluids (such as urine) and tumor tissues may represent a kind of gold biomarkers for such cancers as liver carcinoma.

However, the specificity of a single microRNA identifying hepatocarcinoma is relatively poor. For example, the serum level of aforementioned liver-specific microRNA-122 is upregulated not only among cases with hepatocarcinoma but also among these with chronic virus hepatitis, liver cirrhosis, and fatty liver diseases caused by alcohol or non-alcohol [49, 50]. Evidence has shown that serum microRNA-122 does not discriminate patients with hepatocarcinoma from these with chronic hepatitis, although higher expression is observed among cancer cases [40, 41]. This indicates that more investigations on the basis of large size of samples and the prospective randomized controlled trials should help us for addressing these concerns.

### 3.2. Diagnostic potential of microRNA panel for hepatocarcinoma

Because hepatocarcinoma is a multifactor-induced highly complex malignant disease with heterogeneous feature, a combination of multiple microRNAs in place of a single microRNA may have higher accuracy for hepatocarcinoma discrimination. Several circulating microRNA panels have been reported to have higher early diagnostic value for hepatocarcinoma (**Table 2**) [47, 51, 52, 64–70]. For example, Lin et al. [70] preformed a three-stage study consisting of the discovery stage (including 6 cases with hepatocarcinoma and 8 cases with chronic hepatitis B), the training stage (including 108 cases with hepatocarcinoma, 51 cases with chronic hepatitis B, 47 cases with liver cirrhosis, and 51 healthy controls), and the validation stage (including 229 patients with hepatocarcinoma and 424 controls with or with nontumor liver diseases). In the first stage, they identified 31 different serum microRNAs between individuals with hepatocarcinoma and those with chronic hepatitis B using the TaqMan Array technique. Next, they validated these different microRNAs and constructed diagnostic panel containing miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505 on the basis of logistic regression model. Finally, the established serum microRNA panel was tested among

| MicroRNA panel | Source | AUC (95% CI) | Sen (%) | Spe (%) | Diagnostic relevance | Refs |
|---|---|---|---|---|---|---|
| miR-10b + miR-106b + miR-181a | Serum | 0.94 (0.89–0.99) | / | / | HCCs (n = 27) vs. HCs (n = 50) | [51] |
| miR-10b + miR-106b + miR-181a | Serum | 0.91 (0.80–0.97) | / | / | HCCs (n = 27) vs. CLDs (n = 31) | [51] |
| miR-206 + miR-143-3p + miR-433-3p + miR-1228-5p + miR-199a-5p + miR-122-5p + miR-192-5p + miR-26a-5p | Serum | 0.89 (0.85–0.94) | 82.8 | 83.3 | HCCs (n = 261) vs. HCs (n = 173) | [52] |
| miR-206 + miR-143-3p + miR-433-3p + miR-1228-5p + miR-199a-5p + miR-122-5p + miR-192-5p + miR-26a-5p | Serum | 0.89 (0.84–0.94) | 81.6 | 84.6 | HCCs (n = 261) vs. CCs (n = 233) | [52] |
| miR-122 + miR-192 + miR-21 + miR-223 + miR-26a + miR-27a + miR-801 | Plasma | 0.86 (0.83–0.90) | 68.6 | 90.1 | HCCs (n = 204) vs. CTLs (n = 303) | [64] |
| miR-122 + miR-192 + miR-21 + miR-223 + miR-26a + miR-27a + miR-801 | Plasma | 0.89 (0.85–0.92) | 81.8 | 83.5 | HCCs (n = 196) vs. CTLs (n = 194) | [64] |
| miR-27b-3p + miR-192-5p | Serum | 0.84 (0.78–0.89) | 0.7 | 0.9 | HCCs (n = 91) vs. CTLs (n = 91) | [65] |
| miR-92-3p + miR-107 + miR-3126-5p | Serum | 0.97 (0.95–0.99) | / | / | HCCs (n = 115) vs. HCs (n = 40) | [66] |
| 88-miRNA | Serum | 1.00 (0.97–1.00) | 100.0 | 99.2 | HCCs (n = 261) vs. CCs (n = 233) | [67] |
| miR214-5p + miR-125b + miR-1269 + miR-375 | Serum | 0.95 | 96.9 | 83.2 | HCCs (n = 224) vs. HCs (n = 84) | [68] |
| miR-122 + miR-885-5p + miR-29b | Serum | 1.00 | / | / | HCCs (n = 192) vs. HCs (n = 96) | [69] |
| miR-29a + miR-29c + miR-133a + miR-143 + miR-145 + miR-192 + miR-505 | Serum | 0.82 (0.77–0.87) | 74.5 | 89.9 | HCCs (n = 153) vs. CTLs (n = 199) | [70] |
| miR-29a + miR-29c + miR-133a + miR-143 + miR-145 + miR-192 + miR-505 | Serum | 0.88 (0.82–0.95) | 85.7 | 91.1 | HCCs (n = 49) vs. CTLs (n = 90) | [70] |
| miR-618 + miR-650 | Urine | 0.69 | 58.0 | 75.0 | HCCs (n = 32) vs. CTLs (n = 74) | [47] |

Abbreviation: miR, microRNA; HCCs, cases with hepatocarcinoma; CTLs, non-HCC controls (including healthy control and other nontumor controls); HCs, healthy controls; CCs, controls with liver cirrhosis; CHBs, patients with chronic hepatitis B; CLDs, cases with nontumor chronic liver diseases; Sen, sensitivity; Spe, specificity; AUC, the area under the receiver operating characteristic curve; CI, confidence interval; Refs, references.

**Table 2.** The microRNA panel as diagnostic biomarkers for hepatocarcinoma.

individuals from the training and validation cohorts. These data identified seven microRNAs and constructed a serum microRNA panel with an increasing diagnostic accuracy for hepatocarcinoma [AUC = 0.826 (0.771–0.880) for training set and 0.817 (0.769–0.865) and 0.884 (0.818–0.951) for two different validation sets, respectively]. Interestingly, a nest case-control study has further proved that this panel could be used to detect preclinical hepatocarcinoma as well as small-size, early-stage, and $\alpha$-fetoprotein-negative disease.

Similarly, Jiang et al. [59] and Zhou et al. [64] also attempted to identify possible combination of different microRNAs for increasing diagnostic accuracy of hepatocarcinoma on the basis of different controls with or without liver diseases. They found that the panel consisting of miR-10b, miR-106b, and miR-181a as well as the combination of miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801 can improve detection of hepatocarcinoma. These reports indicate that the panel of microRNAs may have better performance than a single-microRNA assay.

### 3.3. Diagnostic potential of microRNAs binding with AFP for hepatocarcinoma

AFP has been regarded as the most important marker for hepatocarcinoma screening and diagnosis, ever since it was identified in the peripheral blood samples from patients with hepatocarcinoma in 1964 [8, 71, 72]. However, this marker is relatively unsatisfactory because of its low sensitivity and specificity. This is mainly because only 60–80% of cases with hepatocarcinoma show positive AFP, whereas about 40% of cirrhotic patients also exhibit different degree increasing level of serum AFP [73, 74]. Thus, AFP may not be a reliable hepatocarcinoma marker, especially for early-stage and/or AFP-negative hepatocarcinoma. On the basis of low sensitivity and specificity of AFP for hepatocarcinoma diagnosis, the American Association for the Study of Liver Disease Practice Guidelines has thrown it away for prognostic surveillance and tumor diagnosis [75]. However, recent studies have displayed that the combination of AFP in the peripheral blood and microRNAs in body fluids may improve the sensitivity and specificity of hepatocarcinoma diagnosis and increase their diagnostic potential [14, 47, 55, 58, 65–67, 69, 70, 76].

For example, Wu et al. [55] investigated the joint diagnostic value of serum microRNA-4651 and AFP for hepatocarcinoma in 279 hepatocarcinoma patients, 324 controls with liver injury, and 338 healthy controls. Their results imply that serum microRNA-4651 has higher expression level among cases with hepatocarcinoma (AUC of 0.85; sensitivity of 78.1% and specificity of 92.1%); this increasing expression also displays higher diagnostic potential than AFP at cutoff of 20 ng/mL (AFP20) (AUC = 0.80, sensitivity = 61.3%, and specificity = 98.8%) and of 400 ng/mL (AFP400) (AUC = 0.72, sensitivity = 43.0%, and specificity = 100.0%). Noticeably, the combination of serum microRNA-4651 with AFP significantly improves the discrimination power between patients with hepatocarcinoma and with chronic nontumor liver injury (AUC = 0.90, sensitivity = 83.2%, and specificity = 97.1%). Similar findings have also been observed in the analyses of combination of serum AFP and other microRNAs, such as miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, miR-505, miR-16, miR-195, and miR-199a [14, 47, 58, 65–67, 69, 70, 76]. Altogether, these data suggest that the combination of microRNAs with AFP may improve diagnostic potential of hepatocarcinoma.

## 4. Prognostic potential of microRNA for hepatocarcinoma

In the past decades, growing evidence has exhibited that microRNAs can act as prognostic biomarkers for hepatocarcinoma [56, 77–101], and **Table 3** summarizes these significantly affecting hepatocarcinoma outcomes. Functionally, the microRNAs affect hepatocarcinoma prognosis

| MicroRNAs | Source | Expression level | Prognostic significance | HR (95%CI) | Refs |
|---|---|---|---|---|---|
| miR-122 | Serum | Upregulated | Increasing levels correlate with poor OS | OS, 0.08 (0.03–0.22) | [120] |
| miR-1 | Serum | Upregulated | Increasing levels correlate with poor OS | OS, 0.45 (0.23–0.86) | [121] |
| miR-122 | Serum | Upregulated | Correlated with clinical chemistry parameters of hepatic necroinflammation, liver function, and synthetic capacity | / | [121] |
| miR-221 | Serum | Upregulated | (1) Correlated with tumor size, cirrhosis, and tumor stage; (2) increasing levels decreased survival rate | / | [122] |
| miR-4651 | Serum | Upregulated | Increasing levels correlate with poor OS and RFS | OS, 2.67 (1.61–4.42)<br>RFS, 3.62 (1.49–8.81) | [55] |
| miR-1268a | Tumor tissues | Upregulated | Increasing levels correlate with poor OS and RFS | OS, 2.44 (1.82–3.23)<br>RFS, 2.86 (2.08–3.85) | [115] |
| miR-24 | Tumor tissues | Upregulated | Increasing levels correlate with poor OS and RFS | OS, 3.58 (2.34–5.46)<br>RFS, 4.75 (2.66–8.47) | [77] |
| miR-429 | Tumor tissues | Upregulated | Increasing levels correlate with poor OS and RFS | OS, 4.64 (2.56–8.41)<br>RFS, 6.94 (3.19–15.08) | [78] |
| miR-143 | Serum | Downregulated | Decreasing levels correlate with poor OS and RFS | / | [56] |
| miR-9 | Tumor tissues | Upregulated | Increasing levels correlate with poor OS and RFS | / | [123] |
| miR-92b | Tumor tissues, serum | Upregulated | Increasing levels promoting tumor metastasis | / | [102] |
| miR-150 | Serum | Downregulated | Increasing levels correlate with poor OS | 0.45 (0.23–0.85) | [58] |
| miR-21 | Serum | Upregulated | Increasing levels correlate with poor OS | 2.23 (1.33–3.74) | [76] |
| 20-miRNA signature | Tumor tissues | 10 downregulated and 20 upregulated miRNAs | 6 were risk factors and 14 were protective factors | OS, 2.75 (1.58–4.79) | [124] |
| miR-221 | Tumor tissues | Upregulated | Increasing expression promotes metastasis-free survival | / | [125] |

| MicroRNAs | Source | Expression level | Prognostic significance | HR (95%CI) | Refs |
|---|---|---|---|---|---|
| miR-96 | Tumor tissues | Upregulated | Increasing expression correlates with poor RFS | / | [126] |
| miR-92a | Tumor tissues | Downregulated | Decreasing expression correlates with poor RFS | RFS, 1.60 (1.00–2.50) | [79] |
| miR-22 | Tumor tissues | Downregulated | Decreasing expression correlates with poor RFS | / | [80] |
| miR-500 | Serum | Upregulated | Decreasing expression correlates with tumor resected | / | [127] |
| miR-375 | Tumor tissues | / | Decreasing expression correlates with poor RFS | RFS, 3.273 | [81] |
| miR-148b | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS | OS, 1.86 (1.23–2.98) | [82] |
| miR-101 | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS and RFS | RFS, 2.56 (1.32–5.69)<br>OS, 3.27 (1.18–6.92) | [83] |
| miR-19a | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS and RFS | / | [84] |
| miR-210 | Tumor tissues | Upregulated | Increasing expression correlates with poor OS | / | [80] |
| miR-224 | Tumor tissues | Upregulated | Increasing expression correlates with poor OS and RFS | / | [85] |
| miR-29 | Tumor tissues | Downregulated | Decreasing expression correlates with poor and RFS | / | [86] |
| miR-139-5p | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS | / | [87] |
| miR-1 | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS and RFS | OS, 2.79 | [88] |
| miR-199b-5p | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS | / | [89] |
| miR-130b | Tumor tissues | Upregulated | Increasing expression correlates with poor OS and RFS | RFS, 4.00 (1.58–7.90)<br>OS, 2.52 (1.02–7.90) | [90] |
| miR-9 | Tumor tissues | Upregulated | Increasing expression correlates with poor OS and RFS | / | [91] |
| miR-25 | Tumor tissues | Upregulated | Increasing expression correlates with poor OS and RFS | RFS, 1.62<br>OS, 2.18 | [92] |
| let-7 | Tumor tissues | Upregulated | Increasing expression correlates with poor OS | / | [93] |

| MicroRNAs | Source | Expression level | Prognostic significance | HR (95%CI) | Refs |
|---|---|---|---|---|---|
| miR-30a | Tumor tissues | Downregulated | Decreasing expression correlates with poor RFS | RFS, 3.2 (1.5–6.8) | [94] |
| miR-99a | Tumor tissues | Downregulated | Decreasing expression correlates with poor RFS | RFS, 1.60 (1.00–2.50) | [79] |
| miR-106b | Tumor tissues | Upregulated | Increasing expression correlates with poor OS | OS, 2.00 (1.13–6.98) | [95] |
| miR-130a | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS | OS, 2.22 (1.10–4.46) | [96] |
| miR-19b | Tumor tissues | / | Increasing expression correlates with good OS | OS, 0.45 (0.24–0.85) | [97] |
| miR-148a | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS | / | [98] |
| miR-372 | Tumor tissues | Upregulated | Increasing expression correlates with poor OS and RFS | RFS, 6.83<br>OS, 9.53 | [99] |
| miR-630 | Tumor tissues | Downregulated | Increasing expression correlates with good OS and RFS | OS, 0.71 (0.26–1.92)<br>RFS, 0.66 (0.33–1.35) | [100] |
| miR-100 | Tumor tissues | Downregulated | Decreasing expression correlates with poor OS | OS, 1.66 (1.32–2.82) | [101] |

Abbreviation: miR, microRNA; OS, overall survival; RFS, tumor recurrence-free survival; HR, hazard ratio; CI, confidence interval; Refs, references.

**Table 3.** The microRNAs as prognostic biomarkers for hepatocarcinoma.

via the following pathways: (1) promoting cancerous growth and proliferation [77, 78, 80, 83, 89, 98, 99, 102–113], (2) inhibiting cancerous apoptosis [77, 78, 86, 99, 101, 107–109, 111, 112, 114], (3) increasing microvessel density in the tumor tissues [77, 115], (4) affecting cell cycles [24, 25, 27, 28, 116–119], (5) increasing the risk of tumor metastasis [77, 115], and (6) decreasing the sensitivity of cancer cells to anticancer drugs [115]. For example, Lu et al. [115] investigated the prognostic potential of microRNA-1268a for hepatocarcinoma in 411 patients with hepatocarcinoma. Their results imply that microRNA-1268a expression in the cancerous tissues is significantly related to tumor features including tumor volume, stage and grade, and microvessel density. Results from multivariable factors analyses based on Cox regression models show that microRNA-1268a expression is independent of other known prognostic factors for hepatocarcinoma. Furthermore, transarterial chemoembolization (TACE) treatment can improve the prognosis of hepatocarcinoma patients with low microRNA-1268a expression, but not for those with high microRNA-1268e expression. These data imply that the dysregulation of microRNA-1268a can modify the response of cancer cells to antidrugs. Their following studies prove that upregulated microRNA-1268a inhibited while its downregulation enhanced doxorubicin

(an anticancer drug)-induced the death of tumor cells. Similarly, Liu et al. [77] and Huang et al. [78] investigated the roles of microRNAs, such as microRNA-24 and microRNA-429, in the tumorigenesis of liver cancer on the basis of analyses of hepatocarcinoma samples and genic toxicity induced by aflatoxin B1 and found the dysregulation of these microRNAs increased microvessel density and mutation frequency of TP53 gene possibly resulting from the loss of DNA repair capacity. Taken together, these reports indicate that microRNAs in body fluids and cancerous tissues may be important candidate biomarkers for hepatocarcinoma prognosis.

## 5. Further direction

In the past decades, the advance in pathological mechanisms of microRNAs regulating tumorigenesis and procession of hepatocarcinoma holds great promise for identifying whether microRNAs in body fluids (such as blood and urine) act as novel early diagnostic and prognostic biomarkers for this malignancy. However, we are still far from a comprehensive view of this kind of potentials. Although some hepato-specific microRNAs have been identified, microRNAs in body fluids may be from hemocytes and vascular endothelial cells and others from tissues and organs with high blood flow as well as hepatocarcinoma. This kind of heterogeneous origin indicates that the dysregulation of tumor-specific microRNA signatures may be concealed by microRNAs from other origins. Furthermore, well-standardized protocols of testing microRNAs have not been constructed or confirmed on the basis of the prospective, randomized controlled trials. Disclosing the different diagnostic and prognostic potential of microRNAs will greatly benefit our constructing high accurate diagnostic and prognostic models for hepatocarcinoma and will shed important light on the early diagnosis, tumor monitoring, and prognosis prediction for individuals with risk factors.

## 6. Summary

To conclude, the advances in technologies, including microarray PCR technology, high-throughput sequencing, and mass spectrometry, make it possible to identify new markers for hepatocarcinoma diagnosis and prognosis. On the whole, the microRNAs are a class of attractive markers and may replace known traditional serum markers such as AFP on the basis of the following reasons. First, because many circulating microRNAs is highly stable and readily detected in patients with hepatocarcinoma, they may have higher diagnostic potential (with high AUCs, sensitivity, and specificity) for hepatocarcinoma than AFP. Second, some microRNAs appear in the urine and can be utilized for screening patients with high-risk factors of hepatocarcinoma. Third, some dysregulated microRNAs in the body fluids can change with the different stages of hepatocarcinoma, indicative of their potential in monitoring tumor recurrence. Finally, different expressions of microRNAs are useful for treatment strategies such as TACE selection. Taken together, the dysregulated microRNAs in body fluids (including urine and blood) may be a kind of promised biomarkers for liver carcinoma diagnosis and prognosis because they are early detected and easily monitored.

However, there are several issues to be noted. First, research on the diagnostic and prognostic potential of microRNAs is still in the early stages, and challenges are noticeable in the clinical utilization of significant microRNAs. Second, in spite of these biomarkers that are discussed well, their therapeutic potential still remains unclear. Finally, although the diagnostic and prognostic potential of microRNAs is well evaluated on the basis of retrospective case-control studies, results from the prospective, randomized controlled trials are absent. Finally, because of the polygenic feature for hepatocarcinoma development, it is essential for a panel of biomarkers to determine high-risk individuals. Thus, the advances in the fields of microRNAs including their origins, stability, detection strategies, variant characteristics, and biofunctions in hepatocarcinoma will progress microRNAs in body fluids to become possible tools for hepatocarcinoma diagnosis and prognosis in the future.

## Conflicts of interest and source of funding

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## Abbreviations

| | |
|---|---|
| AFP | $\alpha$-fetoprotein |
| AFB1 | aflatoxin B1 |
| AUC | the area under the receiver operating characteristic curve |
| CT | computed tomography |
| HBV | hepatitis virus B |
| HCC | hepatocellular carcinoma |
| HCV | hepatitis virus C |
| MRI | magnetic resonance imaging |

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