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REVIEW ARTICLE

Identification of circulating MicroRNAs as novel potential biomarkers for hepatocellular carcinoma detection: a systematic review and meta-analysis

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Abstract
Background MicroRNAs (miRNAs) in body fluids such as serum and plasma can be stably detected and used as potential biomarkers in hepatocellular carcinoma (HCC) diagnosis.
Objective To systematically evaluate circulating miRNAs from HCC expression profiling studies and to determine miRNA biomarkers for HCC detection.
Methods A systematic review and meta-analysis of published studies were carried out for comparing the circulating miRNA expressions between HCC patients and healthy people, hepatitis, or cirrhosis patients. A miRNA ranking system that considered the number of comparisons in agreement and total number of samples was used. Then the summary receiver-operating characteristic curve (sROC) results of the top miRNAs were combined to further evaluate their diagnostic value using Meta-disc 1.4.
Results In the 17 included studies, three circulating miRNAs (miR-21, miR-122, and miR-223) were repeatedly reported three times or more in both HCC patients vs. healthy controls and vs. other hepatitis or cirrhosis patients. In further analysis, the area under curve (AUC) of sROC for miR-21, miR-122 and miR-223 in discriminating HCC patients from healthy people are 0.9293, 0.8128, and 0.8597, respectively.
Conclusions Circulating miR-21 has highest level of diagnostic efficiency among three miRNAs candidate biomarkers (miR-21, miR-122, and miR-223) for detection of HCC.

Keywords MicroRNA · Hepatocellular carcinoma · Systematic review · Meta-analysis

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignant disease and the third leading cause of cancer-related mortality worldwide [1]. Although diagnostic and therapeutic improvements, HCC incidence and mortality rates have obviously increased in recent years, especially in Asian countries [2, 3]. The rapid progression of HCC, to a large degree, leads to the poor prognosis of this disease, and the five-year survival rate lower than 5 %. Therefore, the key to reducing the mortality rate and improving the prognosis for HCC patients is an early and accurate diagnosis. Biomarker detection as a noninvasive, convenient, and low-price diagnostic method has been widely applied in clinics. Although several potential biomarkers, such as Golgi protein-73 (GP73), des-gamma-carboxy prothrombin (DCP) and the circulating AFP isoform AFP-L3, have been used for early detection of HCC in the clinic [4–6], the practical diagnostic value of these markers has yet to be fully evaluated. Therefore, it is necessary to investigate potential biomarkers that can diagnose HCC in a sensitive and specific way.

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MicroRNAs (miRNAs), a group of small noncoding RNA molecules that range in length from 18 to 25 nucleotides, have been proposed as promising biomarkers of early cancer detection, accurate prognosis and efficient therapeutic targets [7, 8]. They play important roles in the regulation of translation of many genes and the degradation of mRNAs by base pairing to partially complementary sites, predominately in the 3′ untranslated region (3′UTR) [9, 10]. The miRNAs that appear in cell-free body fluids such as serum and plasma are called circulating miRNAs, which are protected from degradation by ribonucleases in blood and thereby can be stably detected [11]. Circulating miRNAs were demonstrated first by Mitchell as potential biomarkers in cancer detection [12], and later their effects in diagnosis and prognosis of cancers were proved in numerous studies. Such as the deregulation of microRNAs has been revealed to greatly influence HCC development, invasiveness, prognosis, and treatment response [13]. However, the results on expressions of miRNAs are inconsistent, and it is hard to select a suitable miRNA as a cancer biomarker. Although abundant studies on circulating miRNAs, few relevant measures have been applied in clinics.

In this article, a vote-counting strategy proposed by Griffith and Chan [14, 15] was used to identify the circulating miRNAs with consistently reported expression, aiming to find suitable miRNAs from multiple independent studies as novel potential biomarkers for HCC detection. Then a diagnostic meta-analysis was conducted on the test data, and the screened miRNAs were evaluated for their diagnostic efficiency. Such an analytical method is independent of the difference between analytical methods, but aims to find miRNAs with consistently reported expressions. But further studies are needed to verify the application values of these miRNAs using high-throughput molecular technology [14].

Methods

Search strategy

A systematic search of PubMed and Embase was performed for studies published between January 2000 and April 2014 (cut-off date 23 April 2014) and relevant to HCC microRNA biomarkers in blood, urine or saliva. Literature search was initiated based on the search terms listed in Table 1.

Study selection

Studies were evaluated for their relevance to our present topic. A study was included if it met the following inclusion criteria: (1) studies on circulating miRNA expressions in HCC patients; (2) the sample types are serum, whole blood, plasma, urine, or saliva; (3) studies comparing circulating miRNA expressions between HCC patients and healthy people, hepatitis, or cirrhosis patients; (4) each group contains more than 10 patients. Additionally, studies exclusion criteria are: (1) duplicate publications; (2) review, letters, case report, editorials, or comments; (3) studies on local expressions in HCC tissues or cell lines. Two reviewers (Gang Li and Qingrong Shen) independently read the titles and abstracts of the included studies, and after the studies that evidently did not meet the inclusion criteria had been excluded, the full text of potential studies was read to confirm whether they really met the inclusion criteria. The unpublished studies were not searched. The two researchers crosschecked the included tests, and any disagreement was solved through discussion to reach a consensus.

Data extraction

Data were retrieved from each study independently by two reviewers (Gang Li and Qingrong Shen) including the following characteristics: study details (first author, publication year, study of country, and studied period), description of study population (number of patients, age, gender, and infection of HBV or HCV, and cirrhosis), RNA detection methods, studied miRNA, and its expression (up- or down-regulation, and normalization for RT-PCR), data needed for diagnostic meta-analysis (sensibility and specificity data), and information needed for methodological quality assessment.

Ranking

Each included studies comparing circulating miRNA expressions between HCC patients and healthy people, hepatitis, or cirrhosis patients resulted in a list of differentially expressed miRNAs. Then, a method of ranking potential molecular biomarkers based on Griffith's and Chan's studies [14, 15] was used. The differentially expressed miRNAs reported by each study were ranked according to the following order of importance: (1) number of the studies that consistently reported the miRNA as differentially expressed and with a consistent direction of change; (2) total number of samples for comparison in agreement; (3) average fold change reported by the studies in agreement. Total sample size was considered more important than average fold change because many studies do not report a fold change. Griffith proposed this vote-counting strategy-based method for ranking potential molecular biomarkers.

Table 1 Terms used in the systematic search

Database	Search terms
PubMed	(Hepatocellular carcinoma[mesh] OR hepatoma*[tw] OR liver cell neoplasm*[tw] OR hepatocellular neoplasm*[tw] OR liver cell cancer*[tw] OR hepatocellular cancer*[tw] OR liver cell tumo*[tw] OR hepatocellular tumo*[tw] OR liver cell carcinoma*[tw] OR hepatocellular carcinoma*[tw])
	AND (biological markers[mesh] OR Biomarker*[tw] OR Biological Marker*[tw] OR Biologic Marker*[tw] OR Biochemical Marker*[tw] OR Immunologic Marker*[tw] OR Immune Marker*[tw] OR Laboratory Marker*[tw] OR Serum Marker*[tw] OR Clinical Marker*[tw])
	AND (blood[mesh] OR blood[sh] OR blood[tw] OR serum*[tw] OR plasm[tw] OR plasma[tw] OR urine[mesh] OR urine[sh] OR urine*[tw] OR saliva[tw])
	AND (microRNA OR microRNAs OR miRNA OR miRNAs OR miR)
	NOT (animals[mesh] NOT humans[mesh])
Limits	Publication date: 2000–3000
	Language: English OR chinese
Embase	((('liver cell' OR hepatocell*) NEAR/3 (neoplasm* OR cancer* OR tumo* OR carcinoma*)) :ti,ab.de)
	AND (marker/exp OR (biological NEAR/3 marker*) :ti,ab.de OR biomarker* :ti,ab,de)
	AND (blood/exp OR blood :ti,ab,de OR serum* :ti,ab,de OR plasm :ti,ad,de OR plasma :ti,ab,de OR urine* :ti,ab,de OR saliva :ti,ab,de)
Limits	Publication date: 2000–present
	Language: English OR chinese

Quality assessment

Based on the above ranking method, the top miRNAs were used in further statistical analysis. The quality of each study was assessed independently by two reviewers (Gang Li and Qingrong Shen) according to the QUADAS (Quality Assessment of Diagnostic Accuracy Studies, an evidence-based quality assessment tool for use in systematic reviews of diagnostic accuracy studies, maximum score 14) [16].

Statistical analysis

Sensitivities and specificities of the included studies were logistically transformed, and a linear regression line was fitted through the resulting points. This line was then back-transformed to obtain the summary receiver-operating characteristic curve (sROC), according to the method described by Littenberg and Moses in 1993 [17]. A conventional ROC curve describes the impact of threshold in a single patient population. The sROC curve, a compact description of the accuracy of the diagnostic test, describes the test in many populations. The area under curve (AUC) of sROC and the maximum point of intersection between sensitivity and specificity (Q value) were calculated. The AUC represents an analytical summary of test performance and displays the trade-off between sensitivity and specificity. An AUC of 1.0 (100 %) indicates perfect discriminatory ability to distinguish cases from non-cases. The Q value reflects the closeness of sROC to the upper left corner and the accuracy of diagnostic tests. All diagnostic meta-analysis was performed using Meta-disc 1.4 for Windows. The possibility of publication bias was assessed with Begg and Egger funnel plots [18]. The results of these tests are not separately reported, however, because this method is known to be unreliable when there are fewer than 10 studies in the meta-analysis [19].

Results

Study selection

In total, 148 studies on PubMed an Embase were preliminarily included. Of these, 68 studies were excluded after the first screening based on titles or abstracts, because they were duplicates, or not relevant to the miRNA expressions in HCC. Then, we further reviewed 80 full-text articles to determine whether they met our inclusion and exclusion criteria. 33 studies were excluded for reviews, 2 studies were excluded for no control group or other cancer types, 27 studies were excluded for other simple types, and one study was excluded for each group contains $\leq$10 patients. Finally, 17 studies [20–36] were included in this systematic review. The flow of search strategy is shown in Fig. 1.

Study characteristics

In the 17 included studies, 11 studies were completed in China and 6 studies in Egypt, Germany, Turkey, American, or Japan. Male HCC patients were much more than female ones in all the included studies. HBV/HCV infection status

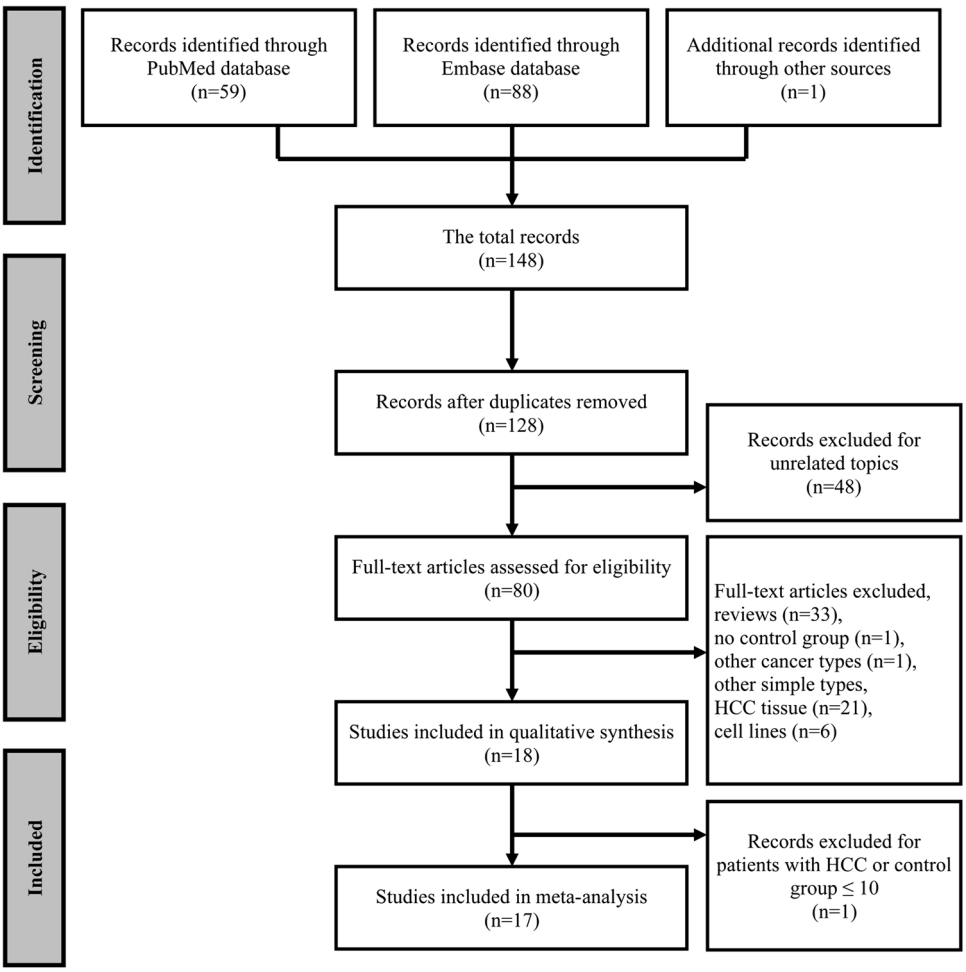

Fig. 1 Search strategy flow diagram. Figure shows the process of literature identification, screening, eligibility, and included

was reported in most studies, and only 2 studies reported the number of cirrhosis cases. The characteristics of these studies are listed in Table 2.

Comparison of circulating miRNA expressions between HCC patients and healthy people

In 15 studies [20, 22–24, 26–36] comparing the circulating miRNA expressions between HCC patients and healthy people, the miRNA detection methods, sample type, total sample size, miRNA expressions and their direction of changes are listed in Table 3. Most studies used quantification real-time PCR to measure the expressions level of miRNA, 2 studies [20, 24] used microarray combined with PCR method, and one study [28] used direct sequencing method. The sample types were also not uniform, including serum (13 studies), plasma [32, 36], whole blood [23], or urine [20]. Since no article confirmed which sample is the best for detection of miRNA levels, the four types were all included in this review.

In the included studies, miR-21, miR-122, and miR-223 were repeatedly reported three times or more. Comprehensive data are listed in Table 4, including the number of studies with consistent regulation features and total sample size. Table 4 shows that miR-21 was the hottest miRNA and reported in 6 studies [26, 28, 30, 32, 33, 36] with a total of 863 samples, where miR-21 was consistently up-regulated among 5 studies [26, 28, 32, 33, 36]. The expressions of miR-122 and miR-223 were each reported in 3 studies [30, 33, 36], where they were consistently up-regulated in serum among 2 studies [30, 33]. Based on the above ranking method, miR-21, miR-122, and miR-223 can be used as the candidate biomarkers for HCC detection, so their diagnostic efficiency should be further evaluated.

In the all studies consistently reporting the up-regulation of miR-21, miR-122, and miR-223, only part of studies provide complete diagnostic test data (e.g., sensitivity and specificity). Based on QUADAS, these studies underwent methodological quality assessment, and the results and sample size, and detection results of miR-21, miR-122, and miR-223 [true positive (TP), false positive (FP), true negative (TN), false negative (FN)] are listed in Table 5.

A graph of the summary receiver-operating characteristic (sROC) curve for comparing the diagnostic efficiency

Table 2 MicroRNA expression profiling studies included in the systematic review

References	Year	Country	Hepatocellular carcinoma patient				
			Period	Age	Sex (M/F)	HBV/HCV	Fibrosis/Cirrhosis
[20]	2012	Egypt	NR	50 ± 8	26/6	HCV $n = 32$	NR
[21]	2011	Germany	NR	61.4 ± 9.1	22/7	HCV $n = 29$	F4-F6 $n = 13$
[22]	2013	China	NR	50.16	19/6	HBV $n = 20$	NR
[23]	2014	Turkey	NR	60.4 ± 2.57	14/6	HBV $n = 20$	NR
[24]	2011	China	Nov. 2008-Jan. 2010	54.2 ± 9.2	39/7	HBV $n = 33$	$n = 46$
[25]	2013	Germany	Feb. 2009-Jul. 2012	62.6 ± 10.4	153/42	HBV $n = 33$ HCV $n = 87$	Child-Pugh A, $n = 124$ B, $n = 50$ C, $n = 21$
[26]	2011	China	NR	<50 $n = 18$ ≥50 $n = 28$	33/13	HBV $n = 30$	$n = 24$
[27]	2012	China	NR	NR	NR	HBV $n = 86$	NR
[28]	2010	China	NR	52.8 ± 7.9	46/9	HBV $n = 55$	NR
[29]	2012	China	NR	<60 $n = 47$ ≥60 $n = 10$	49/8	HBV $n = 57$	Child-Pugh A, $n = 54$ B, $n = 2$
[30]	2011	China	NR	NR	NR	HBV $n = 48$	NR
[31]	2011	America	NR	55 (25–80)	88/17	HBV $n = 20$ HCV $n = 66$	NR
[32]	2012	Japan	Jan. 2001–Dec 2005	63 ± 10	99/27	HBV $n = 28$ HCV $n = 87$	NR
[33]	2011	China	NR	57.03	78/23	HBV $n = 76$	NR
[34]	2012	China	2008-2010	54.4 ± 12.9	80/32	NR	NR
[35]	2013	China	Jan 2007–May 2010	51.7 ± 11.7	58/29	NR	NR
[36]	2011	China	Aug 2008–Jun 2010	53 ± 12	168/36	NR	NR

NR not reported, *HBV* hepatitis B virus, *HCV* hepatitis C virus

of three miRNAs (miR-21, miR-122, and miR-223) in discriminating HCC patients from healthy people is shown in Fig. 2. The area under curve (AUC) of sROC for miR-21, miR-122 and miR-223 candidate biomarkers are 0.9293, 0.8128, and 0.8597, respectively. Therefore, it is indicated that miR-21 has highest level of diagnostic efficiency among three miRNAs candidate biomarkers.

Comparison of circulating miRNA expressions between HCC patients and other hepatitis or cirrhosis patients

The expressions of circulating miRNAs in HCC patients and other hepatitis or cirrhosis patients were compared in 10 studies [21, 25, 27, 29–34, 36]. The detailed test method, sample type, sample size, and miRNA expressions are listed in Table 6. By ranking by literature number and sample size, coincidentally, three same miRNAs as above (miR-21, miR-122, and miR-223) were repeatedly reported three times or more, and comprehensive data are listed in Table 4. However, no study provided complete diagnostic test data, so we cannot compare the diagnostic efficiency of three miRNAs (miR-21, miR-122, and miR-223) in discriminating HCC patients from other hepatitis or cirrhosis patients.

Discussion

At present, the lack of agreement among several miRNA expression profiling studies is the common drawback. Differences in lab protocols, measurement platforms and small sample sizes can lead to gene expression levels incomparable. With reference to Griffith's and Chan's studies [14, 15], a logical solution is to determine the overlap among many miRNA profiling studies and to observe which differentially expressed miRNAs are consistently reported. These miRNAs likely reveal biological relevance

Table 3 Fifteen microRNA expression profiling studies (HCC patients vs. healthy controls)

References	Method	Sample types	Total sample size (HCC/C)	MicroRNAs	Status	Normalization control
[20]	Array/PCR	Urine	44 (32/12)	miR-625, miR-532, miR-618	↗	5S rRNA
				miR-516-5p, miR-650	↘	
[22]	PCR	Serum	45 (25/20)	miR-101	↗	U6RNA
[23]	PCR	Blood	48 (20/28)	miR-125b-5p	↗	RNU48
				miR-223-3p	↘	
[24]	Array/PCR	Serum	70 (46/24)	miR-885-5p	↗	U6RNA/miR-16
[26]	PCR	Serum	66 (46/20)	miR-221, miR-222, miR-21, miR-224	↗	mmu-miR-295
[27]	PCR	Serum	131 (86/45)	miR-18a	↗	U6RNA
[28]	SD	Serum	105 (55/50)	miR-1, let-7f, miR-25, miR-92a, miR-206, miR-375	↗	miR-168
[29]	PCR	Serum	87 (57/30)	miR-15b, miR-21, miR-130b, miR-183	↗	–
[30]	PCR	Serum	72 (48/24)	miR-122, miR-222, miR-223	↗	miR-16
				miR-21	↘	
[31]	PCR	Serum	176 (105/71)	miR-16, miR-199a	↘	U6RNA
[32]	PCR	Plasma	176 (126/50)	miR-21	↗	miR-16
[33]	PCR	Serum	190 (101/89)	miR-21, miR-122, miR-223	↗	miR-181a/miR-181c
[34]	PCR	Serum	168 (112/56)	miR-483-5p, miR-500a	↗	standard curve
[35]	PCR	Serum	183 (87/96)	miR-29b	↘	U6RNA
[36]	PCR	Plasma	272 (204/68)	miR-192, miR-21, miR-801	↗	miR-1228
				miR-122, miR-223, miR-26a, miR-27a	↘	

HCC/C hepatocellular carcinoma patients/healthy controls, *Array* microRNA microarray, *PCR* polymerase chain reaction, *DS* direct sequencing

Table 4 The candidate microRNA biomarkers for HCC detection

MicroRNA name	HCC patients vs. healthy controls				HCC patients vs. other hepatitis or cirrhosis patients			
	Status	No. of studies		Total sample size (HCC/C)	Status	No. of studies		Total sample size (HCC/O)
miR-21	↗	5	[26, 29, 32, 33, 36]	791 (534/257)	↗	3	[29, 32, 36]	581 (387/194)
	↘	1	[30]	72 (48/24)	↘	1	[33]	149 (101/48)
	→	0		–	→	2	[21, 30]	144 (77/67)
miR-122	↗	2	[30, 33]	262 (149/113)	↗	1	[30]	96 (48/48)
	↘	1	[36]	272 (204/68)	↘	2	[33, 36]	488 (305/183)
	→	0		–	→	1	[25]	249 (149/54)
miR-223	↗	2	[30, 33]	262 (149/113)	↗	0		–
	↘	1	[36]	272 (204/68)	↘	1	[36]	339 (204/135)
	→	0		–	→	2	[30, 33]	245 (149/96)

HCC/C HCC patients/healthy controls, *HCC/O* HCC patients/other hepatitis, or cirrhosis patients

to the tumorigenesis of HCC, as opposed to sporadically reported miRNAs, which maybe false positives.

Previous studies [37, 38] have made meta-analysis about microRNAs as biomarkers for HCC. However, in our study, we first made a meta-analysis with a vote-counting strategy proposed by Griffith's and Chan's studies to evaluate the diagnostic value of miRNAs for HCC. This strategy can find suitable miRNAs from multiple independent studies by the ranking method, and these miRNAs can be analyzed separately. Besides, our study only focused on circulating miRNAs from body fluids such as serum, plasma or urine samples, rather than from tissue

Table 5 Characteristics of studies with ROC curve analysis of three microRNAs and methodological quality assessment (HCC patients vs. healthy controls)

MicroRNA name	Study	Status	Sample size (HCC/C)	TP	FP	FN	TN	QUADAS
miR-21	[29]	↗	116 (57/59)	51	17	6	42	8
	[32]	↗	176 (126/50)	110	4	16	46	11
	[33]	↗	190 (101/89)	85	24	16	65	10
miR-122	[30]	↗	72 (48/24)	39	4	9	20	9
	[33]	↗	190 (101/89)	71	28	30	61	10
miR-223	[33]	↗	190 (101/89)	81	20	20	69	10

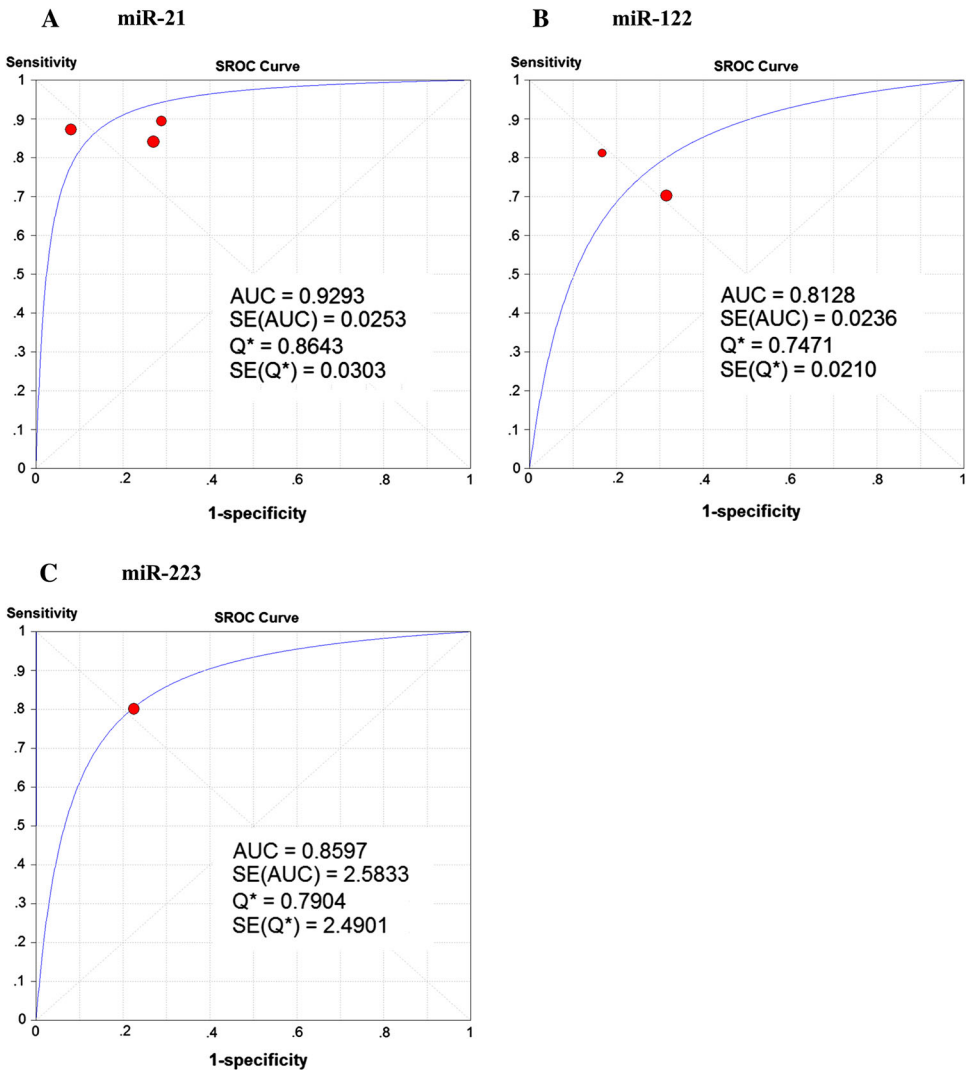

Fig. 2 The symmetric receiver-operation characteristic (sROC) curve using miR-21, miR-122, and miR-223 as marker for HCC detection versus healthy people. The AUC of sROC for miR-21, miR-122, and miR-223 are 0.9293, 0.8128, and 0.8597, respectively, indicating miR-21 has highest level of diagnostic efficiency

samples and cell lines. Therefore, we included 17 independent circulating miRNA profiling studies into this meta-analysis.

A ranking method was used in a comprehensive collection of 15 circulating miRNA profiling studies comparing miRNA expressions between HCC patients and normal people, and we found miR-21, miR-122, and miR-223 were repeatedly reported three times or more. And miR-21 was the hottest miRNA and reported in 6 studies with a total of 863 samples, where miR-21 was consistently

Table 6 Ten microRNA expression profiling studies (HCC patients vs. other hepatitis or cirrhosis patients)

References	Method	Sample types	Total sample size	MicroRNAs	Status	Normalization control
[21]	PCR	Serum	48 (29/19) (HCC/HCV)	miR-21	→	miR-16
[25]	PCR	Serum	249 (195/54) (HCC/Cirrhosis)	miR-1, miR-122	→	miR-16
[27]	PCR	Serum	116 (86/30) (HCC/HBV or Cirrhosis)	miR-18a	↗	U6RNA
[29]	PCR	Serum	86 (57/29) (HCC/HBV)	miR-15b, miR-21, miR-130b, miR-183	↗	–
[30]	PCR	Serum	96 (48/48) (HCC/HBV)	miR-122	↗	miR-16
				miR-21, miR-222, miR-223	→	
[31]	PCR	Serum	212 (105/107) (HCC/CLDs)	miR-16, miR-199a	↘	U6RNA
				miR-195	→	
[32]	PCR	Plasma	156 (126/30) (HCC/CH)	miR-21	↗	miR-16
[33]	PCR	Serum	149 (101/48) (HCC/HBV)	miR-21, miR-122	↘	miR-181a/miR-181c
				miR-223	→	
[34]	PCR	Serum	197 (112/85) (HCC/CH)	miR-483-5p, miR-500a	↗	standard curve
[36]	PCR	Plasma	339 (204/75 + 60) (HCC/HBV + Cirrhosis)	miR-192, miR-21, miR-801	↗	miR-1228
				miR-122, miR-223, miR-26a, miR-27a	↘	

PCR, polymerase chain reaction; HCC, hepatocellular carcinoma patients; HBV, hepatitis B virus; HCV, hepatitis C virus; CLDs, chronic liver diseases; CH, chronic hepatitis

up-regulated in HCC detection among 5 studies [26, 28, 32, 33, 36]. MiR-21, which is located in 17q23, has oncogenic activity in humans and often has altered expression, and is one of the most studied miRNAs in many types of cancers, such as HCC [39], breast cancer [40, 41], lung cancer [42, 43], and colorectal cancer [44]. The up-regulated miR-21 may lead to cell growth, survival, motility, invasion, and metastasis [39, 45–47]. The participation of miR-21 in regulation of key oncogenic and new pathways affecting liver cancer, such as the MAPK, TGFβ and cell cycle pathways that drive tumorigenic transformations of somatic and stem cells [48]. Suppression of miR-21 in MCF-7 cell, which overexpressed miR-21, could decrease cell proliferation and increase apoptosis, and knockdown of the miR-21 in glioblastoma cells also revealed that miR-21 has an antiapoptotic function [49, 50]. Moreover, two direct targets of miR-21, maspin, and PDCD4, which could cause the reduction of the malignancies metastasis, have been found [51]. Taken other line of evidence together, the possible role of miR-21 as an oncogene has been hypothesized, including cell cycle, proliferation, metastasis, and chemosensitivity of tumor cells by targeting several tumor suppressor genes such as MARCKS, PTEN, Cdc25A, and PDCD4 [52–55].

We further analyzed the diagnostic efficiency of miR-21, miR-122, and miR-223, and the AUC of sROC are 0.9293, 0.8128, and 0.8597, respectively. The diagnostic efficiency of miR-21 is largely higher than miR-122 and miR-223, and higher than the serum markers such as AFP in clinics, indicating that miR-21 has high clinical significance for diagnosis of HCC. However, there are only 3 studies [29, 32, 33] with a total sample size of 482 provide complete diagnostic test data, so this meta-analysis is methodologically limited in validating the diagnostic potency of miR-21. A small number of included studies may result in low fitting efficiency in SROC. But we hope this part of analysis may have a certain reference value for future studies. Moreover, most of current circulating miRNA expression profiling studies are retrospective, and their sample size is small. Further large-size clinical studies such as a double cohort study and prospective cohort study are required to validate their clinical significance.

Moreover, miR-122 and miR-223 were inconsistently reported as differentially expressed. A potential explanation for these observed inconsistencies is the heterogeneity in the samples used. MiR-122 and miR-223 were reported as down-regulated in plasma in one article [36], which was inconsistent with two other articles [30, 33] using serum as sample. It was already known that miRNA expression profiles were considerably different between plasma and serum. Recent studies show that many miRNAs in plasma can combine with some proteins such as Argonaute2 and

high-density lipoproteins (HDL) and thereby generate high stability [56, 57]. McDonald et al. [58] found higher concentrations of circulating endogenous miRNAs in plasma samples compared to serum samples. Clearly, in different sample types, miRNA expressions are not necessarily the same. There is no consensus regarding whether plasma or serum is preferable for use as a sample, and there is a limitation to analyzing the microRNA expression results of both plasma and serum. Second, the miRNA expressions in circulation system are affected by various physiologic modulations and pathologic disruptions, suggesting that individual variability may affect the expression of miRNAs. Besides, it should be noted that a single miRNA may have many targets, and each miRNA is regulated by several different miRNAs contributing to the biological complexity of finding a miRNA biomarker, the present type of approach is unable to solve this problem.

Conclusions

In conclusion, this systematic review identified one up-regulated miRNA (miR-21) as a novel potential biomarker for HCC. Further clinical studies focusing on miR-21 are required to verify its clinical significance in HCC detection. However, the number of circulating miRNA expression profiling studies is not large, and these studies are retrospective, and their sample size is small. More well-designed and larger sample trials using microarrays and direct sequencing are required to provide more valuable miRNA biomarkers for hepatocellular carcinoma detection.

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Conflict of interest The authors report no declarations of interest.

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