Biomarker MicroRNAs for Diagnosis, Prognosis and Treatment of Hepatocellular Carcinoma: A Functional Survey and Comparison

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Hepatocellular Carcinoma (HCC) is one of the most common malignant tumors with high incidence and mortality rate. Precision and effective biomarkers are therefore urgently needed for the early diagnosis and prognostic estimation. MicroRNAs (miRNAs) are important regulators which play functions in various cellular processes and biological activities. Accumulating evidence indicated that the abnormal expression of miRNAs are closely associated with HCC initiation and progression. Recently, many biomarker miRNAs for HCC have been identified from blood or tissues samples, however, the universality and specificity on clinicopathological features of them are less investigated. In this review, we comprehensively surveyed and compared the diagnostic, prognostic, and therapeutic roles of HCC biomarker miRNAs in blood and tissues based on the cancer hallmarks, etiological factors as well as ethnic groups, which will be helpful to the understanding of the pathogenesis of biomarker miRNAs in HCC development and further provide accurate clinical decisions for HCC diagnosis and treatment.

Hepatocellular Carcinoma (HCC) is the sixth most common cancer worldwide in terms of number of cases and the second major contributor to cancer mortality in man. The survival rates in the United States and developed countries are only 3% to 5%. There are still no effective biomarkers for the early diagnosis and prognosis of HCC. Currently, only about 30% to 40% patients with HCC can get effective treatment at the right time. It is extremely necessary to discover new biomarkers for precision diagnosis, prognosis and treatment of HCC.

MicroRNAs (miRNAs) are small endogenous non-coding RNAs with 22–24 nucleotides in length. They play important roles in regulating human genes by inhibiting translation or cleavage. Recent studies showed that miRNAs were associated with a variety of important biological processes such as cell proliferation, development, and apoptosis. Accumulating evidence indicated that miRNAs could be latent biomarkers in human cancers, including gastric cancer, lung cancer, prostate cancer, and breast cancer etc. Nowadays, extensive research efforts have demonstrated the biomarker role of miRNAs in HCC. For example, Jiang and his colleagues confirmed that miRNA panel assay (miR-10b, miR-106b and miR-181a) could be potential biomarkers for HCC preliminary screening. He et al. focused on the applications of miRNAs from 13 studies and 21 sets of data and the association between the risk of HCC and miRNAs polymorphisms. Another review summarized the function of circulating miRNAs, and a meta-analysis included 14 studies involving 1,848 cases with HCC and 1187 controls concluded that the miRNA panels can be biomarkers for HCC with AUC = 0.99 (96% sensitivity and 96% specificity). Many comprehensive reviews recommend to pay attentions to the role and function of miRNAs in disease diagnosis, prognosis and therapy. However, the differences in biological features of miRNAs...
between blood and tissues are still unclear, which limits the investigation on understanding clinical implications of miRNAs in different specimen.

In this review, we performed comprehensive functional analyses and comparisons of miRNA biomarkers in blood and tissues. The miRNA biomarkers in “tissues” were mainly extracted from liver tissues, adjacent non-cancerous tissues or human HCC tissues whereas those in “blood” were collected from plasma, serum or whole blood samples. This review aims at comprehensively understanding the pathogenic mechanism and clinical value of HCC biomarker miRNAs, and providing insights into precision diagnosis and treatment of HCC.

Methods

Data collection. We systematically collected HCC biomarker miRNAs from citations in NCBI PubMed by retrieval formula “(liver cancer OR intrahepatic bile duct OR hepatocellular carcinoma OR hepatoblastoma OR cholangiocarcinoma) AND (miRNA* OR microRNA*) AND (biomarker* OR indicator*)”. Here, studies in which miRNAs were exactly defined as markers or biomarkers were mainly considered, and those identified from body fluids such as saliva, urine and sweat were excluded as we only focused on miRNA biomarkers in blood and tissues. Besides, for further comparing the differentiation between HCC and cirrhosis and providing valuable strategies for the early detection of HCC, we also collected diagnostic miRNA biomarkers for liver cirrhosis using retrieval formula “cirrhosis AND diagnos* OR indicator* OR predictor*”. Here, studies in which miRNAs were exactly defined as markers or biomarkers were mainly considered, and those identified from body fluids such as saliva, urine and sweat were excluded as we only focused on miRNA biomarkers in blood and tissues. For computationally predicted miRNA pairs, they should reside in no fewer than two of the three prediction databases. Meanwhile, we unitized miRNA IDs according to the latest nomenclature in miRBase (release 21).

Target genes of miRNA biomarkers. The miRNA targets used in this study were integrated from both experimentally validated, i.e. miR2Disease, TarBase (version 6.0), miRTarBase (version 4.5), miRecords (version 4.0) and computationally predicted, i.e. HOCTAR (version 2.0), ExprTargetDB, and starBase (version 2.0) miRNA-target databases. To reduce false positives, we mainly selected miRNA-miRNA pairs validated by low-throughput experiments, i.e. real-time quantitative PCR, Western blot, etc. For computationally predicted pairs, they should reside in no fewer than two of the three prediction databases. Meanwhile, we unitized miRNA IDs according to the latest nomenclature in miRBase (release 21).

Functional survey of HCC biomarker miRNAs. The functions of HCC biomarker miRNAs are summarized based on the hallmarks of cancers. Since some of the miRNAs are associated with liver injury and few of the miRNAs’ functions are unclear, we therefore grouped their functions into 12 categories as antigrowth signals, resisting cell death, avoiding immune destruction, tissue invasion and metastasis, tumor promotion inflammation, sustained angiogenesis, limitless replicative potential, genome instability and mutation, other clinicopathological features, liver injury, tumor suppressor/onco-miR, and unclear. Moreover, we compared the pathogenesis of HCC biomarker miRNAs based on etiological factors as well as ethnic groups, i.e. the effects of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and ethnic variation on HCC development.

Pathway enrichment analyses. For better understanding the association between miRNAs and HCC pathogenesis, we mapped the targets of biomarker miRNAs onto signaling pathways using IPA (Ingenuity Pathway Analysis) program. The top 10 significantly enriched pathways (p-value < 0.01) were selected and further validated the correlation with HCC by PubMed literature exploration.

Results

Overview of the collected HCC biomarker miRNAs. After manually searching and checking in PubMed citations, a total of 50 and 18 diagnostic miRNA biomarkers in blood and tissues, respectively, were extracted from 44 articles (see Tables 1 and 2) and their clinicopathological features of HCC were further compared based on the hallmarks of cancers, etiological factors and ethnic groups, respectively. As for prognostic and therapeutic biomarkers, respectively, 16 and 32 prognostic miRNAs in blood and tissues together with 8 therapeutic markers were collected according to records in 34 articles (see Tables 3, 4 and 5) and their clinicopathological features as well as functions were then explored.

Functional characterization of HCC biomarker miRNAs based on cancer hallmarks. The functional characterization of HCC biomarker miRNAs are summarized from the primary references and classified into 12 categories as shown in Fig. 1. It indicates that the biomarker miRNAs are associated with all aspects of hallmarks of cancers and all the hallmarks lead to the cancer. Therefore, the personalized biomarkers are needed to precision diagnosis, prognosis and treatment of the complex HCC. The functions of the biomarker miRNAs are summarized as follows.

Insensitivity to Antigrowth Signals. Although it is unclear for the units and interconnections between the different kinds of antigrowth and differentiation—including signals and the core cell cycle machinery, an antigrowth signaling must be exist to circumvent developing HCC. MiR-125b-5p and miR-15b-5p were the circulating diagnostic miRNA biomarkers associated with insensitivity to antigrowth signals and all of them were up-regulated and highly expressed in early-stage HCC cases. Liu et al. combined miR-15b-5p and miR-130b-3p as a classifier for HCC detection, yielding a receiver operating characteristic curve area of 0.98 in their validation study, the same was found in tissue samples, miR-15-5p was also reported highly expressed. As for prognostic biomarkers, three miRNAs related to insensitivity to antigrowth signals in the tissue samples were identified, including miR-137, miR-185-5p and miR-26a-5p. All of them were down-regulated in poor prognostic group which had a lower survival rate and shorter time to recurrence.
Resisting Cell Death. Cancer cells evolve various ways to circumvent or restrict apoptosis. The diversity of apoptosis-avoiding machinery and program reflects the multiplicity of apoptosis-including signals that tumor cell populations experienced while their evolution to the malignant state. In tissues, miR-101-3p, miR-224-5p and miR-483-5p were associated with resisting cell death. Among them, miR-101-3p was down-regulated whereas the remaining two were reported to be up-regulated. Resisting cell death was significantly associated with lower expression of miR-101-3p, miR-16-5p, miR-195-5p, miR-203a-3p and miR-221-3p in blood samples. Increased miR-221-3p, miR-224-5p, miR-483-5p and miR-122-3p expression were also detected in blood of HCC patients. These above diagnostic biomarkers as classifiers for HCC detection, yielding a receiver operating characteristic curve area of 0.635 to 0.884 (see Tables 1 and 2).

### Table 1. Diagnostic biomarkers in tissues for hepatocellular carcinoma.

| Reported ID | Official ID | Sample | Ethnicity | Features | Expression | AUC | PMID | Validated Targets |
|-------------|-------------|--------|-----------|----------|------------|-----|------|------------------|
| miR-101     | miR-101-3p  | 30 HC 67 CHB 61 HBV-LC 67 HBV-HCC | China | 1.inhibit HCC cell proliferation 2.tumor suppressor 3.promote apoptosis | down | CHB from HC 0.635 HBV-LC from HC 0.884 HBV-HCC from HC 0.788 | 24971953 | Mcl-1, SOX9 |
| miR-126     | miR-126-3p  | 19 HCC 6 HCC | Germany | tumor suppressor | down | NA | 25500075 | NA |
| miR-127     | miR-127-3p  | 33 HCC | China | tumor suppressor | down | NA | 24854842 | NA |
| miR-130b    | miR-130b-3p | 97 HCC | China | onco-miR | up | 0.914 | 22403344 | RUNX3 |
| miR-139     | miR-139-5p  | 31 CHB 31 HCC | China | 1.suppress metastasis and progression of cancer cells 2.tumor suppressor | down | HCC from CH 0.761 (0.770) | 24549282 | Rho-kinase 2 |
| miR-148a    | miR-148a-3p | 19 HCC | China | onco-miR | up | NA | 22496917 | NA |
| miR-150     | miR-150-5p  | 15 HCC 15 ICC | China | tumor suppressor | up | 0.764 | 25482320 | NA |
| miR-15b     | miR-15b-5p  | 96 HCC | China | preventing replicative stress in response to mitogensignalling | up | 0.98 | 22403344 | NA |
| miR-182     | miR-182-5p  | HCC | China | proliferation | up | NA | 24653623 | IGF1R and GSK3B |
| miR-18b     | miR-18b-5p  | 110 HCC | Japan | 1.proliferation 2.loss of cell adhesion ability | up | NA | 23496901 | TNRC6B |
| miR-199a    | miR-199a-5p | 17 CH 23 HCC | Egypt | NA | down | 0.856 | 26302751 | Mitogen-activated protein kinase (MAPK) |
| miR-200a    | miR-200a-3p | 29 HCC | Germany | suppress cancer cell migration | up | NA | 24895326 | ZEB1/ZEB2 |
| miR-200b    | miR-200b-3p | 29 HCC | Germany | suppress cancer cell migration | up | NA | 24895326 | ZEB1/ZEB2 |
| miR-21      | miR-21-5p   | 50 HC 30 LC 136 HCC | Japan | excessive secretion by primary cancer cells | CH from HC 0.773 HCC from HC 0.953 | 21749846 | NA |
| miR-21      | miR-21-5p   | 17 CH 23 HCC | Egypt | 1.cell growth 2.migration 3.invasion | up | NA | 26302751 | phosphatase and tensin homolog (PTEN) |
| miR-21      | miR-21-5p   | 30 HC 97 HCC | China | 1.promote cell proliferation 2.tumor invasion | up | NA | 25973032 | PDCD4 and PTEN |
| miR-21      | miR-21-5p   | 74 ICC | China | intrahepatic cholangiocarcinoma proliferation and growth | up | NA | 25803229 | PTPN14 and PTEN |
| miR-214     | miR-214-3p  | 9 HC 10 HCC | China | tumor suppressor | down | NA | 24789420 | EZH2, CTNNB1 and CDH1 |
| miR-224     | miR-224-5p  | 9 HC 10 HCC | China | 1.cell proliferation 2.migration 3.invasion 4.anti-apoptosis | up | NA | 24789420 | CD40 |
| miR-29a-5p  | miR-29a-5p  | 266 HCC | China | 1.tissue invasiveness and metastasis r 2.tumor suppressor | up | 0.746 | 23285022 | NA |
| miR-483-5p  | miR-483-5p  | 69 HC 69 HCC | America | anti-apoptotic oncogene | HCC from HC 0.827 | 24172413 | NA |
miR-155-5p and miR-21-5p were up-regulated whereas others were down-regulated\textsuperscript{45-48}. Circulating miR-122-5p and miR-16-5p could be used as putative biomarkers for HCC. Among them, miR-122-5p and miR-16-5p were shown to be up and down-regulated, respectively\textsuperscript{49,50}.

Avoiding Immune Destruction. According to the long-standing theory of immune surveillance proposes, most of solid tumors such as HCC appeared to have somehow controlled to avoid detection by the different kinds of arms of the immune system or could limit the extent of immunological killing, thus they could evade eradication by immune system\textsuperscript{32}. Motawi and his colleagues overviewed that serum miR-146p-5p was up-regulated in HCC and showed the clinical value for HCV-related HCC diagnosis. This circulatory biomarker miRNA was reported to exerted negative effects on anti-tumor immune response\textsuperscript{42}.

Tissue Invasion and Metastasis. Invasion and metastasis, complex and multi-step processes, are elementary factors that affects HCC patients survival rate and their genetic and biochemical mechanisms remain poorly understood\textsuperscript{31}. In tissues, high expression of miR-18b-5p, miR-200a-3p, miR-200b-3p, miR-21-5p, miR-224-5p and miR-29-5p were most frequently to be detected in HCC, and miR-139-5p was down-regulated. Therefore, they were valuable for diagnosis of HCC\textsuperscript{39,51-57}. Several circulating miRNA biomarkers also displayed signally correlation with tissue invasion and metastasis, including highly expressed miR-146a-5p, miR-181b-5p, miR-182-5p, miR-21-5p, miR-215, miR-23-5p, miR-224-5p, miR-296-5p, miR-331-3p and miR-96-5p and low expressed miR-125b-5p, miR-199a-3p, miR-122-5p, miR-139-5p, miR-150-5p, miR-195-5p and miR-19a-3p. The above...
| Reported ID | Official ID | Sample | Source | Ethnicity | Features | Expression | AUC | PMID | Validated Targets |
|------------|-------------|--------|--------|-----------|----------|------------|-----|------|------------------|
| miR-199a-3p | miR-199a-3p | 156 HC, 78 HCC | serum | China | invasion capability | down | 0.883 | 25618599 | phosphorlated-S6 protein |
| miR-223 | miR-223-3p | 167 HC, 169 CHB, 141 LC, 457 HCC | blood | China | NA | down | 0.864(training set), 0.888(validation set) | 22105822 | Statsmin1 |
| miR-101 | miR-101-3p | 30 HC, 79 CHB, 61 HBV-LC, 67 HBV-HCC | serum | China | 1.inhibit HCC cell proliferation 2.tumor suppressor 3.promote apoptosis | down1 | CHB from HC 0.635 HBV-LC from HC 0.884 HBV-HCC from HC 0.788 | 24971953 | Mcl-1, SOX9 |
| miR-106b | miR-106b-5p | 50 HC, 31 CLD, 27 HCC | blood | China | Proliferation | up | HCC from HC 0.89 CLD from HC 0.81 CLD from HC 0.63 | 25761179 | p21/E2F5 |
| miR-122 | miR-122-5p | 167 HC, 169 CHB, 141 LC, 457 HCC | blood | China | 1.tumor size 2.differentiation grade 3.poor prognosis 4.distance metastasis | down1 | CHB from HC 0.635 HBV-LC from HC 0.884 HBV-HCC from HC 0.788 | 22105822 | Mcl-1, SOX9 |
| miR-122 | miR-122-5p | 15 HC, 30 DN, 120 HCC | serum | China | 1.induce apoptosis 2.suppress proliferation | up | HCC from HC 0.85 CLD from CLD 0.73 CLD from HC 0.66 | 25761179 | NA |
| miR-122 | miR-122-5p | 34 HC, 70 HBV-HCC, 48 CHB | serum | China | liver injury | up | HCC from HC 0.79 CHB from HC 0.93 | 21296103 | NA |
| miR-122 | miR-122-5p | 173 HC, 233 LC, 261 HCC | serum | China | 1.regulating hepatocyte development and differentiation 2.apoptosis and suppress proliferation | down | 0.887(training sets), 0.879(validation sets) | 25238238 | HepG2 and Hep3B cells |
| miR-122-5p | miR-122-5p | 173 HC, 233 LC, 261 HCC | serum | China | 1.induce apoptosis 2.suppress proliferation | up | 0.629 | 26264553 | NA |
| miR-122 | miR-122-5p | 85 volunteers matched | serum | China | tumor suppressor | down | 0.707(0.943)2 | 23723713 | NA |
| miR-125-5p | miR-125-5p | 28 HC, 24 CHB, 22 HBV-LC, 20 HBV-HCC | plasma | Turkey | suppress the cell growth | up | NA | 24595450 | AKT |
| miR-130a | miR-130a-3p | 42 HC, 125 HCV-CLD, 112 HCV-HCC | blood | Egypt | NA | up | HCV-HCC from HC 0.691 | 26352740 | NA |
| miR-130b | miR-130b-3p | 97 HCC | serum | China | onco-miR | up | 0.914 | 22403344 | RUNX3 |
| miR-139 | miR-139-5p | 31 CHB, 31 HC | plasma | China | 1.suppress metastasis and progression of cancer cells 2.tumor suppressor | down | HCC from CH 0.761 (0.770)3 | 24549282 | Rho-kinase 2 |
| miR-143 | miR-143-3p | 127 HC, 118 CH, 95 HCC | serum | China | differentiation | up | CH from HC 0.617 HCC from CH 0.795 | 24993656 | FND3B |
| miR-146a | miR-146a-5p | 42 HC, 125 HCV-CLD, 112 HCV-HCC | blood | Egypt | 1.suppresses HCC invasion 2.exerted negative effects on anti-tumor immune response | up | HCV-HCC from HC 0.787 HCV-HCC from HCV-CLD 0.85 | 26352740 | VEGF |
| miR-146a | miR-146a-5p | 313 HC, 294 HCC | serum | China | onco-miR | NA | NA | 24816919 | NA |
| miR-150 | miR-150-5p | 120 HC, 110 CHB, 120 HCC | serum | China | 1.tumor suppressor 2.metastasis 3.BCLC stage 4.advanced TNM stages | down | NA | 26215970 | NA |
| miR-150 | miR-150-5p | 15 HC, 15 ICC | plasma | China | tumor suppressor | up | 0.764 | 25482320 | NA |
| miR-15b | miR-15b-5p | 96 HCC | serum | China | preventing replicative stress in response to mitogenicsignalling | up | 0.984 | 22403344 | NA |
| miR-16 | miR-16-5p | 107 CLD, 105 HCC | serum | America | 1.tumor suppressor 2.apoptosis | down | NA | 21278583 | BCL2, MCL1, CCND1, WNT3A |
| miR-17-5p | miR-17-5p | 28 HC, 26 CHC, 30 HCV-positive cirrhotic 8 HCC | blood | Turkey | NA | up | NA | 25391771 | NA |

Continued
| Reported ID | Offical ID | Sample Source | Ethnicity | Features | Expression | AUC | PMID | Validated Targets |
|-------------|------------|----------------|-----------|----------|------------|-----|------|-------------------|
| miR-181a    | miR-181a-5p| 50 HC 31 CLD 27 HCC | blood China | tumor suppressor | down | HCC from HC 0.82 HCC from CLD 0.71 CLD from HC 0.64 | 25761179 | NA |
| miR-182     | miR-182-5p| 40 HC 95 BLD 103 HCC | serum China | 1.metastasis | up | 0.911 | 25903466 | TP53INP1 |
| miR-18a     | miR-18a-5p| 60 HC 30 HBV CH 101 HBV-HCC | serum China | 1.liver injury 2.onco-miR | up | NA | 22865399 | NA |
| miR-192     | miR-192-5p| 167 HC 169 CHB 141 LC 457 HCC | blood China | NA | up | 0.864(training set) 0.888(validation set) | 22105822 | NA |
| miR-192     | miR-192-5p| 42 HC 125 HCV-CLD 112 HCV-HCC | blood Egypt | liver injury | up | HCV-HCC from HC 0.875 HCV-HCC from HCV-CLD 0.693 | 26352740 | NA |
| miR-192-5p  | miR-192-5p| 173 HC 233 LC 261 HCC | serum China | NA | down | 0.887(training sets) 0.879(validation sets) | 25238238 | NA |
| miR-195     | miR-195-5p| 42 HC 125 HCV-CLD 112 HCV-HCC | blood Egypt | 1.onco-miR 2.evading apoptosis 3.tissue invasion and metastasis | down | HCV-HCC from HC 0.653 HCV-HCC from HCV-CLD 0.784 | 26352740 | FGFR and GHR |
| miR-196a    | miR-196a-5p| 313 HC 294 HCC | serum China | onco-miR | NA | NA | 24816919 | NA |
| miR-199a-5p | miR-199a-5p| 173 HC 233 LC 261 HCC | serum China | tumor suppressor | down | 0.887(training sets) 0.879(validation sets) | 25238238 | NA |
| miR-19a     | miR-19a-3p| 42 HC 125 HCV-CLD 112 HCV-HCC | blood Egypt | 1.PV thrombosis 2.invasion, satellite nodules and progression 3.recurrence | down | HCV-HCC from HC 0.714 HCV-HCC from HCV-CLD 0.866 | 26352740 | NA |
| miR-206     | miR-206   | 173 HC 233 LC 261 HCC | serum China | NA | up | 0.887(training sets) 0.879(validation sets) | 25238238 | NA |
| miR-21      | miR-21-5p | 89 HC 48 CHB 101 HCC | blood China | liver injury | up | HCC from HC 0.87 CHB from HC 0.91 | 21229610 | NA |
| miR-21      | miR-21-5p | 167 HC 169 CHB 141 LC 457 HCC | blood China | tumor suppressor | up | 0.864(training set) 0.888(validation set) | 22105822 | PTEN |
| miR-21      | miR-21-5p | 50 HC 30 LC 136 HCC | serum Japan | excessive secretion by primary cancer cells | up | CH from HC 0.773 HCC from HC 0.955 | 21749846 | NA |
| miR-21      | miR-21-5p | 30 HC 97 HCC | blood China | 1.promote cell proliferation 2.tumor invasion | up | NA | 25973032 | PDCD4 and PTEN |
| miR-21      | miR-21-5p | 74 HCC | serum China | intrahepatic cholangiocarcinoma proliferation and growth | up | NA | 25803229 | PTPN14 and PTEN |
| miR-215     | miR-215   | 127 HC 118 CH 95 HCC | serum China | metastasis | up | CH from HC 0.802 HCC from HC 0.816 | 24993656 | NA |
| miR-221     | miR-221-3p| 10 HC 30 HCV 30 HCV-LC 30 HCV-HCC | serum Egypt | anti-apoptotic | down | 0.655 | 25429320 | NA |
| miR-223     | miR-223-3p| 89 HC 48 CHB 101 HCC | blood China | liver injury | up | HCC from HC 0.86 CHB from HC 0.88 | 21229610 | NA |
| miR-223-3p  | miR-223-3p| 28 HC 26 CHC 30 HCV-LC 8 HCV-HCC | blood Turkey | NA | down | NA | 25391771 | NA |
| miR-223-3p  | miR-223-3p| 28 HC 24 CHB 22 HBV-LC 20 HBV-HCC | plasma Turkey | NA | down | NA | 24595450 | NA |
| miR-24-3p   | miR-24-3p | 46 HC 31 CLD 84 HCC | serum China | 1.vascular invasion | up | HCC from CLD 0.636 (0.834) | 25129312 | NA |
| miR-26a     | miR-26a-5p| 167 HC 169 CHB 141 LC 457 HCC | blood China | lower miR-26a expression experienced worse survival but better response to interferon therapy | down | 0.864(training set) 0.888(validation set) | 22105822 | NA |
| miR-26a-5p  | miR-26a-5p| 173 HC 233 LC 261 HCC | serum China | NA | down | 0.887(training sets) 0.879(validation sets) | 25238238 | NA |
| miR-27a     | miR-27a-3p| 167 HC 169 CHB 141 LC 457 HCC | blood China | onco-miR | down | 0.864(training set) 0.888(validation set) | 22105822 | NA |
| miR-296     | miR-296-5p| 42 HC 125 HCV-CLD 112 HCV-HCC | blood Egypt | 1.metastasis 2.tumor angiogenesis | up | HCV-HCC from HC 0.792 HCV-HCC from HCV-CLD 0.645 | 26352740 | NA |

Continued
| Reported ID | Official ID | Sample Source | Ethnicity | Features | Expression | AUC | PMID | Validated Targets |
|------------|------------|---------------|-----------|----------|------------|-----|------|-------------------|
| miR-302c-3p | miR-302c-3p | 28 HC 26 CHC 30 HCV-positive cirrhosis 8 HCC | blood | Turkey | NA | up | NA | 25391771(41) | NA |
| miR-30c-5p | miR-30c-5p | 28 HC 26 CHC 30 HCV-positive cirrhosis 8 HCC | blood | Turkey | 1.HCV-positive cirrhosis | up | NA | 25391771(41) | NA |
| miR-331-3p | miR-331-3p | 40 HC 95 BLD 103 HCC | serum | China | 1.proliferation | up | 0.89 | 25903466(41) | PH |
| miR-34a | miR-34a-5p | 42 HC 125 HCV-CLD 112 HCV-CLD | blood | Egypt | child stage and BCLC score | up | HCV-HCC from HC 0.98 HCV-HCC from HCV-CLD 0.67 | 26352740(42) | NA |
| miR-375 | miR-375 | 156 HC 78 HCC | serum | China | tumor suppressor | down | 0.637 | 25618599(46) | NA |
| miR-375 | miR-375 | 210 HC 135 HBV 48 HCV 120 HCC | serum | China | 1.interferon-beta therapy | up | 0.96 | 21098710(49) | NA |
| miR-433-3p | miR-433-3p | 173 HC 233 LC 261 HCC | serum | China | NA | up | 0.887(training sets) 0.879(validation sets) | 25283283(46) | NA |
| miR-483-5p | miR-483-5p | 69 HC 69 HCC | serum | America | anti-apoptotic onco-gene | up | HCC from HC 0.827 | 24127413(39) | NA |
| miR-885-5p | miR-885-5p | 24 HC 23 CHB 26 LC 17 GC 9 ICC 6 FHN 46 HCC | serum | China | cholesterol reverse transport | up | 0.904 | 20815808(111) | NA |
| let-7b | let-7b-5p | 15 HC 30 DN 120 HCC | serum | China | tumor suppressor | up | 0.645 | 26264553(44) | NA |
| miR-203 | miR-203a-3p | 10 HC 30 non-cirrhotic HCV 25 HCV-related cirrhosis 23 HCV-CLD | serum | Egypt | 1.tumor-suppressive | down | HCC from non-HCC 0.76 | 27268654(41) | NA |
| miR-885-5p | miR-885-5p | 192 HCC 96 LC 96 CHC 95 HC | serum | Egypt | 1.onco-miR 2.liver injury | up | HCC from HC 0.63 HCC from LC 0.775 | 27271989(40) | ISRE |
| miR-122 | miR-122-5p | 193 HCC 96 LC 96 CHC 95 HC | serum | Egypt | 1.tumor suppressor 2.regulate lipid and cholesterol metabolism | up | HCC from HC 0.617 HCC from LC 0.617 | 27271989(40) | ADAM17 |
| miR-29b | miR-29b-3p | 194 HCC 96 LC 96 CHC 95 HC | serum | Egypt | tumor suppressor | down | HCC from HC 0.766 | 27271989(40) | NA |
| miR-221 | miR-221-3p | 195 HCC 96 LC 96 CHC 95 HC | serum | Egypt | 1.onco-miR 2.apoptosis | up | HCC from LC 0.702 | 27271989(40) | CDKN1B/p27 CDKN1C/p57 |
| miR-181b | miR-181b-5p | 196 HCC 96 LC 96 CHC 95 HC | serum | Egypt | 1.onco-miR 2.migration and invasion | up | HCC from LC 0.679 | 27271989(40) | TIMP3 |
| miR-22 | miR-22-3p | 197 HCC 96 LC 96 CHC 95 HC | serum | Egypt | tumor suppressor | down | HCC from CHC 0.586 | 27271989(40) | HDAC4 |
| miR-199a-3p | miR-199a-3p | 198 HCC 96 LC 96 CHC 95 HC | serum | Egypt | tumor suppressor | down | HCC from CHC 0.7 | 27271989(40) | mTOR |
| miR-125b | miR-125b-5p | 56 HC 63 CHB 59 HBV LC 64 HBV-HCC | plasma | China | 1.tumor suppressor 2.migration and invasion 3.cellular proliferation and cell cycle progression | down | HBV-HCC from HC 0.891 | 27152953(41) | LIN28B |
| miR-96 | miR-96-5p | 104 HCC 100 CHB 90 LC 120 HC | serum | China | 1.onco-miR 2.migration and invasion | up | HCC from CHB 0.803 | 26770453(42) | NA |
| miR-126 | miR-126-3p | 28 HC 20 LC 59 HCC | plasma | India | NA | up | low AFP HCC from non-HCC 0.765 low AFP HCC from LC 0.643 | 26756996(43) | APAF1, APC2, VEGFA, IRS1, CDKN2A |
| miR-224 | miR-224-5p | 26 HCC 22 LC 23 CHB 22 HC | serum | China | 1.migration and invasion 2.suppress apoptosis | up | 0.88 | 26724963(44) | NA |

**Table 2.** Diagnostic biomarkers in blood for hepatocellular carcinoma. Abbreviations and note: HC: healthy controls; CHB: patients with chronic type B hepatitis; CLD: chronic liver disease; HCV-CLD: non-malignant HCV-associated CLD patients; DN: chronic hepatitis B patients with pathologically proven DN; ICC: intraphepatic cholangiocellular carcinoma; LC: liver cirrhosis; HCV: hepatitis C virus HBV: hepatitis B virus; NA: not available; 1: upregulated in the HBV-LC group; 2: combined classifier (AFP and miRNA-122a); 3: combination of plasma miRNA-139 with serum AFP; 4: combined miR-15b and miR-130b; 5: Combined serum alpha-fetoprotein (AFP) and miR-24-3p.
diagnostic biomarkers could be used as classifiers for HCC detection, yielding a receiver operating characteristic curve area of 0.645 to 0.943. In tissues, with regard to up-regulated microRNAs in HCC tissues, high expression of miR-106b-5p, miR-125b-5p, miR-17-5p, miR-183-5p, miR-18b-5p, miR-21-5p, miR-25-3p, miR-331-3p, miR-9-5p and miR-96-5p were significantly correlated with invasion and metastasis. The expression level of miR-1269a in HCC patients without portal vein tumor embolus was reduced. In addition, the low expression of miR-125a-5p, miR-128-3p, miR-137, miR-185-5p, miR-188-5p, miR-26a-5p, miR-503-5p and miR-744-5p were detected in HCC tissues compared with their non-tumor livers and were involved in the multi-step processes. There were six circulating prognostic biomarker miRNAs reported to be associated with tissue invasion and metastasis, including miR-122-5p, miR-17-5p, miR-182-5p, miR-21-5p, miR-24-3p and miR-331-3p, all of them were up-regulated in the group with low survival rate. Meanwhile, the serum miR-150-5p was shown highly expressed in HCC patients after surgical operation and then low expressed after tumor relapsed.

**Tumor Promoted Inflammation.** Inflammation has been proved to be existed at the earliest stage of tumor processes and to be capable of fostering the progression of incipient neoplasia into advanced tumors. Besides chemicals, particularly reactive oxygen species were positively mutagenic for adjacent cancer cells, accelerating their genetic evolution towards the high malignant carcinoma. In blood, the increased expression of miR-30c-5p could be used as a new classifier for HCV-positive HCC in early-stage. In addition, hepatic necroinflammatory activity was associated with the high expression of miR-122-5p in plasma. The over expression of circulating miR-122-5p was a prognostic biomarker predicting the poor survival rate of patients underwent radio frequency ablation.

**Sustained Angiogenesis.** Both oxygen and nutrients transported by vasculature are essential for cell survival and function. All cells in tissues obligate to live within 100 μm of a capillary blood vessel. The evidence showed that cells with aberrant proliferative lesions tended to lack angiogenic ability at first, and led to the capability for expansion. The development of angiogenic ability is vital for incipient neoplasia growth. The over expression of circulating miR-296-5p was significantly associated with tumor angiogenesis. In tissues, high expression of miR-26a-5p could suppress tumor angiogenesis in HCC by targeting HGF-cMet signaling, and it was a novel prognostic biomarker for HCC.

**Limitless Replicative Potential.** There are three factors can lead to an uncoupling of the growth of a cell process from signals in their microenvironment, including insensitivity to antigrowth signals, resistance to apoptosis, and growth signal autonomy. Senescence, just like apoptosis, is as a protective system that could be activated by opposite growth signals or shortened telomeres that drives abnormal cells irreversibly into a G0-like state, and it could prevent further proliferation. The high expression of miR-182-5p, miR-18b-5p, miR-21-5p and miR-224-5p, together with the down-regulated expression of miR-101-3p and miR-139-5p not only played important roles in the regulation of cell proliferation and limitless replicative potential, but also were diagnostic signals for HCC.

**Genome Instability and Mutation.** Multi-step cancer progression could be described as a series of genic clonal expansions. Acquiring the chance of an enabling mutant gene triggered these clonal expansions. The widespread destabilization of genome is inherent to the vast majority of HCC cells. The high expression of miR-122-5p and low expression of miR-143-3p in blood were prominently correlated with differentiation and genome instability. They could be used as noninvasive circulating biomarkers for diagnosis of HCC. Up-regulated expression of miR-21-5p has been observed to be associated with genome instability and mutation, and it was a novel prognostic biomarker for HCC. Patients with high serum concentrations of miR-1-3p and miR-122-5p showed a long overall survival time and these miRNAs could be used to assess the HCC staging scores.

**Liver injury.** Biochemical molecules including miRNAs can be released into the circulation system due to the hypoxia and damage of liver cells. Accumulating reports indicated that serum miR-10b-5p, miR-122-5p, miR-18-5p, miR-192-5p, miR-223-3p and miR-885-5p were up expressed in patients with chronic hepatitis or HCC and they could serve as diagnostic biomarkers for liver injury but not specific for HCC.

**Tumor suppressor/onco-miR.** Genetic suppressor and carcinogenicity interpreted the function of miRNAs from another perspective. In tissues, high expression of miR-150-5p and miR-29a-3p and low expression of miR-101-3p, miR-126-3p, miR-127-3p, miR-139-5p and miR-214-3p played tumor-suppressor roles and could be used as diagnostic biomarkers for HCC. The circulating miR-101-3p, miR-125b-5p, miR-139-5p, miR-150-5p, miR-16-5p, miR-181a-5p, miR-199a-3p, miR-199a-5p, miR-203a-3p, miR-21-5p, miR-22-3p, miR-29b-3p, miR-375, let-7b-5p correlated with tumor suppressor and could be potential biomarkers to differentiate HCC from healthy controls.
miR-125b-5p, miR-130a-3p, miR-146a-5p, miR-214-3p and miR-99a-5p were considered as tumor suppressors in HCC and served as prognostic indicators for HCC\cite{38,99–104}. Serum miR-1-3p, miR-101-3p, miR-122-5p, miR-150-5p, miR-203a-3p and miR-30c-5p were associated with suppressing tumorigenicity and new independent parameters of overall survival in HCC\cite{38,49,60,77,91,105}.

The high expression of miR-130b-3p, miR-148a-3p, miR-181b-5p, miR-221-3p, miR-885-5p and miR-96-5p were functional in tumorigenicity and could be served as early diagnostic biomarkers for different tumor type\cite{34,106}. Meanwhile, miR-10b-5p, miR-130b-3p, miR-146a-5p, miR-18-5p, miR-195-5p, miR-196a-5p and miR-27a-3p were related to carcinogenicity and played vital roles in HCC detection\cite{10,34,42,59,94,107}. There were six miRNAs associated with oncogenicity and could be potential biomarkers for the overall survival of patients with HCC, including miR-1269a, miR-155-5p, miR-182-5p, miR-183-5p, miR-96-5p and miR-128-2\cite{66,72,108,109}.

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**Figure 2.** The Venn diagram of miRNA biomarkers for liver cirrhosis and HCC. Here circles in blue and red, respectively, represent miRNAs for cirrhosis and HCC. The miRNAs in red and green represent the up- and down-regulated expression, respectively. The miRNAs in purple means they showed inverse expression patterns in cirrhosis and HCC samples and those in black means their expressions were inconsistently up- or down-regulated according to different literature reports.

**Figure 3.** The Venn diagram of miRNA biomarkers for HBV/HCV-related HCC. Here miRNA biomarkers for HBV/HCV-related HCC were extracted from our collected dataset. Circles in blue and red, respectively, represent miRNAs for HBV-related HCC and HCV-related HCC. The miRNAs in orange and dark green represent the diagnostic and prognostic markers, respectively. The miRNAs in brown means they had both diagnostic and prognostic role according to different literature reports.
| Reported ID | Official ID | Sample | Ethnicity | Features | Expression | PMID | Validated Targets |
|------------|------------|--------|-----------|----------|------------|------|------------------|
| miR-101    | miR-101-3p | 20 HC 25 HBV-HCC | China | 1. HBsAg, HBV DNA level and tumor size | up | 24260081 | NA |
| miR-101    | miR-101-3p | 130 HCC | China | tumor suppressor | down | 23178713 | SOX9 |
| miR-101    | miR-101-3p | 30 HC 79 CHB 61 HBV-LC 67 HBV-HCC | China | 1. inhibit HCC cell proliferation | up | 24971953 | NA |
| miR-106b   | miR-106b-5p | 104 HCC | China | 1. tumor size | up | 25466449 | NA |
| miR-122    | miR-122-5p | 60 HCC | China | 1. tumor suppressor | down | 26252254 | PKM2 |
| miR-125b   | miR-125b-5p | 49 HCC | China | tumor suppressor | down | 24811246 | Eaf5a2 |
| miR-1269   | miR-1269a | 95 HCC | China | 1. tumor nodes | up | 25785048 | AGAP1, AGK, BPTE C160r74, DACT1, LIX1, RBMS3, ZNF706 and BMPER |
| miR-128-3p | miR-128-3p | 72 HCC | China | 1. suppress proliferation | down | 25962360 | PIK3R1 PI3K/AKT |
| miR-130a   | miR-130a-3p | 102 HCC | China | 1. gender, HBsAg status, tumor size, and TNM stage | down | 25218269 | NA |
| miR-137    | miR-137   | 136 HCC | China | 1. vein invasion | down | 24970808 | AKT2 |
| miR-146a   | miR-146a-5p | 85 HCC | China | tumor suppressor | down | 24172202 | ROCK1 |
| miR-155    | miR-155-5p | 100 HCC | China | 1. metastasis | up | 23863669 | NA |
| miR-155    | miR-155-5p | 216 HCC | China | 1. onco-miR | up | 22629365 | NA |
| miR-17-5p  | miR-17-5p | 120 HCC | China | 1. onco-miR | up | 22583011 | p38 MAPK-HSP27 |
| miR-182    | miR-182-5p | 81 HCC | China | 1. motility and invasiveness | up | 25813403 | FOXO1 |
| miR-183    | miR-183-5p | 81 HCC | China | 1. onco-miR | up | 25813403 | FOXO1 |
| miR-185    | miR-185-5p | 41 NTR 54 TR | China | 1. suppress the tumor cell growth | down | 23648054 | NA |
| miR-188-5p | miR-188-5p | 250 HCC | China | 1. suppress tumor cell proliferation | down | 25998163 | FGF5 |
| miR-18b    | miR-18b-5p | 110 HCC | Japan | 1. proliferation | up | 23496901 | TNRC6B |
| miR-199a-5p| miR-199a-5p | 120 HCC | China | 1. Negatively Associated With Malignancies | down | 26054020 | Hexokinase 2 |
| miR-206    | miR-206   | 147 HCC | China | 1. suppresses cell proliferation | down | 25513086 | NA |
| miR-21     | miR-21-5p | 50 HC 36 CH 136 HCC | Japan | 1. tumor differentiation | down | 21749846 | NA |
| miR-21     | miR-21-5p | 112 HCC | China | 1. tumor differentiation | up | 26261620 | NA |

Continued
Other clinicopathological features. Besides the above ten clinicopathological features and the hallmarks of cancer, biomarker miRNAs were also correlated with other clinicopathological features, such as secretion by primary cancer cells, child stage, cholesterol reverse transport, tumor size and recurrence, etc. Tomimaru et al. found that miR-21-5p was excessively secreted by primary cancer cells and could be a potential diagnostic biomarker for HCC110. Motawi and his colleagues identified that serum miR-34a-5p was correlated with child stage and BCLC score and could be used as an early biomarker for HCC in high-risk group42. The miR-885-5p and miR-122-5p in serum was reported related to cholesterol reverse transport and assessment of liver pathologies111.

In addition, miR-101-3p, miR-106b-5p, miR-130a-3p, miR-16-5p, miR-199a-5p, let-7f-5p and miR-34a-5p were found to have a significant correlation with tumor size in the tissue and serum of HCC patients50,64,102,112–114. The present literature also provided evidence that miR-130a-3p, miR-21-5p, miR-25-3p, miR-17-5p were independent prognostic factors and were associated with the TNM classification which is a universally accepted cancer staging system based on extension and size of the primary tumor (T), the adjacent lymph node (N), and the distant metastasis (M)68,69,78,102. The down-regulated expression of miR-774-5p and let-7f-5p can be considered as noninvasive biomarkers for predicting of the recurrence of HCC76,114.

Comparison of HCC biomarker miRNAs based on etiological factors and ethnic groups. Recently, accumulating evidence indicated that the occurrence and development of HCC are closely associated with etiological factors as well as ethnic groups. The differentiation between HCC and liver cirrhosis, for instance,
Table 4. Prognostic biomarkers in blood for hepatocellular carcinoma. Abbreviations and note: PVTT: portal vein tumor thrombosis; LC: liver cirrhosis; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; HC: healthy controls; CHB: patients with chronic type B hepatitis; BLD: benign liver diseases; ICC: intrahepatic cholangiocellular carcinoma; CH: chronic hepatitis; NA: not available.

| Reported ID | Official ID | Sample | Source | Ethnicity | Features | Expression | PMID | Validated Targets |
|-------------|-------------|--------|--------|-----------|----------|------------|------|-------------------|
| miR-1       | miR-1-3p    | 54 LC 195 HCC serum | Germany |           | 1. differentiation  
               2. tumor suppressor | up | 238102473 | NA |
| miR-101     | miR-101-3p  | 20 HC 25 HBV-HCC serum | China |           | 1. HBsAg, HBV DNA level and tumor size | up | 242600813 | NA |
| miR-101     | miR-101-3p  | 30 HC 79 CHB 61 HBV-LC 67 HBV-HCC serum | China |           | 1. inhibit HCC cell proliferation  
               2. tumor suppressor | up | 249719534 | NA |
| miR-122     | miR-122-5p  | 122 HCC blood | China |           | 1. tumor suppressor  
               2. proliferation  
               3. differentiation  
               4. regulation of cholesterol and lipid metabolisms  
               5. stability and propagation of hepatitis C virus and hepatitis B infection | up | 256364487 | NA |
| miR-122     | miR-122-5p  | 120 HCC plasma | South Korea |           | 1. hepatic necroinflammatory activity  
               2. cell death  
               3. tumor suppressor | up | 261298789 | NA |
| miR-150     | miR-150-5p  | 120 HC 110 CHB 120 HCC serum | China |           | 1. tumor suppressor  
               2. metastasis  
               3. BCLC stage  
               4. advanced TNM stages | down | 262159709 | NA |
| miR-16      | miR-16-5p   | 60 HC 90 HCC serum | China |           | 1. tumor size  
               2. liver dysfunction and coagulation defect | down | 2469711914 | NA |
| miR-16      | miR-16-5p   | 40 HCV 40 HCC serum | Egypt |           | 1. apoptosis  
               2. bilirubin | down | 261337254 | NA |
| miR-17-5p   | miR-17-5p   | 96 HCC blood | China |           | 1. metastasis  
               2. TNM stage | up | 231080867 | NA |
| miR-182     | miR-182-5p  | 40 HC 95 BLD 103 HCC serum | China |           | metastasis | up | 259034666 | TP53INP1 |
| miR-199a    | miR-199a-5p | 40 HCV 40 HCC serum | Egypt | tumor size | down | 261337259 | NA |
| miR-203a    | miR-203a-3p | 90 HCV 152 HCV-HCC serum | China | tumor suppressor | down | 2621045351 | NA |
| miR-21      | miR-21-5p   | 50 HC 30 CH 136 HCC serum | Japan |           | intrahepatic cholangiocarcinoma proliferation and growth | up | 258032299 | PTPN14 and PTEN |
| miR-21      | miR-21-5p   | 74 ICC serum | China |           | intrahepatic cholangiocarcinoma proliferation and growth | up | 258032299 | PTPN14 and PTEN |
| miR-24-3p   | miR-24-3p   | 46 HC 31 CLD 84 HCC serum | China | vascular invasion | up | 251293124 | NA |
| miR-30c     | miR-30c-5p  | 90 HCV 152 HCV-HCC serum | China | tumor suppressor | down | 2621045351 | EMT |
| miR-331-3p  | miR-331-3p  | 40 HC 95 BLD 103 HCC serum | China | 1. proliferation  
               2. metastasis | up | 259034666 | NA |
| miR-335     | miR-335-5p  | 125 HC 125 HCV/ HBV 125 HCC serum | China | response to TACE and clinical outcome | down | 263050264 | NA |
| let-7f      | let-7f-5p   | 60 HC 90 HCC serum | China | 1. tumor size  
               2. early recurrence | down | 2469711914 | NA |

is one of the main problems for the early detection of HCC. Moreover, different etiological factors such as HBV (Hepatitis B Virus) and HCV (Hepatitis C Virus) can also contribute to the HCC carcinogenesis. On the other hand, the incidence and mortality of HCC often showed different patterns among different ethnic groups. Hence it is necessary to compare HCC biomarker miRNAs based on etiological factors and ethnic groups.
Biomarker miRNAs for classifying of HCC and liver cirrhosis. After manually searching for citations in PubMed, a total of 13 miRNA biomarkers for liver cirrhosis diagnosis were collected (see Table S1). We then compared them with HCC diagnostic miRNA biomarkers in order to screen key signatures for HCC early detection. As shown in Fig. 2, eight miRNAs, i.e. miR-106b-5p, miR-122-5p, miR-141-3p, miR-181b-5p, miR-18a-5p, miR-19a-3p and miR-21-5p, were shared by cirrhosis and HCC. Interestingly, three of them (miR-106b-5p, miR-18a-5p and miR-21-5p) showed inverse expression patterns in cirrhosis and HCC groups. For example, the expression of miR-106b-5p (miR-106b) was down in cirrhosis samples whereas it turned out to be up-regulated in the blood of HCC patients. In addition, miR-19a-3p (miR-19a) was reported as a useful molecular marker for monitoring the progression of liver fibrosis to cirrhosis and finally, to HCC. Han et al. found that two miRNAs, i.e. miR-224 (miR-224-5p) and miR-214 (miR-214-3p), were significantly up- and down-regulated in HCC tissue samples respectively, which provided novel biomarker signatures for HCC diagnosis and treatment.

It can be concluded that biomarker miRNAs revealed the pathogenesis of cirrhosis and HCC at the post-transcriptional level and could help deeply understand the differentiation between cirrhosis and HCC. From the perspective of precision medicine, HCC miRNA biomarkers, especially those specific to HCC, were indicators for capturing the early diagnostic signatures at the time of HCC initiation.
| Reported ID | Official ID | Sample | Source | Ethnicity | Features | Expression | PMID     | Validated Targets |
|------------|------------|--------|--------|-----------|----------|------------|----------|------------------|
| miR-335    | miR-335-5p | 62 HCC | tissue | China     | inhibit the proliferation and migration invasion | down      | 25804796 | ROCK1           |
| miR-192    | miR-192-5p | 59 HC 59 HCC | tissue | South Korea | increase tumor cell migration and invasion | down      | 25065598 | NA               |
| miR-224    | miR-224-5p | 9 HC 10 HCC | tissue | China     | cell proliferation s 2. migration 3. invasion 4. anti-apoptosis | up        | 24789420 | CD40             |
| miR-214    | miR-214-3p | 9 HC 10 HCC | tissue | China     | tumor suppressor | down      | 24789420 | EZH2, CTNNB1 and CDH1 |
| miR-148a   | miR-148a-3p | 19 HCC | tissue | China     | onco-miR | up        | 22496017 | NA               |
| miR-206    | miR-206    | 147 HCC | tissue | China     | 1. suppress cell proliferation 2. promote apoptosis. | down      | 25510886 | NA               |
| miR-331-3p | miR-331-3p | 457 HCC | tissue | China     | 1. promote proliferation 2. metastasis | up        | 24825302 | Leucine-Rich Repeat Protein Phosphatase |
| miR-26a    | miR-26a-5p | 120 HCC | tissue | China     | 1. cell Cycle 2. angiogenesis | up        | 24259426 | CDK6, cyclin D1   |
| miR-26a    | miR-26a-5p | 130 HCC | tissue | China     | 1. suppress the tumor cell growth 2. suppress invasive | down      | 23389848 | interleukin-6-Stat3 |

Table 5. Therapeutic biomarkers for hepatocellular carcinoma. Abbreviations and note: HC: healthy controls; NA: not available.

Biomarker miRNAs for monitoring the development of HBV/HCV-related HCC. It has been widely acknowledged that the progression of HCC is closely affected by the infection of etiological factors, such as HBV, HCV, etc. On the other hand, miRNAs are reported to play crucial roles in HBV/HCV replication and pathogenesis117–119, i.e. they regulated HBV by directly binding to HBV transcripts or changing HBV gene expression at the transcriptional level118. For better investigating the influence of HBV/HCV on HCC development, miRNA biomarkers for HBV/HCV-related HCC were extracted from our collected dataset. As illustrated in Fig. 3, several miRNAs, i.e. miR-122-5p, miR-126-3p, miR-143-3p, miR-192-5p, etc., were functional in both HBV- and HCV-related HCC evolutionary progression. For example, Tan et al. found that serum miR-122-5p could be used as the diagnostic biomarker for detecting HBV-related HCC. Both the area under the receiver operating characteristic curve (AUC) and logistic regression model convinced the predictive power86. Meanwhile, the miRNA was also turned out to be effective for early detection of HCC on top HCV infection. Using the miRNA panel where miR-122-5p included, HCC patients could be classified from healthy controls and liver cirrhosis patients with high diagnostic accuracy120.

There is still a large number of biomarker miRNAs that could be specifically used for monitoring the development of HBV/HCV-related HCC. Chen et al. analyzed the plasma samples from 242 individuals and uncovered that the expression of miR-125b-5p (miR-125b) was significantly down-regulated in HBV-induced HCC (HBV-HCC) patients compared to healthy controls as well as HBV groups without HCC121. Moreover, the low plasma level of miR-125b-5p also reflected the higher possibility of metastasis. Therefore, the miRNA held promise as a valuable diagnostic biomarker for HBV-HCC and HBV-infected patients with high HCC risks could be early detected by dynamically monitoring the changes of this miRNA. Liu et al. demonstrated that the expression levels of miR-30c-5p (miR-30c) and miR-203a-3p (miR-203a) were crucial indicators for predicting the poor prognosis of HCV-related HCC because the core protein of HCV could down-regulate the expression of miR-30c-5p and miR-203a-3p, resulting in the activation of epithelial-mesenchymal transition in normal hepatocytes as well as HCC tumor cells. As reported before, the activation process may contribute to the carcinogenesis of HCC108.

Understanding the pathogenesis of miRNA biomarkers in HBV/HCV-related HCC provided insights to evaluate the potential effects of HBV/HCV on HCC development, which will be helpful to the early and personalized detection of HCC.

HCC miRNA biomarkers within different ethnic groups. Genomic profiling of HCC tumors showed that HCC patients in different geographic regions tended to have specific recurrent molecular aberrations122. Asians, on the whole, achieved the highest HCC incidence according to the report by Wong et al.123. In terms of prognosis, the overall survival rate was also disparate among different ethnic groups124. Here we reorganized HCC miRNA biomarkers based on the ethnicity of patients described in each citation. As illustrated in Fig. 4a, most of the reported HCC miRNA biomarkers were related to Chinese population, which indirectly indicated the high risk or high incidence of HCC in China. For further exploring the ethnic specificity of HCC miRNA biomarkers, we then partitioned miRNAs into two categories based on the patient race, i.e. Asian-related (Chinese, Japanese, South Korean, Indian and Iranian) and non-Asian-related (Egyptian, American, Turk and German) HCC miRNA biomarkers. As shown in Fig. 4b, the number of Asian-specific HCC miRNA biomarkers is far more than that of non-Asian. We noticed that some miRNAs were reported to be functional in both Asian and non-Asian group.

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However, the expression pattern of them was sometimes quite different when they were involved in different pathogenic processes or belonged to different ethnic groups. For example, miR-125b-5p was associated with the biological behavior of HCC and had the diagnostic value of HCC for both Turks and Chinese. As in plasma samples of Chinese patients, it was found to be down-regulated whereas in Turks samples, its expression level was

Figure 5. Top 10 pathways significantly enriched with targets of different biomarker miRNAs from HCC tissue and blood. Sub-figure (a), (b), and (c) represent pathways enriched by targets of diagnostic, prognostic and therapeutic biomarker miRNAs, respectively. The statistical significance level (p-value) was negative 10-based log transformed.

However, the expression pattern of them was sometimes quite different when they were involved in different pathogenic processes or belonged to different ethnic groups. For example, miR-125b-5p was associated with the biological behavior of HCC and had the diagnostic value of HCC for both Turks and Chinese. As in plasma samples of Chinese patients, it was found to be down-regulated whereas in Turks samples, its expression level was
up. For comparison of Egyptian and Chinese, the down-regulation of miR-146a-5p was correlated with HCC carcinogenesis and deterioration in Chinese population, but in samples of Egyptian patients, it was inverse.

This ethnic difference may be caused by the heterogeneous pathogenesis, lifestyles and various factors including diet, environmental exposures, etc. Moreover, the incidence of HBV/HCV infection in different countries is also inconsistent. Therefore, more in-depth researches on ethnically specific miRNA biomarkers is of clinical significance, which would provide personalized strategies for HCC diagnosis and treatment in the era of precision medicine.

Pathway enrichment analysis for targets of HCC miRNA biomarkers. We performed the pathway enrichment analysis for targets of different types of reported miRNA biomarkers using IPA program. Here the targets of miRNA biomarkers originated from seven publicly available miRNA-target databases, including four experimentally validated databases and three computationally predicted databases (see Methods). For the three categories, i.e. the diagnostic, prognostic and therapeutic biomarker miRNAs, the top 10 significantly enriched pathways (p-value < 0.01) were chosen and shown in Fig. 5. The common enriched pathways among them were Molecular Mechanisms of Cancer, Glucocorticoid Receptor Signaling, HGF Signaling, NGF Signaling, p53 Signaling etc. Most of them are well-studied cancer associated pathways. Das et al. reported that the pathway Molecular Mechanisms of Cancer was potentially associated with recurrent HCC secondary to HCV following liver transplantation. Glucocorticoids are involved in controlling many essential biological processes that are related to energy supply and growth control. The Glucocorticoid Receptor often functions as a cofactor of transcription factor STAT5 for growth hormone induced genes and Glucocorticoid Receptor Signaling has been turned out to be important in body growth, steatosis and metabolic liver cancer development. The experimental result in mouse model demonstrated that the metabolic dysfunction and impairment of Glucocorticoid Receptor Signaling could cause steatosis and HCC in mice. Wu et al. revealed that the HGF signaling could be activated by over expression of gene C1GALT1 in HCC via modulation of MET O-glycosylation and dimerization, which offered new insights into O-glycosylation and HCC pathogenesis. Lin et al. indicated that p53 Signaling pathway was significantly dysregulated in HCC and it could reflect the development and progression of HCC. Moreover, a number of genes participated in regulating human HCC by interacting with p53 Signaling pathway. For instance, the key gene RASSF10, which is located on chromosome 11p15.2, could suppress the growth of HCC via activating p53 Signaling pathway. EGR1 is one of the key components in p53 Signaling, the re-expression of gene BCL6B in HCC cells could increase its expression and finally contribute to the activation of p53 Signaling.

Discussion
In this review, we made comprehensive functional survey and comparison of HCC diagnostic, prognostic and therapeutic miRNAs in blood and tissues. The number of diagnostic miRNA biomarkers in blood is approximately twice as much as those in tissues and meanwhile, the number of prognostic miRNA biomarkers in tissues is twice as much as those in blood. The reason for the statistical difference may be that many studies are inclined to investigate the noninvasive diagnostic miRNA biomarkers and researchers tend to use relatively stable hepatogenic biomarkers as prognostic indicators because miRNAs may be released into the blood selectively. Most of the diagnostic, prognostic and therapeutic miRNA biomarkers are associated with one or two clinic pathological features in blood and tissues. A great number of prognostic biomarkers with high expression levels were detected in patients with shorter overall survival. Since the etiological factors as well as ethnic groups are closely associated with HCC carcinogenesis, we analyzed miRNA biomarkers by taking the HBV/HCV infection as well as regional variations into account in order to provide better clues for HCC pathogenic research. We mainly selected miRNAs which were explicitly reported as HCC markers/biomarkers in our current study. Besides, several miRNAs are still common and important during HCC development. For example, miR-142-3p was functional in HCC tumorigenesis and played a key role in regulating human RAC1 gene. The upregulation of miR-142-3p inhibited the expression level of RAC1 mRNA, suppressing the migration and invasion of HCC cells. Interferon regulatory factor-1 (IRF-1) is a tumor-suppressor in HCC and its down-expression would help HCC tumors evade death. Yan et al. found that miR-23a was a negative regulator of IRF-1 in HCC, which highlighted its importance in HCC initiation and progression. Zhang et al. demonstrated that miR-99a could directly regulate AGO2 and control tumor growth in HCC, indicating the potential strategies for HCC treatment.

HCC is a complex disease which is difficult for early diagnosis and treatment. The death rate of HCC remains high due to its poor prognosis. To some extent, miRNAs are effective biomarkers for HCC because of the noninvasive detection, good specificity and sensitivity. More systematic investigations and clinical experiments need to be done for better understanding the role and function of miRNA biomarkers in HCC pathogenesis.

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S.S. and Y.L. contributed equally to the work. S.S. and Y.L. collected and analyzed the data; X.Y., L.S. and L.C. performed the computational analyses; S.S., Y.L., J.C. and B.S. wrote the manuscript; B.S. and L.Q. conceived and supervised the work jointly.

**Additional Information**

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