Review Article

Dysregulated microRNAs with prognostic significance in human hepatocellular carcinoma: a study based on high-throughput microarray

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Abstract: Hepatocellular carcinoma (HCC) is the fifth most widespread type of malignancies with only 18% of post-operative 5-year survival. However, the prognostic value of microRNAs (miRNAs) for HCC has not been fully studied. In this study, we aimed to identify the potential candidates of dysregulated miRNAs for HCC prognosis in the profiling studies based on high-throughput microarray. The literature and NCBI Gene Expression Omnibus (GEO) database were used to identify HCC-related miRNAs. Based on the ranking results of a vote-strategy method, the most constantly stated dysregulated miRNAs were chosen as the predictive biomarkers for HCC patients’ survival. For meta-analysis on prognosis, eligible studies were searched and sufficient data were collected adequately. Pooled hazard ratios (HRs) with 95% confidence intervals (CIs) were used to estimate the effect size. The top six upregulated and six downregulated miRNAs were identified based on 43 published miRNA profiling studies. Additional 30 studies involving 2735 HCC patients with follow-up data were also included. Among the top 12 dysregulated miRNAs, miR-222, miR-25, miR-221, miR-21, miR-214, miR-199a-3p and miR-199a-5p were closely associated with HCC patients’ survival. Both univariate and multivariate results suggested that the low quality of life for HCC patients was significantly related with high miR-21, miR-221, miR-25 and low miR-199a-3p, miR-214. In conclusion, miR-222, miR-221, miR-21 and miR-25 were significantly related with dismal prognosis for HCC patients, and miR-199a-3p and miR-214 emerged as significant predictors of unfavorable prognosis for HCC patients. These miRNAs may serve as potential candidates for HCC prognosis and therapy.

Keywords: MicroRNAs, gene expression profiling, hepatocellular carcinoma, prognosis, biological markers, meta-analysis

Introduction

Hepatocellular carcinoma (HCC) ranks the fifth in the most common types of malignant tumors all over the world and HCC is the second leading cause of cancer-related death globally as well [1]. Almost 500,000 new cases of HCC occurred every year in the Asia-Pacific region, with estimated 360,000 deaths in Far East countries only [2]. China alone accounted for over 60% of the total HCC cases, most of which were related to chronic hepatitis B virus (HBV) infection [3, 4]. The high mortality with almost 0.7 million deaths from HCC each year is probably attributed to the failure of survival prediction and the delayed treatment for patients [5-8]. Although surgical resection, radiation and chemotherapy are optional treatments for a potential cure currently, many cases of patients may still develop recurrence, which converts the situation to a poor prognosis, leading to a postoperative 5-year survival of 18% [9-13]. Due to the lack of potential predictor for diagnosis and prognosis in early stages, a majority of HCC patients miss the best operation time and suffer a poor postoperative survival [14-17]. It is thus urgent to identify novel prognostic targets and biomarkers for HCC and demonstrate the mechanisms underlying HCC in molecular level.

MicroRNAs (miRNAs), which belong to a cluster of endogenously expressed, non-coding small
Dysregulated microRNAs with prognostic significance in HCC

RNAs with approximately 22 nucleotides, control gene expression by targeting mRNA and trigger either RNA degradation or translation repression [18]. These multistep biological programs are closely associated with the regulation of various cellular processes such as cell proliferation, death and tumor carcinogenesis [19]. Growing evidence indicates that dysregulation of miRNAs can be involved in the invasion and metastasis in various cancers, especially in HCC [20-26]. Therefore, the roles of miRNAs in liver cancers made it possible to be promising biomarkers to accurately predict prognosis and to be potential targets for high-efficient treatment.

Nowadays high-throughput technologies were globally used to explore miRNA expression across diverse tumor and corresponding non-tumor tissues, which contributed to the identification of specific aberrant miRNAs involved in oncogenesis. However, with more complex information on the importance of miRNAs in HCC provided by accumulating findings, some miRNAs were reported to express in an inconsistent direction among the profiling studies partly because of small sample size. To overcome the limitations in current studies and increase the statistical persuasiveness, our meta-analysis was performed, which combined the results of several individual studies and improved the precision and accuracy of estimation. Firstly, we performed a systematic review of 43 published studies that compared the miRNAs expression in HCC tissues with that in normal liver tissues, and then made efforts to get complicated information of miRNA expression data from these disparate original profiling datasets and integrated them systematically. Subsequently, our meta-analysis was carried out to identify the miRNAs correlated with HCC prognosis and their targeting molecules with relevant pathways, which may provide potential biomarkers for HCC development.

Materials and methods

Search strategy

MiRNA profiling studies on prognosis of HCC were searched on databases including PubMed, Web of Science, Wiley Online Library, EMBASE, Ebso, Chinese National Knowledge Infrastructure (CNKI), Chinese Chong Qing Yangtze Island Club (VIP) and Chinese Wan Fang. The search strategy was built by means of the MeSH terms up to date Aug 16th, 2015: 'miRNA OR microRNA OR miR' in combination with the keyword 'neoplas* OR tumor OR malignant* OR carcinoma OR cancer' and ‘HCC OR hepatocellular OR liver OR hepatic’.

Inclusion criteria of the literature

For microarray studies, eligible studies were included if they met the principles: (i), miRNA profiling studies in HCC; (ii), tissue samples gained surgically including resected liver tumor...
Dysregulated microRNAs with prognostic significance in HCC

Table 1. Characteristics of 43 miRNA profiling studies included in this systemic review

<table>
<thead>
<tr>
<th>First author (reference)</th>
<th>Year</th>
<th>Origin</th>
<th>Period</th>
<th>No. of collected tissue samples (tumor/normal)</th>
<th>Cut-off Criteria</th>
<th>Total</th>
<th>Up-regulated miRNAs in HCC</th>
<th>Down-regulated miRNAs in HCC</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang [1]</td>
<td>2014</td>
<td>Shanghai, China</td>
<td>NR</td>
<td>85 (75/10)</td>
<td>FC&gt;3</td>
<td>13</td>
<td>8</td>
<td>5</td>
<td>MRCURY™ LNA Array (v.11.0)</td>
</tr>
<tr>
<td>Zhang [2]</td>
<td>2015</td>
<td>Hong Kong SAR, China</td>
<td>Up to Feb.2015</td>
<td>327 (327/43)</td>
<td>FDR&lt;0.001, FC&gt;3</td>
<td>50</td>
<td>13</td>
<td>37</td>
<td>Illumina HiSeq 2000 miRNA sequencing Platforms (Illumina Inc., San Diego, CA)</td>
</tr>
<tr>
<td>Park [3]</td>
<td>2015</td>
<td>Korea</td>
<td>NR</td>
<td>16 (9/7)</td>
<td>FC&gt;1.5, P&lt;0.05</td>
<td>267</td>
<td>10'</td>
<td>10'</td>
<td>MiRNA 3.0 Array; DNA Link</td>
</tr>
<tr>
<td>Li [4]</td>
<td>2013</td>
<td>Tianjin, China</td>
<td>Apr.2003 to Aug.2010</td>
<td>22 (11/11)</td>
<td>FC&gt;2, P&lt;0.05</td>
<td>18</td>
<td>9</td>
<td>9</td>
<td>RT² miRNA PCR array</td>
</tr>
<tr>
<td>Wei [5]</td>
<td>2013</td>
<td>Guangzhou, China</td>
<td>2004 to 2007</td>
<td>220 (110/110)</td>
<td>FDR = 0, P&lt;0.1</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>Custom microarray</td>
</tr>
<tr>
<td>Murakami [6]</td>
<td>2006</td>
<td>Japan</td>
<td>NR</td>
<td>46 (24/22)</td>
<td>P&lt;0.001</td>
<td>30</td>
<td>3</td>
<td>5</td>
<td>Human miRNA microarray</td>
</tr>
<tr>
<td>Gramantieri [8]</td>
<td>2007</td>
<td>American</td>
<td>NR</td>
<td>38 (17/21)</td>
<td>FC&gt;1.3, P&lt;0.05</td>
<td>35</td>
<td>1</td>
<td>34</td>
<td>KCC/TJU miRNA microarray chip</td>
</tr>
<tr>
<td>Li [9]</td>
<td>2008</td>
<td>Jiangsu Province, China</td>
<td>NR</td>
<td>177 (78/88)</td>
<td>FRD&lt;0.01</td>
<td>69</td>
<td>29</td>
<td>40</td>
<td>NR</td>
</tr>
<tr>
<td>Li [10]</td>
<td>2014</td>
<td>Xi’an, China</td>
<td>2010 to 2012</td>
<td>6 (3/3)</td>
<td>P&lt;0.05</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>μParaffin microfluidic chip</td>
</tr>
<tr>
<td>Shen [11]</td>
<td>2015</td>
<td>Columbia</td>
<td>NR</td>
<td>20 (10/10)</td>
<td>FRD&lt;0.05</td>
<td>25</td>
<td>6</td>
<td>19</td>
<td>TaqMan Low Density Arrays (TLDA, Applied Biosystems, Foster City, CA)</td>
</tr>
<tr>
<td>Liu [12]</td>
<td>2011</td>
<td>Hong Kong</td>
<td>1990 to 2007</td>
<td>188 (94/94)</td>
<td>P&lt;0.05</td>
<td>9</td>
<td>7</td>
<td>2</td>
<td>Custom Affymetrix array, RM-HU01Aa520485 RSTA Custom Affymetrix 1.0</td>
</tr>
<tr>
<td>Chung [13]</td>
<td>2010</td>
<td>Korea</td>
<td>2001 to 2004</td>
<td>50 (25/25)</td>
<td>FRD&lt;0.05, P&lt;0.05</td>
<td>21</td>
<td>14</td>
<td>7</td>
<td>MiRNA microarray slide consisting of 308 probes for human miRNAs from Rosetta Genomics Corp</td>
</tr>
<tr>
<td>Elyakim [14]</td>
<td>2010</td>
<td>South Korea</td>
<td>NR</td>
<td>60 (30/30)</td>
<td>FC&gt;3, P&lt;0.01</td>
<td>35</td>
<td>27</td>
<td>8</td>
<td>Custom microarrays</td>
</tr>
<tr>
<td>Wang [15]</td>
<td>2012</td>
<td>Shanghai, China</td>
<td>NR</td>
<td>6 (3/3)</td>
<td>FC&gt;3</td>
<td>86</td>
<td>10'</td>
<td>13'</td>
<td>Taqman low density miRNA array (TLDA)</td>
</tr>
<tr>
<td>Katayama [16]</td>
<td>2012</td>
<td>Tokyo</td>
<td>Nov.2005 to May.2008</td>
<td>46 (40/6)</td>
<td>FC&gt;1.5, P&lt;0.05</td>
<td>48</td>
<td>18</td>
<td>30</td>
<td>MiRNA microarray using 3D-Genie (Toray Industries, Tokyo, Japan)</td>
</tr>
<tr>
<td>Han [17]</td>
<td>2014</td>
<td>Chongqing, China</td>
<td>NR</td>
<td>19 (10/9)</td>
<td>FRD&lt;0.05, FC(logFC)&gt;1</td>
<td>32</td>
<td>14</td>
<td>18</td>
<td>Homo sapiens miRNA profiling platform version 4 (Luminex, New York, NY, USA)</td>
</tr>
<tr>
<td>Gao [18]</td>
<td>2015</td>
<td>Guangxi, China</td>
<td>Jan.2012 to Dec.2013</td>
<td>60 (30/30)</td>
<td>FC&gt;2.0</td>
<td>33</td>
<td>21</td>
<td>12</td>
<td>Agilent 8x60K microarray</td>
</tr>
<tr>
<td>Huang [19]</td>
<td>2007</td>
<td>Shenzhen, China</td>
<td>Mar.2007 to Jul.2007</td>
<td>20 (10/10)</td>
<td>FC&gt;2, P&lt;0.05</td>
<td>16</td>
<td>15</td>
<td>1</td>
<td>MiRNA microarray chips</td>
</tr>
<tr>
<td>Meng [20]</td>
<td>2007</td>
<td>American</td>
<td>NR</td>
<td>6 (3/3)</td>
<td>P&lt;0.05</td>
<td>15</td>
<td>9</td>
<td>6</td>
<td>MiRVana miRNA Bioarrays</td>
</tr>
<tr>
<td>Su [21]</td>
<td>2009</td>
<td>Guangzhou, China</td>
<td>2005 to 2006</td>
<td>8 (5/3)</td>
<td>FC&gt;2, P&lt;0.05</td>
<td>29</td>
<td>14</td>
<td>15</td>
<td>CapitalBio human/mouse/rat non-coding RNA microarray</td>
</tr>
<tr>
<td>Burchard [22]</td>
<td>2010</td>
<td>Hong Kong</td>
<td>1990 to 2007</td>
<td>192 (96/96)</td>
<td>P&lt;0.05 OR P&lt;0.01</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>Custom Affymetrix array, RM-HU01Aa520485 RSTA Custom Affymetrix 1.0</td>
</tr>
</tbody>
</table>
### Dysregulated microRNAs with prognostic significance in HCC

**Shih [23]**
- **2012**
- **Taiwan**
- **1999 to 2000**
- **89 (68/21)**
- **FC>1.5, FDR<10^-3, P<0.01**
- **Sentrix Array Matrix (Illumina, San Diego, CA)**

**Yang [24]**
- **2010**
- **China**
- **NR**
- **13 (8/5)**
- **FC>2, P<0.05**
- **Taqman low density miRNA array (TLDA)**

**Wang [25]**
- **2012**
- **Shanghai, China**
- **NR**
- **6 (3/3)**
- **FC>3, P<0.05**
- **GeneChip miRNA 2.0 Array (Affymetrix, Santa Clara, CA, USA)**

**Xia [26]**
- **2013**
- **Singapore**
- **NR**
- **30 (20/10)**
- **NR, P<0.05**
- **Taqman Low Density Array (TLDA)**

**Li [27]**
- **2014**
- **Shanghai, China**
- **Jun.2010**
- **106 (53/53)**
- **P<0.05**
- **MiRNA array analysis (Western Technology, Inc., Chongqing, China)**

**Yang [24]**
- **2010**
- **China**
- **NR**
- **13 (8/5)**
- **FC>2, P<0.05**
- **23 MiRNA microarrays by LC sciences**

**Wang [25]**
- **2012**
- **Shanghai, China**
- **up to Jan.2010**
- **486 (272/214)**
- **P<0.05, FR>0.05**
- **CapitalBio Mammalian miRNA Array Services V1[1].0; CapitalBio human/mouse/rat non-coding RNA microarray**

**S.El-Halawany [34]**
- **2015**
- **Egypt**
- **2006 to 2010**
- **15 (15/10)**
- **FC>3, P<0.05**
- **Taqman MirNA assays human panel early access kit (Applied Biosystems)**

**Yu Wang [35]**
- **2008**
- **Singapore**
- **NR**
- **38 (19/19)**
- **FRD<0.05**
- **Bead-based miRNA expression profiling**

**Huang [36]**
- **2009**
- **Guangzhou, China**
- **2004 to 2006**
- **40 (20/20)**
- **P<0.05**
- **Agilent human miRNA microarray release 14.0**

**Murakami [37]**
- **2014**
- **Japan**
- **NR**
- **17 (11/6)**
- **p<0.05**
- **Illumina BeadArray (human v2 miRNA panel)**

**Hung [38]**
- **2014**
- **Taiwan**
- **NR**
- **58 (29/29)**
- **FC>2 or <0.5, P<0.05**
- **LNA™ miRNA array (11.0)**

**Ma [39]**
- **2009**
- **China**
- **Sept.2007 to Aug.2008**
- **2 (1/1)**
- **NR**
- **Multi-analyte suspension array**

**Sun [40]**
- **2006**
- **China**
- **Jan.2005 to Jul.2005**
- **40 (20/20)**
- **P<0.01**
- **Paraflom™ MiRNA array by LC sciences**

**Wang [41]**
- **2010**
- **China**
- **Jun.2006 to Oct.2008**
- **50 (25/25)**
- **FC>4, FRD<0.05**
- **Affymetrix GeneChip miRNA Arrays (Genisphere, FT30AFYB)**

**Xiao [42]**
- **2012**
- **China**
- **NR**
- **FC>2, P<0.01**
- **213 MiRNA microarrays by LC sciences**

**Fan [43]**
- **2013**
- **China**
- **Jul.2011 to Sept.2013**
- **18 (9/9)**
- **FC>2 or <0.5, P<0.05**
- **References* here were shown in Supplementary Files.**
Table 2. Deregulated miRNAs (n = 12) consistently reported in 43 profiling studies (HCC tissues versus normal tissues)

<table>
<thead>
<tr>
<th>Direction of expression</th>
<th>MiRNA name</th>
<th>No. of studies with same direction (reference*)</th>
<th>No. of tissue samples tested</th>
<th>Subset of studies with fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of studies</td>
<td>No. of tissue samples tested</td>
<td>Mean FC</td>
</tr>
</tbody>
</table>
| Upregulated             | MiR-222     | 17 (7, 9, 14, 16, 17, 20, 21, 23, 24, 28, 30, 31, 34, 35, 36, 40, 43) | 712 | 14 | 455 | 5.54 | 1.57–17.42 
|                         | MiR-221     | 15 (5, 7, 8, 9, 10, 14, 16, 20, 30, 31, 34, 35, 36, 41, 43) | 845 | 13 | 428 | 5.67 | 1.49–39.43 
|                         | MiR-224     | 14 (1, 2, 6, 9, 16, 17, 21, 26, 30, 31, 32, 35, 36, 40) | 982 | 12 | 487 | 27.80 | 1.81–117.46 
|                         | MiR-21      | 14 (5, 7, 10, 14, 16, 17, 19, 20, 30, 31, 32, 34, 35, 43) | 592 | 12 | 312 | 6.05 | 1.67–31.63 
|                         | MiR-25      | 12 (4, 9, 13, 16, 21, 28, 30, 31, 35, 42, 43) | 524 | 10 | 336 | 2.38 | 1.32–6.55 
|                         | MiR-18a     | 10 (4, 5, 9, 14, 16, 21, 29, 30, 34, 43) | 679 | 8 | 286 | 3.49 | 1.43–9.05 
| Downregulated           | MiR-199a-5p | 23 (2, 3, 6, 8, 9, 13, 14, 15, 20, 21, 23, 24, 25, 30, 31, 32, 34, 36, 38, 39, 40, 41, 42) | 1173 | 12 | 530 | 0.28 | 0.00–0.57 
|                         | MiR-99a     | 16 (9, 10, 11, 14, 17, 18, 21, 24, 29, 30, 31, 33, 34, 39, 42, 43) | 1037 | 11 | 916 | 0.38 | 0.13–0.78 
|                         | MiR-195     | 16 (2, 6, 8, 9, 13, 14, 17, 21, 24, 30, 31, 32, 36, 39, 40, 43) | 964 | 11 | 488 | 0.43 | 0.10–0.75 
|                         | MiR-130a    | 15 (2, 8, 9, 11, 13, 14, 17, 24, 28, 29, 30, 31, 32, 34, 39) | 899 | 11 | 456 | 0.34 | 0.10–0.65 
|                         | MiR-199a-3p | 14 (4, 6, 9, 11, 13, 14, 23, 29, 30, 31, 32, 34, 41, 43) | 713 | 9 | 348 | 0.37 | 0.16–0.67 

Abbreviations: FC, fold change. References* here were shown in Supplementary Files. 

For prognostic studies, two independent investigators (Zhong Tan, Huo-Jie Xiong) reviewed the literature quantitatively with the same multi-step procedure as mentioned above. Firstly, the abstracts helped eliminate the duplicated or irrelevant studies. Then, full-text of the rest articles were further reviewed by the authors individually to make a decision whether to include based on the inclusion criteria listed as follows: (i), samples should be accumulated from HCC patients; (ii), studies published in either English or Chinese should investigate the relationship between miRNAs and survival data; (iii), presented data to analyze hazard ratios (HRs) value and its 95% confidence interval (95% CI) should be offered. Additionally, trails with either animals or cell lines, case reports, letters and reviews were excluded. If survival analyses were presented in the studies with inadequate information to analyze the HR value, we would make efforts to contact the authors to acquire primary survival data wherever and whenever possible.

Data abstraction

Differentially expressed miRNAs were identified from each included profiling study. The subsequent information was gathered from each study: first author, year of publication, characteristics of recruited HCC patients, defined cut-off criteria, fold change (if available) of differentially expressed miRNAs, and platform of miRNA detected. Then, the miRNAs ranking-based method for ranking potential molecular markers by Chan and Griffith [28, 29], was adopted in the systematic review, which included: (i), number of the studies that reported the same miRNA in an accordant direction; (ii), total number of patients in the study; (iii), mean fold change calculated from the originally reported fold change. After that, data of miRNAs associated with prognosis were extracted cautiously from included studies by another two investigators (Xiao-Na Liang, Yue-Qi Cao) independently and all items reached a consistency. The
Dysregulated microRNAs with prognostic significance in HCC

Study selection and characteristics

In the matter of miRNAs ranking, a total of 43 studies (Figure 1; Table 1, Supplementary Files) were included according to our inclusion criteria and identification. Based on the frequency of miRNAs mentioned in involved studies and the overall sample size as described previously, we screened the top 12 most dysregulated miRNAs with corresponding microarray studies (Table 2). When referring to expression of miRNAs with HCC patients’ prognosis, there were 7362 studies identified in both English and Chinese databases with the search strategies (Figure 2). We took 4848 studies into consideration within this meta-analysis after duplicates were removed. One hundred and eight full-text articles were obtained for further identification after the assessment of initial studies reviewed through titles and abstracts that whether or not appropriate for evaluation of prognostic miRNA biomarkers in HCC. Amongst studies with full-text, 78 studies were eliminated with definite reasons for either absence of survival data or insufficient data to determine HR. Lastly, thirty independent studies published from 2008 to 2015, which ana-
## Table 3. Summary of hazard ratios of miRNA expression in HCC

<table>
<thead>
<tr>
<th>MiRNA</th>
<th>Author</th>
<th>Year</th>
<th>Region</th>
<th>Patients</th>
<th>Age</th>
<th>Samples</th>
<th>Assay</th>
<th>Cut-off (ΔCt)</th>
<th>Follow-up (mon)</th>
<th>Survival analysis</th>
<th>HR/RR (95% CI)</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td>miR-21</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wang WY</td>
<td>2014</td>
<td>China</td>
<td>119</td>
<td>NR</td>
<td>Tissue</td>
<td>RT-PCR</td>
<td>Median</td>
<td>60</td>
<td>OS</td>
<td>1.830 (1.150-2.910)</td>
</tr>
<tr>
<td></td>
<td>Gyöngyösi B</td>
<td>2014</td>
<td>Italy</td>
<td>20</td>
<td>68</td>
<td>Tissue</td>
<td>RT-PCR</td>
<td>Median</td>
<td>35</td>
<td>OS</td>
<td>1.030 (0.360-2.960)</td>
</tr>
<tr>
<td></td>
<td>Liu M</td>
<td>2014</td>
<td>China</td>
<td>136</td>
<td>NR</td>
<td>Serum</td>
<td>qRT-PCR</td>
<td>Median</td>
<td>50</td>
<td>OS</td>
<td>1.429 (1.080-1.890)</td>
</tr>
<tr>
<td></td>
<td>He XD</td>
<td>2014</td>
<td>China</td>
<td>109</td>
<td>74</td>
<td>Tissue</td>
<td>qRT-PCR</td>
<td>Median</td>
<td>50</td>
<td>DFS</td>
<td>2.110 (1.296-3.508)</td>
</tr>
<tr>
<td></td>
<td>Wang W</td>
<td>2015</td>
<td>China</td>
<td>97</td>
<td>50</td>
<td>Serum</td>
<td>RT-PCR</td>
<td>NR</td>
<td>60</td>
<td>DFS</td>
<td>2.257 (1.420-5.570)</td>
</tr>
<tr>
<td></td>
<td>Shi QK</td>
<td>2015</td>
<td>China</td>
<td>107</td>
<td>57</td>
<td>Tissue</td>
<td>qRT-PCR</td>
<td>Optimal</td>
<td>80</td>
<td>OS</td>
<td>1.416 (1.057-1.897)</td>
</tr>
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<td>72</td>
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<td>Median</td>
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<td>China</td>
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<td>Tissue</td>
<td>RT-PCR</td>
<td>Median</td>
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<td>OS</td>
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<td>Median</td>
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<td>qRT-PCR</td>
<td>Median</td>
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<td>OS</td>
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<tr>
<td></td>
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<td>China</td>
<td>120</td>
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<td>Optimal</td>
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<td>Tissue</td>
<td>RT-PCR</td>
<td>Median</td>
<td>60</td>
<td>OS</td>
<td>0.310 (0.160-0.620)</td>
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<td>miR-214</td>
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<td>2015</td>
<td>China</td>
<td>135</td>
<td>NR</td>
<td>Tissue</td>
<td>RT-PCR</td>
<td>median</td>
<td>60</td>
<td>OS</td>
<td>0.781 (0.717-0.830)</td>
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<td>RT-PCR</td>
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<td>DFS</td>
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<td>55.1</td>
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<td>qRT-PCR</td>
<td>median</td>
<td>120</td>
<td>OS</td>
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<td>miR-221</td>
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<td>Italy</td>
<td>51</td>
<td>68</td>
<td>Tissue</td>
<td>qRT-PCR</td>
<td>median</td>
<td>130</td>
<td>OS</td>
<td>1.220 (0.685-2.174)</td>
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<td>Li JP</td>
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<td>46</td>
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<td>Serum</td>
<td>qRT-PCR</td>
<td>Mean</td>
<td>60</td>
<td>OS</td>
<td>1.940 (1.030-3.780)</td>
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<td>Rong MH</td>
<td>2013</td>
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<td>48</td>
<td>52</td>
<td>Tissue</td>
<td>RT-qPCR</td>
<td>Median</td>
<td>25</td>
<td>OS</td>
<td>1.066 (0.912-1.245)</td>
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</table>
Dysregulated microRNAs with prognostic significance in HCC

Table 4. Summary of hazard ratios of miRNA expression in HCC

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of studies</th>
<th>Pooled HR</th>
<th>95% CI</th>
<th>P</th>
<th>Heterogeneity test</th>
<th>Statistical method</th>
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<td></td>
<td>Q</td>
<td>P</td>
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<tr>
<td>Overall Survival (OS)</td>
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<td></td>
</tr>
<tr>
<td>Univariate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MiR-21</td>
<td>10</td>
<td>1.598</td>
<td>1.418-1.803</td>
<td>&lt;0.001</td>
<td>14.310</td>
<td>0.112</td>
</tr>
<tr>
<td>MiR-221</td>
<td>6</td>
<td>1.623</td>
<td>1.155-2.281</td>
<td>0.005</td>
<td>28.030</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MiR-222</td>
<td>5</td>
<td>2.992</td>
<td>2.144-4.177</td>
<td>&lt;0.001</td>
<td>1.380</td>
<td>0.847</td>
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<tr>
<td>MiR-214</td>
<td>3</td>
<td>0.777</td>
<td>0.723-0.836</td>
<td>&lt;0.001</td>
<td>2.370</td>
<td>0.306</td>
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<tr>
<td>MiR-199a-3p</td>
<td>3</td>
<td>0.419</td>
<td>0.322-0.545</td>
<td>&lt;0.001</td>
<td>0.380</td>
<td>0.945</td>
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<tr>
<td>MiR-199a-5p</td>
<td>3</td>
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<td>0.275-0.532</td>
<td>&lt;0.001</td>
<td>3.160</td>
<td>0.206</td>
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<td>MiR-25</td>
<td>2</td>
<td>2.153</td>
<td>1.347-3.442</td>
<td>&lt;0.001</td>
<td>0.130</td>
<td>0.714</td>
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<tr>
<td>Multivariate</td>
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</tr>
<tr>
<td>MiR-21</td>
<td>3</td>
<td>2.425</td>
<td>1.639-3.589</td>
<td>&lt;0.001</td>
<td>0.540</td>
<td>0.762</td>
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<tr>
<td>MiR-221</td>
<td>3</td>
<td>1.618</td>
<td>1.297-2.017</td>
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<td>3.590</td>
<td>0.166</td>
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<td>MiR-199a-3p</td>
<td>2</td>
<td>0.476</td>
<td>0.350-0.648</td>
<td>&lt;0.001</td>
<td>0.000</td>
<td>1.000</td>
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<tr>
<td>MiR-25</td>
<td>2</td>
<td>2.424</td>
<td>1.734-3.390</td>
<td>&lt;0.001</td>
<td>0.690</td>
<td>0.405</td>
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<td>Disease Free Survival (DFS)</td>
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<tr>
<td>Univariate</td>
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<tr>
<td>MiR-21</td>
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<td>1.164-2.498</td>
<td>0.006</td>
<td>9.060</td>
<td>0.028</td>
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<tr>
<td>MiR-214</td>
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<td>0.382</td>
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<td>0.895</td>
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<td>Multivariate</td>
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<tr>
<td>MiR-214</td>
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<td>0.416</td>
<td>0.253-0.684</td>
<td>&lt;0.001</td>
<td>0.600</td>
<td>0.44</td>
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</tbody>
</table>

Abbreviations: OS, overall survival; DFS, disease-free survival; CI, confidence interval.
Dysregulated microRNAs with prognostic significance in HCC

A

<table>
<thead>
<tr>
<th>Study ID</th>
<th>HR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang WY (2014)</td>
<td>1.83 (1.15, 2.91)</td>
<td>6.70</td>
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<tr>
<td>Gyongyosi B (2014)</td>
<td>1.03 (0.36, 2.96)</td>
<td>1.30</td>
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<tr>
<td>Liu M (2014)</td>
<td>1.43 (1.08, 1.89)</td>
<td>18.44</td>
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<tr>
<td>Wang W (2015)</td>
<td>2.26 (1.42, 3.57)</td>
<td>6.80</td>
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<tr>
<td>Shi KQ (2015)</td>
<td>1.42 (1.06, 1.90)</td>
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<tr>
<td>Huang CS (2015)</td>
<td>2.42 (1.52, 3.84)</td>
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<td>Hu LT (2015)</td>
<td>3.82 (1.72, 8.48)</td>
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<tr>
<td>Tomimaru Y (2010)</td>
<td>2.72 (1.13, 6.55)</td>
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<tr>
<td>Karakansis A (2011)</td>
<td>1.98 (1.37, 2.86)</td>
<td>10.69</td>
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<tr>
<td>Yu L (2012)</td>
<td>1.39 (1.13, 1.71)</td>
<td>33.09</td>
</tr>
<tr>
<td>Overall (I² = 37.1%, p = 0.112)</td>
<td>1.60 (1.42, 1.80)</td>
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</table>

B

<table>
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<tr>
<th>Study ID</th>
<th>HR (95% CI)</th>
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<tbody>
<tr>
<td>Wang WY (2014)</td>
<td>1.82 (1.24, 2.67)</td>
<td>29.23</td>
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<td>Hu XD (2014)</td>
<td>2.11 (1.36, 3.31)</td>
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<td>Jiang JM (2008)</td>
<td>3.21 (1.12, 9.16)</td>
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<td>Yu L (2012)</td>
<td>1.18 (0.59, 1.47)</td>
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<tr>
<td>Overall (I² = 66.9%, p = 0.028)</td>
<td>1.71 (1.16, 2.50)</td>
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</table>

NOTE: Weights are from random effects analysis.

C

<table>
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<th>Weight</th>
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<tbody>
<tr>
<td>Wang WY (2014)</td>
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<td>Huang CS (2015)</td>
<td>2.28 (1.39, 3.72)</td>
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<td>Overall (I² = 0.8%, p = 0.762)</td>
<td>2.43 (1.64, 3.59)</td>
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</table>
Dysregulated microRNAs with prognostic significance in HCC

Figure 3. Meta-analysis evaluating the relationships between miR-21 and prognosis in HCC. A. Overall survival (univariate); B. Disease free survival (univariate); C. Overall survival (multivariate).

<table>
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<tr>
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<th>Weight</th>
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<tbody>
<tr>
<td>Gramantieri L (2009)</td>
<td>1.22 (0.69, 2.17)</td>
<td>14.76</td>
</tr>
<tr>
<td>Li JP (2011)</td>
<td>1.94 (1.03, 3.78)</td>
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<td>Rong MH (2013)</td>
<td>1.07 (0.91, 1.25)</td>
<td>24.38</td>
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<tr>
<td>Bae HJ (2015)</td>
<td>2.70 (1.67, 4.35)</td>
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<tr>
<td>Gyongyosi B (2014)</td>
<td>1.92 (0.61, 6.10)</td>
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<tr>
<td>Karakatsanis A (2011)</td>
<td>1.79 (1.50, 2.13)</td>
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</tr>
<tr>
<td>Overall (I² = 82.2%, p = 0.000)</td>
<td>1.62 (1.16, 2.28)</td>
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</tr>
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</table>

NOTE: Weights are from random effects analysis

Figure 4. Meta-analysis evaluating the relationships between miR-221 and survival in HCC. A. Overall survival (univariate); B. Overall survival (multivariate).

<table>
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<tr>
<th>Study</th>
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<th>Weight</th>
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<tr>
<td>Li JP (2011)</td>
<td>1.90 (1.24, 2.98)</td>
<td>25.10</td>
</tr>
<tr>
<td>Rong MH (2013)</td>
<td>1.15 (0.75, 1.75)</td>
<td>27.56</td>
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<td>1.81 (1.32, 2.50)</td>
<td>47.34</td>
</tr>
<tr>
<td>Overall (I² = 44.3%, p = 0.166)</td>
<td>1.62 (1.30, 2.02)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

lyzed dysregulated miRNA levels with patients’ survival in 2735 cases of HCC, were included in this qualitative synthesis (Table 3). In the meantime, this meta-analysis included 26 stud-
Dysregulated microRNAs with prognostic significance in HCC

Figure 5. Meta-analysis evaluating the relationships between miR-222 and univariate overall survival in HCC.

Figure 6. Meta-analysis evaluating the relationships between miR-25 and overall survival in HCC.
Dysregulated microRNAs with prognostic significance in HCC

The follow-up period varied from 25 to 350 months. Assay of all researches was quantitative real-time polymerase chain reaction (qRT-PCR). The sample types included tissue (N = 24), serum (N = 5), and plasma (N = 1). The main characteristics involved in the survival data of the prognostic studies were shown in Table 4.

**Meta-analysis**

Six miRNAs that were most consistently upregulated (miR-222, miR-221, miR-224, miR-21, miR-25 and miR-18a) and six most consistently downregulated miRNAs (miR-199a-5p, miR-99a, miR-195, miR-130a, miR-199a-3p and miR-214) were identified in at least ten profiling studies comparing miRNA dysregulation in HCC tissues and corresponding non-tumor liver tissues (Table 2). A summary of HRs estimated and 95% CIs from the entire pooled analysis for specific miRNAs was showed in Table 4.

**MiR-21**

In summary, 12 studies reported survival data on miR-21 and assessed upregulated miR-21 as a predictor for survival outcome in HCC patients (Table 2). Among them, 10 performed univariate analysis on OS and four on DFS. Besides, multivariate analysis was conducted to process OS data in 3 studies. The results of univariate analysis suggested that higher miR-21 expression was significantly correlated with poorer OS, with a pooled HR of 1.598 (95% CI = 1.418-1.803, \(P<0.001\)) (Figure 3A) [31-40]. Fixed-effect model was applied since the heterogeneity of the 10 studies was low \((P = 0.112, I^2 = 37.1\%)\). On the contrary, miR-21 showed no significant association with OS in the sample set of sorafenib-treated patients \((P = 0.95)\) in the research of Gyöngyösi et al. [36]. Multivariate analysis was employed in 3 studies [32, 34, 37], in which no heterogeneity was found \((P\)
= 0.762, $I^2 = 0.0\%$). The pooled HR was 2.425 and 95% CI was 1.639-3.589 (Figure 3C), which indicated that upregulated miR-21 may have a negative effect on patients’ prognosis. As for the DFS of miR-21 in 4 studies identified, we used a random-effect model due to the existence of significant heterogeneity ($P = 0.028, I^2 = 66.9\%$) (Table 4). As shown in Table 4, overexpression of miR-21 was significantly related with poor DFS by univariate analysis [37, 40-42], given a combined HR of 1.709 (95% CI: 1.164-2.498, $P = 0.006$) (Figure 3B).

**MiR-221**

As one of the most upregulated miRNAs in HCC, miR-221 was mentioned in 15 studies (Supplementary Files) including 845 tissue samples (Table 2). Survival data of OS by either univariate or multivariate analysis in the other 6 studies indicated that high miR-221 level may function as one of the candidate predictors for poor OS in HCC ($n = 379$) (Table 3). Five studies used HCC tissues as test samples, while Li et al. [43] tested serum sample in HCC patients. These 6 studies [36, 38, 43-46] all performed univariate analyses and thus a combined analysis was conducted subsequently. Random-effect model was selected as the heterogeneity of the 6 studies obviously existed ($P<0.001, I^2 = 82.2\%$). Patients with elevated miR-221 expression had a pooled HR of 1.623 (95% CI: 1.155-2.281, $P = 0.005$) (Figure 4A). Multivariate analysis was performed in 3 studies [38, 43, 45] with no significant heterogeneity ($P = 0.166, I^2 = 44.3\%$) in a fixed-effect model, the results of which indicated that miR-221 could be a candidate for poor prognosis in HCC as the pooled HR was 1.618 (95% CI: 1.297-2.017, $P<0.001$) (Figure 4B; Table 4).

**MiR-222**

MiR-222 was consistently reported upregulated in HCC tissues as compared with their adjacent non-cancerous hepatic tissues in 17 microarray studies including 712 tissue samples (Table 2), which aroused our interest that whether miR-222 can serve as a predictive factor for HCC patients’ poor outcome. Five studies available focused on the relationship between overexpression of miR-222 and prognosis of HCC, 3 of which tested tissue samples and another 2 tested serum samples (Table 3). No significant inter-study heterogeneity was found ($P = 0.847, I^2 = 0.0\%$) (Table 4) in the univariate analysis (OS) in the 5 studies [36, 47-50], given a pooled HR estimated of 2.992 (95% CI: 2.144-4.177, $P<0.001$) (Figure 5). It was also proved that upregulated miR-222 can be an independent risk factor for poor HCC patients’ survival. Besides, multivariate analysis on OS by Zhan et al. [48] showed a HR of 2.356 (95% CI: 1.055-5.263). Wong et al. [49] conducted univariate analysis on DFS with a HR of 2.214 (95% CI: 1.188-3.829, $P<0.01$) and highlighted a potential prognostic value of miR-222.

**MiR-25**

Recent studies showed that the miR-25 was notably overexpressed in HCC tissues when compared with adjacent normal tissues ($P<0.001$), suggesting an important role of miR-25 in carcinogenesis and development of HCC [51, 52]. Consistently, twelve studies with 524 samples were found to conform this (Table 2). And a meta-analysis including two studies with survival data was subsequently performed [51, 53]. No significant heterogeneity was found in these 2 studies (univariate: $P = 0.714, I^2 = 0.0\%$; multivariate: $P = 0.405, I^2 = 0.0\%$) and thus the fixed-effect model was used. As described in Table 4, pooled HRs of OS for univariate and multivariate analysis were 2.153 (95% CI: 1.347-3.442, $P<0.001$, Figure 6) and 2.424 (95% CI: 1.734-3.390, $P<0.001$) (Figure 6) respectively. The results above highlighted miR-25 could be a prognostic marker and upregulation of miR-25 might have predictive value for poor survival in HCC patients.

**MiR-199a-3p**

Among the aberrant expressed miRNAs that consistently reported in HCC, miR-199a-3p, one of the most downregulated miRNAs in HCC proved by our ranking strategy, was reported in 14 miRNA profiling studies containing 713 tissue samples (average FC: 0.37) (Table 2). Since miR-199a-3p was considered as a research focus and 3 studies [54-56] pointed out its decrement was closely associated with poor prognosis of HCC patients, and it was thought to be a potential protective factor for HCC. The fixed-effect model was applied since no significant inter-study heterogeneity was detected ($P = 0.945, I^2 = 0.0\%$). The combined univariate HR of OS in these 3 studies was 0.419 (95% CI: 0.206-0.850).
Dysregulated microRNAs with prognostic significance in HCC

![Figure 7](image)

Another study by Hou et al. [55] conducted two independent cohorts of HCC patients (N1 = 142; N2 = 152) to pooled the HR of OS from the multivariate analysis, revealing a combined HR of 0.476 (95% CI: 0.350-0.648, P<0.001) for poor OS (Figure 7). The results above suggested the lower expressed miR-199a-3p featured in pathogenesis and unfavorable prognosis of HCC patients.

**MiR-214**

Based on the miRNA ranking results shown in Table 2, miR-214 was found to be downregulated in HCC tissues of 14 microarray studies with 909 samples. As for its role in HCC progression and prognosis, relevant survival data to calculate the HRs of OS and DFS were provided by 5 studies, all of which tested on HCC tissue samples (Table 3). As stated by our meta-analysis, univariate analysis indicated statistically significant relations of lower expressed miR-214 with OS and DFS. The pooled HRs for OS [36, 54, 57] and DFS [57-59] were 0.777 (95% CI: 0.723-0.836, P<0.001) (Figure 8) and 0.382 (95% CI: 0.249-0.585, P<0.001) (Figure 8), respectively. Moreover, similar results were found in the multivariate analysis of DFS in HCC patients [58, 59] with a pooled HR of 0.416 (95% CI: 0.253-0.684, P = 0.001) (Figure 8). The results of pooled HRs and corresponding heterogeneity test were listed in Table 4. The results indicated that the underexpression of miR-214 in HCC tissues might predict a more frustrating outcome for HCC patients.

**MiR-199a-5p**

For miRNA profiling studies, miR-199a-5p was considered to be the most downregulated miRNA in HCC tissues in our meta-analysis on 23 microarray studies with 1173 samples (Table 2). For prognosis, 3 studies [54, 60, 61] described low tumoral miR-199a-5p expression as predictor of poor survival in HCC. Moderate inter-study heterogeneity (I² = 36.8%; P =...
Dysregulated microRNAs with prognostic significance in HCC

0.206) was found thus the fixed-effect model was employed in the univariate meta-analysis, which gave a combined HR of 0.383 (0.275-0.532, \( P < 0.001 \)).

Publication bias

Finally, the publication bias of miR-21 in 10 studies performed by univariate analysis for OS was evaluated by funnel plots and Begg’s method. No publication bias existed in this meta-analysis since the funnel plots were symmetry, and the \( P \) value of the Begg’s test was 0.180 (greater than 0.1) (Figure 9). We performed no Begg’s test for OS, DFS or RFS of other miRNAs because of the limited number of studies.

Discussion

These days, the independent prognostic biomarkers that can be easily detected in serum or tissues signify medical advancement for a growing number of HCC patients. And the high-throughput microarray has made it possible to predict prognosis for HCC patients more accurately depending on the genetic profile of an individual tumor. Previously, researchers had conducted certain systematic review to evaluate circulating miRNAs from miRNA expression profiling studies as novel potential biomarkers for HCC [9, 14, 18, 62]. Though these studies, to some extent, provided large amounts of information about biological importance of miRNAs in cancer genesis, diagnosis, progression, prognosis and response to treatments, the lack of consistency in the expression direction or the differentially expressed miRNAs in tissues compared with normal tissues still challenged us. To deal with the obscurities of unavailable raw data and various investigating methods, according to the reports of Chan and Griffith [28, 29], a vote-strategy method was applied to ensure the consistency among studies with different microarray platforms. Therefore, we collected information of all miRNA microarrays on HCC by searching literatures in the NCBI GEO database and eventually 43 studies with miRNA profiling data were recruited in this systematic review. Frequencies of miRNAs mentioned were taken into consideration, and we analyzed the expression levels of the top 12 miRNAs known to be dysregulated in HCC, which were then selected for further pooled analysis to uncover their roles in HCC prognosis. By combining data from several independent studies, statistical effects were increased and the consistency in association of miRNA expression with survival prognosis was evaluated, too.

MiR-21 is the well-known oncogenic miRNA in human cancers [63] and its aberrant expression in HCC tissues was on average 6.05 times higher than that in non-tumorous tissues. Previous meta-analyses of miR-21 had been conducted for diagnostic application in HCC [64-67], but relatively a few prognostic researches were published. Wang et al. [68] calculated a pooled HR of 5.77 (95% CI: 2.65-12.52) and pointed out higher expression of miR-21 was significantly correlated with worse outcomes in digestive cancer; nevertheless they failed to highlight its dysregulation significance in HCC. Besides, despite the fact that Zhou et al. [67, 69] consistently proved that overexpression of miR-21 in HCC was correlated with poor overall survival, inadequate raw survival data dented the reliability of the combined results. Based on the microarrays of HCC tissues globally and the results of miRNA ranking sort, miR-21 was selected to perform survival analysis and twelve studies including 1099 patients with comprehensive data were used to calculate the pooled HR, with no significant inter-study heterogeneity existed. Many studies have con-

![Begg's funnel plot for assessing publication bias of miR-21 expression and overall survival (univariate) in HCC.](image-url)
Dysregulated microRNAs with prognostic significance in HCC

confirmed that miR-21 overexpression might enhance HCC migration and invasion through certain mechanisms, resulting in poor survival [70-74]. They elucidated that miR-21 simultaneously regulated multiple programs that promote cell proliferation, apoptosis or tumor invasiveness by targeting PTEN, PDCD4, and RECK in HCC, and the results of Hu et al. [75] suggested that miR-21 participated in HCC development through promoting cell proliferation by post-transcriptionally downregulating HEPN1 expression. Besides, Xu et al. [76] pointed out that miR-21 could also directly target MAP2K3 and inhibit its expression during HCC tumorigenesis. Therefore, high tumoral miR-21 expression in HCC patients was significantly correlated with tumor progression and could be a high risk factor for poor HCC prognosis [32].

MiR-221, encoded on human chromosome X, is overexpressed in various aggressive carcinomas [77-79]. In this meta-analysis, we observed that higher tumoral miR-221, with an average of 5.54-fold change than normal tissues, was significantly associated with poor OS in HCC. Furthermore, Yang et al. [80] found it was more efficient to detect miR-221 in serum/plasma samples than in HCC tissue samples when miR-221 was used for predicting the prognosis. The findings reminded us that circulating miR-221 is more qualified as a biomarker in HCC. It was reported that elevated miR-221 promoted growth of HCC cells by positively regulating the expression of two cyclin-dependent kinase inhibitors (CDKIs): CDKN1B/p27 and CDKN1C/p57, and it inhibited cell apoptosis by suppressing the expression of BMF [46, 81]. Hence, miR-221, considered as a tumor promoter, was likely to be a potential biomarker for HCC patients with poor prognosis and it could also assess therapeutic efficacy in the future.

MiR-222 was identified as one of the top five most upregulated miRNAs in our rank study. The expression of miR-222 in 16 miRNA profiling studies covering 632 HCC patients in HCC tissues was 5.54 times higher than that in adjacent non-tumor tissues. As for prognosis, miR-222 was proved to be an independent risk factor leading to poor outcomes for HCC patients, since the univariate pooled HR of 2.992 was calculated through combining OS data in five studies. This finding attracted our attention to the complex molecular mechanisms in liver carcinogenesis. As reported by Wong et al. [49], increased miR-222 expression was related to advanced stage of HCC and poor DFS, and then it was confirmed to accelerate HCC cell motility via enhancing PI3K/AKT signaling, the same oncogenic pathway identified by Liu et al. [47], too. Additionally, Zhang et al. [82] indicated that upregulation of miR-222 may cause the down-regulation of GNAI3, a member of the Gi group involved in regulating cellular proliferation, migration, invasion and apoptosis, and this aberrant regulation promoted invasion and metastasis of HCC. Since upregulation of miR-222 can promote HCC cells proliferation and migration, it is expected to be a potential biomarker in HCC development and may provide new therapeutic targets.

MiR-25, belonging to the miR-106b~25 cluster including miR-106b, miR-93 and miR-25, is located within intron 13 of the minichromosome maintenance protein 7 (MCM7) gene on chromosome 7q22.1 [83, 84]. In addition, miR-25 was one of the most frequently miRNAs mentioned in HCC tissues, with a fold change of 2.38 times higher than corresponding non-tumor tissues. Recently, some researchers [85-87] have found the overexpression of miR-25 was associated with the prognosis of various tumors, such as ovary, gastric and prostate cancers. Similarly, Li et al. [52] demonstrated that the miR-106b~25 cluster possessed oncogenic properties and upregulated miR-25 contributed to liver carcinogenesis in HCC tissues and cell lines. Despite the fact that Su et al. [51] had worked for the clinical relevance of miR-25 and its expression on the prognosis of HCC, we combined the study with Sadeghian et al. [53] in the present meta-analysis to pool the survival data and explore the feasibility of miR-25 as a potential prognostic biomarker for HCC. The result that pooled HR>1 in both univariate and multivariate analysis suggested that overexpression of miR-25 in HCC correlated with poor survival after HCC resection, which was considered to be with certain mechanisms in tumor metastasis. Activated by the WNT/β-catenin signaling pathway, overexpressed miR-25 could play its pro-metastatic role through inhibiting the Rho GDP dissociation inhibitor alpha directly, namely RhoGDI1, whose down-regulation stimulates expression of Snail, thereby enhancing epithelial-mesenchymal transition (EMT). Once EMT was activated, miR-
Dysregulated microRNAs with prognostic significance in HCC

25 could promote cell proliferation, tumor progression and invasion. Therefore MiR-25/RhoGDI1 axis promises to be a probable therapeutic target for the treatment of HCC [88]. The results of our meta-analysis combining two prognostic studies, with no significant heterogeneity, were consistent with the researches above. However, further experimental validation in large and homogenous cohorts of HCC patients, adjusting for multiple clinic pathologic parameters, are required urgently to confirm its prognostic role in HCC.

MiR-199a-3p was downregulated in HCC [89] and it was most frequently mentioned in the microarray studies included. The expression of miR-199a-3p in HCC tissues was 0.37 times lower than that in non-tumorous tissues in 14 studies with 713 samples. One study presented that miR-199a-3p could be a novel serum diagnostic biomarker for HCC [90]. Nevertheless, according to the meta-analysis published, only a few studies pointed out that low miR-199a-3p expression was related with the poor prognosis in HCC [55, 91, 92]. In order to strengthen the reliability of the relevance between low miR-199a-3p level and HCC patients' survival, twelve studies were included and sufficient survival data were collected to calculate the pooled HRs. The combined results consistently suggested that miR-199a-3p was downregulated with shorter overall survival for HCC. Up to now, a considerable portion of studies have demonstrated that downregulated miR-199a-3p might participate in direct or indirect cell proliferation, apoptosis, tumor migration and invasion in HCC through different mechanisms, which may predict poor prognosis in HCC patients [89, 93-95]. Shatseva et al. [94] confirmed that miR-199a-3p regulated cell proliferation and survival by targeting caveolin-2 in HCC. Hou et al. [55] pointed out that miR-199a-3p could target tumor-promoting PAK4 to suppress HCC growth through inhibiting the PAK4/RAF/MEK/ERK pathway, and thus the decrement of miR-199a-3p was significantly correlated with poor survival for HCC patients. Besides, Song et al. [61] proposed that FZD7, identified as a functional target of miR-199a-3p, participated in cell proliferation and cell cycle regulation of HCC. To sum up, due to under-expression of miR-199a-3p that was significantly correlated with tumor progression in HCC patients, miR-199a-3p could be considered as a candidate biomarker indicating poor prognosis of HCC.

MiR-214 was known as a tumor suppressor of HCC [96] and its low expression in human HCC was associated with poor prognosis based on our meta-analysis above. Along with the results of fourteen miRNA profiling studies included, downregulation of miR-214 was proved in HCC according to several other studies recently [36, 97-101]. Xia et al. [59] demonstrated that low expression of miR-214 in HCC tissues was connected with the cell invasion, stem-like traits and early recurrence of HCC, whilst two potential downstream targets of miR-214, enhancer of zeste homologue 2 (EZH2) and β-catenin (CTNNB1) were noticeably identified. Besides, Shih et al. [57] had found that hepatoma-derived growth factor (HDGF) was also a target gene of miR-214, which can activate vascular endothelial cells. While Wang et al. [58] observed that the FGFR-1 can be significantly upregulated by miR-214 and thereby promoted the aggressiveness of HCC expression. Moreover, Wang et al. [91] also found that low expression of miR-214 in HCC cells correlated with inhibition of E2F2, CDK3 and CDK6. As a result, miR-214 could significantly disturb the cell-cycle and promote proliferation of HCC cells, leading to tumor angiogenesis invasion and early recurrence in HCC patients. In short, downregulation of miR-214 may provide a new therapeutic approach for cancer treatment.

To sum up, this systematic review had a well-defined search strategy, rigorous selection of studies in literature, abundant cases of HCC patients and massive sample size. The features mentioned above contributed to the improved reliability and increased statistical conviction in our meta-analysis. Moreover, the heterogeneity was decreased by ensuring the consistency of the sample types and detecting methods used in studies, and the most up- or down-regulated miRNAs in HCC in miRNA profiling studies globally were then ranked and selected, so as to perform relevant survival analysis and thus we could explore the top six dysregulated miRNAs and their specific relationships with HCC patients’ prognosis independently.

However, some limitations of this study should be addressed. Above all, the lack of literatures related prognosis with enough survival data such as DFS and PFS might weaken the statisti-
Dysregulated microRNAs with prognostic significance in HCC

Cal persuasiveness of the final results to some extent. Secondly, subgroup analysis was not performed because of the lack of certain clinicopathological characteristics such as histological grade, TNM stages, tumor size and the number of tumor nodes from the prognostic studies. Although the inter-study heterogeneity of some results was proved insignificant, the specific clinical characteristic that these miRNAs related with remained unexplored. Therefore, more clinical studies with detailed clinicopathological parameters were needed to investigate the detailed and accurate functions of dysregulated miRNAs in HCC.

Conclusions

Overall, the top six upregulated miRNAs consistently reported in HCC tissues were miR-222, miR-221, miR-224, miR-21, miR-25 and miR-18a, all of which lead to dismal prognosis for HCC except miR-18a and miR-224. On the contrary, the top six downregulated miRNAs were miR-199a-5p, miR-99a, miR-195, miR-130a, miR-199a-3p, and miR-214, among which miR-199a-3p and miR-214 emerged as potential predictors of favorable prognosis for HCC patients. These miRNAs can be potential candidates for HCC prognosis and therapy.

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Disclosure of conflict of interest

None.

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Dysregulated microRNAs with prognostic significance in HCC


Dysregulated microRNAs with prognostic significance in HCC

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Dysregulated microRNAs with prognostic significance in HCC

2415


Dysregulated microRNAs with prognostic significance in HCC

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Dysregulated microRNAs with prognostic significance in HCC


