Clinical potential role of circulating microRNAs in early diagnosis of colorectal cancer patients

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Current procedures for diagnosis and biomarker examination of colorectal cancer (CRC) are invasive and unpleasant. There is a great need to identify sensitive and specific biomarkers for early diagnosis of CRC. Circulating microRNAs (miRNAs) are promising molecular markers for CRC prediction. We performed a comprehensive meta-analysis to integrate an evaluation index for diagnostic accuracy of circulating miRNAs in diagnosing CRC patients. Furthermore, we conducted an independent validation set of 49 CRC patients and 49 healthy controls. In our meta-analysis, we found that miR-21 yielded a pooled area under ROC curve (AUC) of 0.867 (sensitivity: 76%, specificity: 82%); in discriminating CRC from controls, and miR-92a yielded a summary AUC of 0.803 (sensitivity: 77%, specificity: 68%); miR-21 had a higher diagnostic efficiency than miR-92a. In the further validation, plasma miR-21 levels in CRC patients were significantly higher than levels observed in healthy subjects. A ROC curve analysis showed a consistent result. However, this phenotype was not present in miR-92a. Moreover, the expression trend of miR-21 in plasma samples was in line with that of tissue samples, along with the cellular level. Current evidences suggest that plasma miR-21 could be a reliable and non-invasive biomarker for CRC diagnosis. Studies with larger cohorts that include the diagnostic value of plasma miR-21 for CRC are warranted.

Introduction

Colorectal cancer (CRC) is the third most common malignancy and the fourth main cause of cancer death in the world (1). It has been reported that CRC accounts for ~8.6 and 8.8% of total cancer incidence and deaths in the USA, respectively (1). CRC that is diagnosed at an early stage of development is more likely to be treated successfully. There is convincing evidence that screening can considerably decrease incidence and mortality from CRC (2). Although colonoscopy is the gold standard for CRC screening, embarrassment, fear of pain and feelings of unpleasantness have limited its wide application (3,4). Flexible sigmoidoscopy, double-contrast barium enema and computed tomography imaging techniques have been used for the early detection of CRC; however, these methods have not been widely used for because of wide detectable range, low sensitivity and specificity (5–7). Furthermore, non-invasive examinations, such as the fecal occult blood test and carcinoembryonic antigen test, are not suitable for screening the majority of patients suspected to have CRC because these tests typically present relatively low clinical specificity (8,9). Therefore, an urgent need exists to identify simple and more reliable detection methods, as well as specific molecular markers for the early diagnosis of CRC (10).

Emerging evidence has demonstrated that microRNAs (miRNAs) are an important class of non-coding RNAs involved in human carcinogenesis as tumor oncogenes or suppressors. The ectopic expressions of miRNAs, along with their profiles in human cancers, could be used to classify tumors and improve prediction, prognosis and progression (11,12). In addition, several studies have focused on the identification of new early diagnostic biomarkers for CRC by comparing the differences between miRNAs expression in CRC and paired adjacent colorectal normal tissues (13). Furthermore, it was found that tumor-associated RNAs, especially miRNAs, were easily and readily detected in circulating body fluids (e.g. serum or plasma) from patients suffering from specific diseases, including various types of cancer (14). Accordingly, circulating miRNAs may be utilized as non-invasive biomarkers for the early detection of cancer.

Although numerous studies report on the relationship between circulating miRNAs and CRC diagnosis, inconsistencies or discrepancies about diagnostic accuracy of differentially expressed miRNAs still exist. Therefore, we performed a systematic analysis to evaluate the diagnostic efficiency of circulating miRNAs in patients with CRC from all published CRC-related studies, combined with a validation study, to identify a novel non-invasive biomarker for early diagnosis of CRC.

Materials and methods

Search strategy

We performed a search for all studies that included circulating miRNAs related to CRC in PubMed up to November 2013. Our search was based on the following key terms: ‘circulating’ or ‘serum’ or ‘plasma’, ‘miRNA’ or ‘microRNA’ or ‘miR’, ‘colorectal’ or ‘colon’ or ‘rectal’ and ‘cancer’ or ‘carcinoma’ or ‘neoplasm’; conference summaries and references cited of for each retrieved article were scanned to identify additional potentially relevant studies.

Selection of publications

All publications included in the meta-analysis had to meet the following criteria: (i) patients with any type of CRC, but not advanced adenomas, and identified by the diagnosis of histopathological confirmation; (ii) peripheral blood including plasma or serum for miRNA analyses must have been collected before any treatment; (iii) studies detecting the expression levels of circulating miRNAs and investigating their associations with CRC diagnosis were included; (iv) studies had to present sufficient data to construct a two-by-two table and (v) patients with benign disease or healthy individuals served as the control group. We excluded the following from our analysis: (i) review paper and letters, (ii) duplicate publications with small sample size or the previous report and (iii) non-English articles. The retrieved studies were independently assessed by two reviewers (M.D. and S.L.) according to the prespecified criteria; discrepancies were completely resolved through detailed discussions and consensus.

Data extraction and quality assessment

The following data characteristics were collected for each included article: first author’s name, publication year, country of publication, miRNA type, sample...
type, detecting method, sample size, age, sex, clinical stage, and data for two-by-two tables [area under ROC curve (AUC) and corresponding 95% confidence interval (95% CI), cutoff, sensitivity and specificity].

The Quality Assessment of Diagnostic Accuracy Studies (QUADAS) checklist was used to systematically assess the quality of the articles included in the diagnostic meta-analysis (15). Specifically, 14 items from the Quality Assessment of Diagnostic Accuracy Studies checklist were applied to each article, and an answer of ‘Yes’, ‘No’ or ‘Unclear’ and only ‘Yes’ would result in a score.

Validation of candidate miRNAs expression

The expression levels of candidate miRNAs were measured in 98 plasma samples using quantitative reverse transcription-PCR with TaqMan microRNA probes, according to the manufacturer’s protocols. Plasma samples were randomly selected from 49 healthy volunteers and 49 consecutively recruited patients with CRC on an ongoing study at the First Affiliated Hospital of Nanjing Medical University from September 2010. Controls were selected based on physical examination and frequency matched to cases on age (±2 years) and sex.

To avoid the biological variation, we added 5 nmol of synthetic Caenorhabditis elegans miRNAs (cel-miR-39) into 100 µl of each plasma sample as a spike-in control before performing RNA isolation. Extraction of miRNA from plasma was used by TRIzol LS Reagent (Invitrogen, Carlsbad, CA) with miRNeasy Mini Kits (Qiagen, Valencia, CA) and then reverse transcribed to complementary DNA using Taqman microRNA RT kits with stem-loop RT primers (Applied Biosystems, Foster City, CA). We quantified miRNA expression to spike-in cel-miR-39 using the 2−ΔΔCt method. This study was approved by the institutional review board of Nanjing Medical University.

Statistical analysis

Data analysis was performed using Stata 8.2 (StataCorporation, College Station, TX), Meta-Disc version 1.4 (16) and SAS 9.1.1 (SAS Institute, Cary, NC). We summarized the AUC, sensitivity, specificity, diagnostic odds ratio (DOR), positive likelihood ratio (PLR) and negative likelihood ratio (NLR). Given the ignorance of difference from different diagnostic threshold effect in the conventional summary receiver-operator characteristic, we plotted study-specific sensitivity and specificity by hierarchical summary receiver-operator characteristic (HSROC) model (17). Cells with ‘0’ observations in 2 × 2 contingency tables were replaced with ‘0.5’ for reducing performance in the small studies. The Q* index, derived from the fitted curve, is the point where sensitivity equals specificity. The Spearman correlation coefficient was used to evaluate cutoff threshold effects between sensitivity and specificity. In addition to having a P value <0.05, heterogeneity across studies was assessed using Cochrane’s Q and I² statistics; I² >50% indicated the existence of significant heterogeneity. DerSimonian and Laird’s random-effects model was applied when heterogeneity existed; otherwise, the fixed-effects model using the Mantel–Haenszel method was employed. The presence of publication bias was assessed using the Deeks’ funnel plot asymmetry test: a P value <0.10 was considered statistically significant. Differences in distributions of demographic variables and relative plasma miRNA expression levels between CRC cases and healthy controls, in validation tests, were evaluated with the Student’s t-test and Pearson’s χ² test. We performed a risk-score analysis that calculated the risk score of each candidate miRNA using the weight by the regression coefficient derived from univariate logistic regression analysis of candidate miRNAs. Then, we performed ROC curves analysis and calculated the AUCs to evaluate the associations of candidate miRNAs and CRC. A P value <0.05 for two-tailed tests was considered statistically significant.

Results

Literature search and study characteristics

Supplementary Table S1, available at Carcinogenesis Online, depicts the workflow of this study and Figure 1 provides a detailed description of the study selection process. A total of 77 articles with information on circulating miRNAs and CRC were retrieved from a primary literature search of PubMed. Sixteen articles with information on CRC diagnosis and circulating miRNAs remained after a series of exclusion criteria were applied (e.g. review or letters, title and abstract screening, exclusion of duplicates, etc.). Another five articles were excluded because they lacked sufficient data for statistical and diagnostic analyses. Eleven articles remained; the main characteristics of each article are summarized in Table 1. Among the 11 remaining candidate articles (18–28), 4 articles with miRNA-panels data or single one data for miRNA were not taken into consideration for further meta-analysis (20,21,26,27). One article including two independent tests was considered as two studies. Finally, six studies that focused on miR-21 (22–25,28) and four studies on miR-92a (18,19,24,28) from the remaining seven articles were enrolled into the diagnostic meta-analyses.

There were a total of 548 patients from miR-21-related studies and 470 from miR-92a. Among the seven eligible articles, five articles studied Asian populations and two articles studied Caucasians; the studies had either serum (n = 3) or plasma (n = 4) samples. All enrolled studies utilized quantitative reverse transcription–PCR with TaqMan probes or SYBR green assay to measure the expressions of miR-21 and miR-92a. The quality of the articles was assessed according to Quality Assessment of Diagnostic Accuracy Studies (Supplementary Table S1, available at Carcinogenesis Online). No articles satisfied the criteria of item 1 as the result of the identifiable subjects, who were histopathologically confirmed as either having cancer or being healthy cancer-free individuals. We assigned ‘Unclear’ to item 10 as there were no statements of whether or not the index test results were interpreted with the knowledge of the results of the reference standard. For items 4 and 6, there was one study that did not state whether they met the criteria or not.

Data analysis for diagnostic accuracy of circulating miRNAs in discriminating CRC

Table II illustrates the pooled results of two candidate miRNAs. For miR-21, we summarized AUC, sensitivity, specificity and DOR, which were 0.867 (SE = 0.036), 0.76 (95% CI: 0.61–0.91), 0.82 (95% CI: 0.78–0.86) and 15.59 (95% CI: 6.69–36.33), respectively, and in which heterogeneity existed in pooled sensitivity and DOR (I² heterogeneity < 0.001 for both; F = 93.5 and 78.5%, respectively), but not in specificity (Supplementary Figure S2, a1–a3, available at Carcinogenesis Online). Since likelihood ratios are considered to be more comprehensive and steady diagnostic values of screening tests (29), we calculated PLR and NLR to predict the diagnostic performance of circulating miRNAs and observed that the pooled PLR and NLR were
<table>
<thead>
<tr>
<th>Author/Publication year</th>
<th>Country/ethnicity</th>
<th>Sample</th>
<th>Quantitative RT–PCR</th>
<th>Normalization control</th>
<th>Patients (male/female)</th>
<th>Controls (male/female)</th>
<th>Case/control age (mean ± SD or range)</th>
<th>TNM stage</th>
<th>miRNAs studied</th>
<th>AUC (95% CI)</th>
<th>Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Quality score (QUADSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al.2012</td>
<td>China/Asian</td>
<td>Serum</td>
<td>SYBR</td>
<td>miR-16</td>
<td>17/15</td>
<td>30/9</td>
<td>63 (45–80)/46(6)</td>
<td>I/II 19; II/IV 13</td>
<td>miR-21</td>
<td>0.85 (0.76–0.94)</td>
<td>3.59</td>
<td>87.5</td>
<td>74.4</td>
<td>12</td>
</tr>
<tr>
<td>Luo et al.2013</td>
<td>Germany/Caucasian</td>
<td>Plasma</td>
<td>TaqMan</td>
<td>miR-16</td>
<td>45/35</td>
<td>60/84</td>
<td>68±10.4/62.5±7.5</td>
<td>I/II 22; II 25; III 26; IV 5</td>
<td>miR-20a, miR-106b</td>
<td>0.86 (0.89–0.94)</td>
<td>4.993</td>
<td>53.64</td>
<td>69.88</td>
<td>11</td>
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<tr>
<td>Wang et al.2013</td>
<td>China/Asian</td>
<td>Plasma</td>
<td>TaqMan</td>
<td>miR-1228</td>
<td>23/32</td>
<td>34/23</td>
<td>64±13/44±12</td>
<td>I 7; B 19; C 19; D 10/9; A 5; B 6; C 10; D 15</td>
<td>miR-9a, miR-19a, miR-15b</td>
<td>0.82 (0.73–0.90)</td>
<td>0.84</td>
<td>78.57</td>
<td>77.36</td>
<td>12</td>
</tr>
<tr>
<td>Giráldez et al.2012</td>
<td>Spain/Latino</td>
<td>Plasma</td>
<td>TaqMan</td>
<td>miR-16</td>
<td>42</td>
<td>26/27</td>
<td>62.8±6.3/62.1±3.5</td>
<td>I 8; II 13; III 16; IV 5</td>
<td>miR-9a, miR-19a, miR-15b, miR-601</td>
<td>0.74 (0.67–0.83)</td>
<td>0.788</td>
<td>80.00</td>
<td>72.4</td>
<td>12</td>
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<tr>
<td>Wang et al.2012</td>
<td>China/Asian</td>
<td>Plasma</td>
<td>TaqMan</td>
<td>cel-miR-39</td>
<td>45/45</td>
<td>30/28</td>
<td>62±11/58±12</td>
<td>I 26; II 25; III 29; IV 10</td>
<td>miR-601</td>
<td>0.866 (0.79–0.92)</td>
<td>0.897</td>
<td>NA</td>
<td>82.94</td>
<td>NC</td>
</tr>
<tr>
<td>Kanaan et al.2012</td>
<td>America/Caucasian</td>
<td>Plasma</td>
<td>TaqMan</td>
<td>RNU6B</td>
<td>14/16</td>
<td>14/16</td>
<td>60±10/61±9</td>
<td>I 3; II 4; III 15; IV 4; not clear 4</td>
<td>miR-21</td>
<td>0.82 (0.73–0.90)</td>
<td>0.84</td>
<td>78.57</td>
<td>77.36</td>
<td>12</td>
</tr>
<tr>
<td>Toiyama et al.2013</td>
<td>Japan/Asian</td>
<td>Serum</td>
<td>TaqMan</td>
<td>cel-miR-39</td>
<td>106/80</td>
<td>27/26</td>
<td>67/64±12</td>
<td>I 46; II 62; III 48; IV 44</td>
<td>miR-21</td>
<td>0.919 (0.87–0.96)</td>
<td>0.0013</td>
<td>91.9</td>
<td>81.1</td>
<td>12</td>
</tr>
<tr>
<td>Liu et al.2013</td>
<td>China/Asian</td>
<td>Serum</td>
<td>TaqMan</td>
<td>miR-16</td>
<td>126/74</td>
<td>42/38</td>
<td>57.09 (20–89)/57.71 (28–89)</td>
<td>I 18; I 96; III 64; IV 22</td>
<td>miR-9a, miR-21</td>
<td>0.802 (0.75–0.85)</td>
<td>0.0043</td>
<td>65</td>
<td>85.2</td>
<td>12</td>
</tr>
<tr>
<td>Pu et al.2010</td>
<td>China/Asian</td>
<td>Plasma</td>
<td>SYBR</td>
<td>No control</td>
<td>66/37</td>
<td>19/18</td>
<td>58 (39–84)/32 (17–77)</td>
<td>I 7; II 38; III 40; IV 18</td>
<td>miR-221</td>
<td>0.606 (0.49–0.72)</td>
<td>1.69</td>
<td>86</td>
<td>41.6</td>
<td>NC</td>
</tr>
</tbody>
</table>
Table II. Summary AUC, sensitivity, specificity, DOR, PLR and NLR of circulating miR-21 and miR-92a for diagnosing CRC

<table>
<thead>
<tr>
<th>Variables</th>
<th>Included studies</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>DOR</th>
<th>PLR</th>
<th>NLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>Wang et al.</td>
<td>0.867</td>
<td>0.798</td>
<td>0.82</td>
<td>0.798</td>
<td>15.59</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>Lao et al.</td>
<td></td>
<td>0.87 (0.61–0.91)</td>
<td>0.78 (0.78–0.86)</td>
<td>0.0%</td>
<td>78.5%</td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>Kanaan et al.</td>
<td></td>
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<tr>
<td></td>
<td>Toiyama et al.</td>
<td></td>
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<tr>
<td></td>
<td>Liu et al.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Huang et al.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-92a</td>
<td>Luo et al.</td>
<td>0.803</td>
<td>0.739</td>
<td>0.68</td>
<td>0.95</td>
<td>7.99</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td>Liu et al.</td>
<td></td>
<td>0.77 (0.65–0.89)</td>
<td>0.52 (0.52–0.84)</td>
<td>&lt;0.001</td>
<td>87.3%</td>
<td>90.0%</td>
</tr>
<tr>
<td></td>
<td>Huang et al.</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

The 'Q*' means 'Q* index', indicating the point at which sensitivity = specificity.

*P and P for heterogeneity test.
Circulating miRNAs in diagnosing colorectal cancer

Validation of circulating miRNAs role in diagnosing CRC

To validate whether the reported circulating miR-21 and miR-92a have potential roles in diagnosing CRC, we compared the two miRNAs expressions in plasma samples in a case–control study. There were no statistically significant differences in age, sex, smoking status, drinking status or family history of cancer between CRC cases and healthy controls (P > 0.05 for all, Supplementary Table S2, available at Carcinogenesis Online). Furthermore, 8.2% of CRC patients were in Dukes’ A, 34.7% in B and 28.6% in C and D, along with 85.7% in Grade II and 14.3% in Grade III. As shown in Figure 2, the expression of miR-21 in plasma was significantly increased in cases compared with that in controls (both P < 0.001), whereas miR-92a expression exhibited no statistical difference (P = 0.443).

We performed risk-score analysis to investigate the effectiveness of miRNAs signature for predicting CRC and ROC curve analysis to estimate whether candidate miRNAs could be used as potential diagnostic markers for CRC. The distribution of miRNA expression and risk scores of all samples are shown in Figure 3. Notably, CRC patients tended to have higher risk scores of miR-21. In addition, the AUC of miR-21 was 0.877 (95% CI = 0.795–0.935) and 0.533 (95% CI = 0.430–0.635) for miR-92a. At the cutoff values of 0.00220 for miR-21 and 0.0157 for miR-92a from 2^ΔCt, the sensitivity and the specificity were 76.2 and 93.2% for miR-21, along with 18.4 and 95.9% for miR-92a, respectively.

Test for similarity of miRNA differential expression analysis in tissue and plasma samples

To verify the consistency of miRNA expression in tissue and plasma samples, we compared the expression level of miR-21 in CRC and normal tissues. By screening Gene Expression Omnibus database with the keywords ‘miRNA’ and ‘colorectal cancer’, we downloaded GSE28364 (30) and GSE35602 (31) published miRNA profiling databases and extracted relative expression values of miR-21 for our validation in tissue samples. Furthermore, we acquired the miR-21 expression value of colon adenocarcinoma from miRNA Sequencing database in The Cancer Genome Atlas Data Portal. As shown in Figure 4, miR-21 expression in CRC tissues was significantly higher than that in normal tissues (both P < 0.001; GSE28364 and The Cancer Genome Atlas miRNA Sequencing data for paired tissues and GSE35602 for non-paired tissues). In addition, we measured miR-21 expression level in four CRC cell lines (HT29, SW620, LoVo and SW480) and one normal colon cell line (FHC) and found that miR-21 expression in CRC cell lines was notably higher than that in the normal colon cell line. Overall, miR-21 was overexpressed in both tissue and plasma samples from CRC patients.

Fig. 2. Expression level of miR-21 and miR-92a in the plasma of CRC patients and controls by quantitative reverse transcription–PCR analysis in the validation study. (a) The expression of plasma miR-21 was significantly higher in CRCs than that in controls. (b) No significant difference of plasma miR-92a expression was observed in cases and healthy controls.
Discussion

In this diagnostic meta-analysis, we observed that circulating miR-21 had relatively higher diagnostic accuracy and yielded a combined AUC of 0.867 with 76% pooled sensitivity and 82% pooled specificity in discriminating CRC cases. Similarly, in our independent test, we observed the same diagnostic efficiency of plasma miR-21, and the level was significantly higher in CRC cases than in controls. Furthermore, we identified the differential expressions of miR-21 from CRCs and normal tissues, as well as in CRC and normal cell lines. To our knowledge, this is the first report on comprehensive assessment for diagnostic efficiency of circulating miRNAs in CRC.

Many studies have reported that specific cancer features, both genetics and epigenetic, can be detected in human plasma or serum and be useful in diagnosis to monitor pathological processes, such as prostate-specific antigen for prostate cancer (32) and alpha-feto-protein for liver cancer (33). Since the emerging findings of miRNAs are extensively reported as regulatory molecules, numerous studies have demonstrated the correlation of miRNAs abnormal expression with various diseases, including the development and progression of cancer (34). However, most studies are centered on the examination of miRNA levels in tissue specimens, and a limited number of them pay attention to the practicability of circulating miRNAs as biomarkers in blood, serum or plasma samples. In 2007, Valadi et al. (35) first proved that exosomal shuttle RNAs, including miRNAs, could be detected in total RNA preparation that originated from biological fluids. Mitchell et al. (14) were the first to establish procedures on the examination of tumor-derived miRNAs in serums or plasmas and identified that the serum miR-141 was able to discriminate prostate cancer patients from healthy controls. Since then, although the sources of miRNAs and their functions into circulation remain controversial, potential use of miRNAs as valuable blood biomarkers, owing to their stability, cancer specificity and ease of determination, has attracted the attention of researchers (36). We are looking forward to the development of suitable biomarkers for diagnosing CRC.

To date, many studies have reported the possibility of circulating miRNAs as non-invasive biomarkers for CRC screening (18,19), but diagnostic accuracy values have been inconsistent between these studies and the specific circulating miRNAs were not confirmed. Thus, we performed a systemic meta-analysis to evaluate the diagnostic value of specific circulating miRNAs for CRC from all the possible publications. Eventually, only miR-21 and miR-92a were analyzed for seven articles including six studies for miR-21 and four for miR-92a. We observed that both of them could distinguish CRC patients from healthy controls, but circulating miR-21 with higher combined AUC and specificity demonstrated as a better molecule marker for CRC prediction than miR-92a. Due to the lack of consensus as to whether serum or plasma is a better source of circulating nucleic acids,
including miRNAs, and to validate the above findings, we measured these two miRNAs expressions in our plasma samples among CRCs and healthy individuals. Similarly, we identified that plasma miR-21 expression of CRC patients was markedly higher than that of controls. Furthermore, analysis of risk score and ROC curve showed that it had good discrimination between cases and controls, yielding an AUC of 0.877 with 76.2% sensitivity and 93.2% specificity, which are consistent with meta-analysis results. However, plasma miR-92a expression in our validation set was not observed the similar results, which was possible for the different methods used for miRNA extraction and detection, or the endogenous controls used for normalization (37,38). Our validation test further confirmed that plasma miR-21 was a preferable biomarker in discriminating CRC.

Some researchers have confirmed that the origin of circulating miRNAs is the result of secretion or leakage from normal and tumor tissues (36), and the relationship of extracellular miRNAs expressions to normal/tumor tissue miRNAs levels has been testified. Toiyama et al. (25) found that miR-21 expression in both matched CRC tissues and serum samples was notably higher in CRC patients with clinicopathological features; meanwhile, it exhibited a remarkably positive correlation. Recently, Chen et al. (39), from the perspective of global miRNAs, observed a significantly positive correlation of tissues and circulating miRNAs. Hence, we further analyzed miR-21 level in CRC and normal tissues by utilizing public databases. Reid et al. conducted miRNA expression profiles for 40 CRCs and their paired tissues from GSE28364 data, in which levels of miR-21 prominently overexpressed in tumor tissues. Likewise, the same phenotype was observed in GSE35602 data from 17 CRCs and 8 non-paired normal tissues performed by Nishida et al. Besides, miRNA Sequencing data from The Cancer Genome Atlas also showed the higher expression value of miR-21 in colon cancer tissues. Furthermore, on the cellular level, miR-21 in CRC cell lines also expressed higher than that in normal colon cells. Compared with the former validation results, it is plausible that plasma miR-21 mirrors tissue of miR-21.

Reports have indicated that exosomes containing secreted miRNAs can be delivered from cell to cell and regulate gene expression in recipient cells by canonical binding to their target mRNAs (35) and may even be involved in distant immunoregulation, angiogenesis and vascular mimicry of tumor cells (40). In 2012, Fabbri et al. (41) was the first to provide evidence that suggested tumor-secreted miR-21 and miR-29a were regulators of the tumor microenvironment and were able to interact with the Toll-like receptor family of immune cells to stimulate prometastatic inflammatory processes, tumor growth and spread. Recently, Pritchard et al. (37) demonstrated that some reported circulating miRNA cancer biomarkers reflected a blood cell-based phenomenon and were highly expressed in blood cells, as well as in cancer-specific origin. Also, Zanutto et al. (38) reported that plasma miR-378, not miR-21, was a hemolysis-independent biomarker for CRC, which was not included in our meta-analysis because of the lack of sufficient data. Thus, we intend to conduct future studies to trace the origin of circulating miR-21 and explore its potential function in the development and progression of CRC.

Ever since the presence of miR-21 in serum or plasma of diffuse large B-cell lymphoma patients was first demonstrated upregulation by Lawrie et al. (42), several studies have reported the overexpression of plasma miR-21 in other solid cancers. Kanaan et al. (23) observed

![Fig. 4. Expression level of miR-21 in tissues and cell lines. (a) The miR-21 expression from GSE28364 was detected in 40 CRC tissues with its corresponding normal tissues, compared by the method of paired t-test. (b) The miR-21 expression of GSE35602 was examined in 17 CRC tissues and 8 normal tissues, analyzed by unpaired t-test. (c) The expression value of miR-21 from The Cancer Genome Atlas data was derived in eight colon adenocarcinoma tissues and matched paracarcinoma tissues by miRNA sequencing, compared by paired t-test. RMM, reads per million miRNA mapped. (d) The relative level of miR-21 in four CRC cell lines expressed remarkably higher compared with the normal colon cell line.](image-url)
higher expression of plasma miR-21 in CRCs than that in controls. Wang et al. (22) reported the same result in a systematical study of five solid tumors, including breast cancer, esophageal cancer, gastric cancer, CRC and lung cancer. Furthermore, the controls in our included studies were healthy individuals, who were generally not what would be the intended use population for circulating miR-21 detection, and there is still no sufficient evidence demonstrating that circulating miR-21 could discriminate among the healthy individuals, the colorectal precancerous lesions patients and the CRCs. Thus, these limit the use of miR-21 as a specific molecular marker for CRC diagnosis currently.

In conclusion, our comprehensive analysis of circulating miRNAs in discriminating CRC and further validation in an independent cohort provide compelling evidence for the clinical potential of plasma miR-21 as a promising non-invasive screening tool for the early detection of CRC. Even so, further large-scale prospective studies are warranted.

Supplementary material

Supplementary Tables S1 and S2 and Figures S1–S4 can be found at http://carcin.oxfordjournals.org/

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References


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