Limited Diagnostic Value of microRNAs for Detecting Colorectal Cancer: A Meta-analysis

Xuan-Jun Zhou, Zhao-Gang Dong, Yong-Mei Yang, Lu-Tao Du, Xin Zhang, Chuan-Xin Wang*

Abstract

Background: MicroRNAs have been demonstrated to play important roles in the development and progression of colorectal cancer. Several studies utilizing microRNAs as diagnostic biomarkers for colorectal cancer (CRC) have been reported. The aim of this meta-analysis was to comprehensively and quantitatively summarize the diagnostic value of microRNAs for detecting colorectal cancer. Methods: We searched PubMed, Embase and Cochrane Library for published studies that used microRNAs as biomarkers for the diagnosis of colorectal cancer. Summary estimates for sensitivity, specificity and other measures of accuracy of microRNAs in the diagnosis of colorectal cancer were calculated using the bivariate random effects model. A summary receiver operating characteristic (SROC) curve was also generated to summarize the overall effectiveness of the test. Result: Thirteen studies from twelve published articles met the inclusion criteria and were included. The overall sensitivity, specificity, positive likelihood ratio, negative likelihood ratio and diagnostic odd ratio of microRNAs for the diagnosis of colorectal cancer were 0.81 (95% CI: 0.79-0.84), 0.78 (95% CI: 0.75-0.82), 4.14 (95% CI: 2.90-5.92), 0.24 (95% CI: 0.19-0.30), and 19.2 (95% CI: 11.7-31.5), respectively. The area under the SROC curve was 0.89. Conclusions: The current evidence suggests that the microRNAs test might not be used alone as a screening tool for CRC. Combining microRNAs testing with other conventional tests such as FOBT may improve the diagnostic accuracy for detecting CRC.

Keywords: Colorectal cancer - microRNAs - diagnostic - meta-analysis

RESEARCH ARTICLE

Limited Diagnostic Value of microRNAs for Detecting Colorectal Cancer: A Meta-analysis

Xuan-Jun Zhou, Zhao-Gang Dong, Yong-Mei Yang, Lu-Tao Du, Xin Zhang, Chuan-Xin Wang*

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide. In 2008, over 1.2 million people worldwide were diagnosed with CRC, causing nearly 608,700 deaths (Jemal et al., 2010). CRC is curable if detected at an early stage. The 5-year survival rate for CRC patients is strikingly different by stage, ranging from greater than 93% for stage I disease to less than 8% for stage IV disease (O’Connell et al., 2004). Given improved survival rates seen with stage I and IV CRC, it is necessary to develop a screening test by which the cancer can be diagnosed at an early stage. To date, several screening methods for the early detection of CRC are available, including fecal occult blood testing (FOBT), stool DNA test and colonoscopy (Levin et al., 2008; Mandel 2008). However, none of them has been established as an effective screening tool. Colonoscopy is a reliable screening tool for CRC. However, many patients delay or completely avoid colonoscopy because of its invasive nature and high cost. Thus, new approaches that can complement and improve the current CRC screening methods are urgently needed.

MicroRNAs are a functional class of non-protein-coding RNA molecules with 18-24 nucleotides that negatively modulate the activity of specific mRNA targets. Studies have demonstrated that microRNAs play important roles in the multistep carcinogenesis process through the dysregulation of oncogenes and tumor suppressor genes (Zhang et al., 2007). Aberrant expression of microRNAs is also found in CRC tissue, blood and feces (Ng et al., 2009; Huang et al., 2010; Koga et al., 2010; Pu et al., 2010; Kalimutho et al., 2011; Kanaan et al., 2012; Wang et al., 2012; Wang et al., 2012; Wu et al., 2012; Giraldez et al., 2013; Wang et al., 2013a; Wang et al., 2013b). In recent years, an increasing number of studies utilizing microRNAs in blood or tissue samples as diagnostic biomarkers for CRC have been reported. Meanwhile, several studies also evaluated the feasibility of using microRNAs from fecal specimens as screening biomarkers for CRC. The results of these studies are variable even with...
some encouraging information. Therefore, we performed the present meta-analysis to analyze the diagnostic accuracy of microRNAs for CRC.

Materials and Methods

Search strategy and study selection

A systematic literature search in PubMed, Embase and Cochrane Library for articles published up to February 28, 2013 was performed to achieve the accessible original articles that focused on the diagnostic value of microRNAs for CRC. No start data limit was applied. The search terms used were “colon cancer” OR “colorectal cancer” OR “rectal cancer” AND “microRNA”. A manual search with a reference list of all the relevant publications was also performed.

Two investigators (Xuanjun Zhou and Zhaogang Dong) independently inspected all the article titles and abstracts to identify those studies that likely reported the diagnostic value of microRNAs for CRC and then retrieved the full texts of these published articles to determine whether they were exactly eligible. Disagreements between the two investigators were resolved by consensus. Inclusion criteria for the primary studies were as follows: (1) the study must be published in English; (2) all the participants involved in the study must have been confirmed by standard test (such as colonoscopy or histopathologic analysis); (3) studies evaluated the diagnostic value of microRNAs for detecting human CRC; (4) sufficient data should be included to reconstruct the diagnostic 2×2 contingency table of microRNAs.

Data extraction and quality assessment

The following data were extracted and filled onto standardized data forms: (a) first author, (b) publication year, (c) study of state, (d) specimen, (e) total sample size including numbers of cases and controls, (f) assay method, (g) microRNA expression signature, (h) the diagnostic test results. The methodological quality of each study was assessed by QUADAS tool, which is a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews (Whiting et al., 2003). The QUADAS tool is structured as a list of 14 items, which should be answered with ‘yes’ ‘no’, or ‘unclear’. When a specific item was fulfilled, a score of ‘1’ was given, ‘0’ if this item was unclear and ‘-1’ if the item was not achieved. The same two reviewers (Xuanjun Zhou and Zhaogang Dong) extracted the data and assessed the study quality independently, and disagreements were resolved by consensus.

Statistical Methods

Standard methods recommended for meta-analysis of diagnostic studies were used (Deville et al., 2002). Based on the 2×2 contingency table, we extracted the numbers of participants with true-positive (TP), false positive (FP), true-negative (TN) and false-negative (FN) results from every included study. The chi-square and inconsistency index (I²) were performed to detect statistically significant heterogeneity within studies (Higgins et al., 2003). Heterogeneity was considered significant when I² was greater than 50%. Subgroup analysis and meta-regression were performed to investigate the heterogeneity within the included studies. A bivariate random effects model was used to calculate the pooled sensitivity, specificity and other related indexes across studies (Reitsma et al., 2005). A summary receiver operating characteristic (SROC) curve, which based on the sensitivity and specificity of each included study, was constructed. The area under the SROC curve (AUC) represents an analytical summary of test performance. An AUC close to 0.5 indicates a poor test whereas an AUC of 1.0 indicates that a test can accurately distinguish cases from non-cases. MetaDiSc statistical software version 1.4 (Unit of Clinical Biostatistics, Ramony Cajal Hospital, Madrid, Spain) was used for all the above statistical analyses. Concerning the publication bias for meta-analyses of diagnostic studies, we explored the potential publication bias through Deeks’ funnel plots (Deeks et al., 2005). These statistical analyses were undertaken using STATA 11.0 (Stata Corporation, College Station, TX). All statistical tests were two sided, and p < 0.05 was considered statistically significant.

Results

Characteristics and quality of the included studies

The article selection process used in this study is summarized in Figure 1. A total of 13 studies from 12 English language articles met the inclusion criteria and were included in the present meta-analysis. The main clinical characteristics of the included studies, along with QUADAS scores, are presented in Table 1. Overall, the 13 selected studies included 1,512 individuals and the sample size varied from 32 to 316 individuals with a median size of 116 individuals. The included studies originated from 6 countries or regions (including United States, Italy, Spain, Japan, Hong Kong and China) and were published from 2009 to 2013. The categories of specimens included colorectal tissue (2 studies, 15.4%), plasma (7 studies, 53.8%), serum (1 study, 7.7%) and feces (3 studies, 23.1%). Six studies (Ng et al., 2009; Pu et al., 2010; Kalimutho et al., 2011; Kanaan et al., 2012; Wang et al., 2012; Wu et al., 2012) evaluated a single microRNA as the diagnostic biomarker, while the other seven studies (Huang et al., 2010; Koga et al., 2010; Kanaan et al., 2012; Wang et al., 2012; Giraldez et al., 2013; Wang et al., 2013a; Wang et al., 2013b) focused on multiple microRNAs for detecting CRC. In total, 23 microRNAs (miR-1, miR-7, miR-15b, miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-21, miR-29a, miR-31, miR-92a, miR-92, miR-93, miR-96, miR-100, miR-122, miR-125a, miR-143, miR-145, miR-200a, miR-200b, miR-204, miR-29b, miR-373, miR-574) were included in the present meta-analysis.

Figure 1. Flow Chart of Selection Process for Eligible Articles
miR-133a, miR-135a, miR-135b, miR-144*, miR-21, miR-135b

miR-17-92 cluster included miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a

Wang et al., 2013b) had a QUADAS score < 10. Most studies (10/13) recruited a group known to have the target disorder and a group of healthy controls, which may lead to spectrum bias. Eight studies (Ng et al., 2009; Koga et al., 2010; Pu et al., 2010; Kanaan et al., 2012; Wang et al., 2012; Wang et al., 2013a; Wang et al., 2013b) used histopathologic analysis as the standard test, whereas the remaining five (Huang et al., 2010; Koga et al., 2010; Pu et al., 2010; Kalimutho et al., 2011; Wu et al., 2010; Giraldez et al., 2013) used histopathologic analysis and colonoscopy as the standard test. One study (Pu et al., 2010) did not report the uninterpretable index test results. All studies clearly stated that the results of the index test were interpreted blind to the results of the standard test. However, it was unclear whether the index test results. All studies clearly stated that the results of the index test were interpreted blind to the results of the standard test were interpreted blind to the results of this meta-analysis. The size of each solid circle indicates the size of each study. The regression SROC curve summarizes the overall diagnostic accuracy.

Table 1. Main Characteristics of 13 Studies Included in Meta-analysis

| First author | Publication year | Location | Specimen | Total sample size | Tp | Fn | Assay method | threshold | microRNA | QUADAS scores |
|--------------|-----------------|---------|----------|-------------------|----|----|--------------|-----------|-----------|---------------|
| Ng et al., 2009 | 2009 | Hong Kong | plasma | 140 | 80 | 15 | 10 | 35 | qRT-PCR | Yes | miR-92 | 11 |
| Koga et al., 2010 | 2010 | Japan | feces | 316 | 146 | 25 | 51 | 94 | qRT-PCR | Yes | miR-17-92 cluster*, miR-21, miR-135 | 10 |
| Pu et al., 2010 | 2010 | China | plasma | 140 | 89 | 22 | 14 | 15 | qRT-PCR | Yes | miR-221 | 8 |
| Huang et al., 2010 | 2010 | China | plasma | 159 | 83 | 9 | 17 | 50 | qRT-PCR | Yes | miR-29a, miR-92a | 9 |
| Kalimutho et al., 2011 | 2011 | Italy | feces | 75 | 26 | 5 | 9 | 35 | qRT-PCR | No | miR-144* | 9 |
| Giraldez et al., 2013 | 2012 | Spain | plasma | 95 | 33 | 11 | 9 | 42 | qRT-PCR | No | miR19a, miR19b, miR15b | 9 |
| Wang et al., 2012 | 2012 | China | plasma | 148 | 75 | 4 | 15 | 54 | qRT-PCR | Yes | miR-29a, miR-92a, miR-760 | 9 |
| Kanaan et al., 2012 | 2012 | America | tissue | 32 | 16 | 2 | 0 | 14 | qRT-PCR | No | miR-1, miR-31, miR-133a, miR-135b | 9 |
| Kanaan et al., 2012 | 2012 | America | tissue | 32 | 16 | 2 | 0 | 14 | qRT-PCR | No | miR-1, miR-31, miR-133a, miR-135b | 9 |
| Wang et al., 2012 | 2012 | China | serum | 71 | 28 | 10 | 4 | 29 | qRT-PCR | Yes | miR-21 | 9 |
| Wang et al., 2012 | 2012 | China | serum | 71 | 28 | 10 | 4 | 29 | qRT-PCR | Yes | miR-21 | 9 |
| Wang et al., 2013a | 2012 | China | tissue | 58 | 31 | 4 | 2 | 21 | qRT-PCR | No | miR-92a, miR-375, miR-424 | 9 |
| Wu et al., 2012 | 2012 | Hong Kong | feces | 189 | 63 | 27 | 25 | 74 | qRT-PCR | Yes | miR-92a | 9 |
| Wang et al., 2013b | 2013 | China | plasma | 49 | 18 | 3 | 4 | 24 | qRT-PCR | NO | miR-409-3p, miR-7, miR-93 | 9 |

*Sensitivity (a) and Specificity (b) for microRNAs on Detection of CRC for All Studies

The point estimates of sensitivity and specificity for each study are shown as solid circles and the size of each study are shown as solid circles and the size of each study. Error bars are 95% confidence intervals.

Figure 2. Forest Plot of Estimates of Sensitivity (a) and Specificity (b) for microRNAs on Detection of CRC for All Studies. The point estimates of sensitivity and specificity for each study are shown as solid circles and the size of each solid circle indicates the sample size of each study. Error bars are 95% confidence intervals.

Figure 3. Summary Receiver Operating Characteristic (SROC) Curve for microRNAs on Detection of CRC for All Studies. Solid circles represent each study included in the meta-analysis. The size of each solid circle indicates the size of each study. The regression SROC curve summarizes the overall diagnostic accuracy.
Discussion

MicroRNAs are small non-coding RNA molecules that play a crucial role in the development and progression of CRC (Corte et al., 2012). During the past few years, relevant studies have been carried out to assess the diagnostic use of microRNAs for CRC. However, the exact role of microRNAs for detecting CRC still needs to be analyzed. Hence, we performed a comprehensive meta-analysis on the use of microRNAs in detecting CRC.

We included 13 studies with a total participant population of 1,512. Calculated with the bivariate random effects model, utilizing microRNAs as biomarkers for detecting CRC yielded an overall sensitivity of 85% and an overall specificity of 78%. The AUC represents an overall summary measure of the SROC curve and the test’s overall ability to accurately distinguish cases from non-cases. In this meta-analysis, the AUC was 0.91 for blood-based tests and 0.785 for feces-based tests. Thus, compared with the feces-based microRNAs tests, the blood-based microRNAs tests had a higher level of diagnostic accuracy. The subgroup analysis for feces-based studies showed no significant heterogeneity, whereas the heterogeneity for blood-based studies was still apparent in specificity, PLR and DOR.

Table 2. Summary Results for Diagnostic Accuracy of microRNAs

|                      | CRC vs. non-CRC | Blood-based microRNAs | Feces-based microRNAs |
|----------------------|-----------------|-----------------------|-----------------------|
| Number of studies    | 13              | 8                     | 3                     |
| Sensitivity (95% CI) | 0.81 (0.79-0.84) | 0.85 (0.82-0.88) | 0.73 (0.68-0.78) |
| I2 (p)               | 0.08 (0.0016)   | 0.06 (0.0081)        | 0.05 (0.0000)        |
| Specificity (95% CI) | 0.78 (0.75-0.82) | 0.78 (0.73-0.82) | 0.78 (0.73-0.83) |
| I2 (p)               | 0.08 (0.0000)   | 0.05 (0.0000)        | 0.06 (0.0001)        |
| PLR (95% CI)         | 4.14 (2.90-5.92) | 4.27 (2.42-7.56) | 3.36 (2.39-4.73) |
| I2 (p)               | 0.08 (0.0000)   | 0.10 (0.0166)        | 0.07 (0.0048)        |
| NLR (95% CI)         | 0.24 (0.19-0.30) | 0.21 (0.16-0.26) | 0.24 (0.18-0.31) |
| I2 (p)               | 0.07 (0.0001)   | 0.09 (0.0067)        | 0.05 (0.0028)        |
| DOR (95% CI)         | 19.15 (11.65-31.48) | 21.19 (10.94-41.05) | 9.90 (6.17-15.89) |
| I2 (p)               | 0.07 (0.0001)   | 0.12 (0.0470)        | 0.05 (0.0028)        |

Table 3. Meta-regression of Potential Heterogeneity Within the Included Studies

| Covariates            | Coefficient | SE   | RDOR (95% CI) | p value |
|-----------------------|-------------|------|---------------|---------|
| Single microRNA       | -0.724      | 0.5937 | 0.48 (0.12-1.91) | 0.2572  |
| QUADAS scores ≥10     | 0.169       | 0.5855 | 1.18 (0.31-4.57) | 0.7798  |
| Sample size ≥100      | -0.781      | 0.5302 | 0.46 (0.13-1.55) | 0.1788  |

Meta-regression and publication bias

A meta-regression was performed to investigate the potential heterogeneity within the included studies. We used 3 covariates in the present meta-regression: (1) single or multiple microRNAs used as diagnostic biomarkers; (2) sample size (≥100 or < 100); (3) QUADAS scores (≥10 or < 10). The outcomes of the regression are shown in Table 3. In the present study, none of the above covariates were found to be the significant source of heterogeneity (all p > 0.05).

Since publication bias is concerned for meta-analyses of diagnostic studies, we explored the potential publication bias through Deeks’ funnel plots. The Deeks’ test indicated that there was a significant small publication bias (p = 0.035) (Figure 4).

Discussion

MicroRNAs are small non-coding RNA molecules that play a crucial role in the development and progression of CRC (Corte et al., 2012). During the past few years, relevant studies have been carried out to assess the diagnostic use of microRNAs for CRC. However, the exact role of microRNAs for detecting CRC still needs to be analyzed. Hence, we performed a comprehensive meta-analysis on the use of microRNAs in detecting CRC.
Limited Diagnostic Value of microRNAs for Detecting Colorectal Cancer: A Meta-analysis

Wanjiu Huang, Nan Jiang, Yaping Zhang, Xianwen Gu, Xiyu Jiang, Xingyi Zhang, Xingzhi Liu, Hua Cui, Junjie Wang, and Tiejun Fei

Asian Pacific Journal of Cancer Prevention, Vol 14, 2013

DOI: http://dx.doi.org/10.7314/APJCP.2013.14.8.4699

from 0 to infinity (Glas et al., 2003). The higher value of a DOR indicates greater diagnostic accuracy. A DOR of 1.0 shows that the test can not distinguish individuals with the disease from those without it. In the present meta-analysis we found that the pooled DOR was 19.15, also indicating that the overall accuracy of microRNAs test for detecting CRC is high.

Moreover, likelihood ratios (LRs), which combine the stability of sensitivity and specificity to provide an omnibus index of test performance, are considered to be more clinically meaningful than the SROC curve and DOR (Gallagher 1998). A PLR greater than 10 or a NLR less than 0.1 generates large and often conclusive changes from pre-test to post-test probability. In the present meta-analysis, a pooled PLR of 4.14 (95%CI: 2.90-5.92) suggests that individuals with CRC have about 4.14-fold higher chance of being tested positive using microRNAs compared with individuals without CRC. A pooled NLR of 0.24 (95%CI: 0.19-0.30) means that the probability of the individuals having CRC is 24% when the microRNAs test is negative. Neither the PLR nor the NLR alone was adequate to confirm or rule out the diagnosis of CRC.

We also performed subgroup analysis regarding the clinical specimen used in the studies. Our meta-analysis showed that using blood microRNAs as biomarkers for detecting CRC yielded an overall sensitivity of 85% and an overall specificity of 78%. The AUC was 0.91 and DOR was 21.19, also indicating a relatively high level of diagnostic accuracy. In terms of feces-based microRNAs tests, the overall sensitivity was 73%, specificity was 78%, and AUC was 0.785, indicating a moderate level of diagnostic accuracy. Moreover, AUCs were used in the current meta-analysis to compare diagnostic accuracies between blood-based microRNAs tests and feces-based microRNAs tests. Our data showed that the microRNAs detection had a higher accuracy in blood than in feces, which suggested that microRNAs in blood may be better diagnostic biomarkers for detecting CRC. The pooled PLR was 4.27 (95%CI: 2.42-7.56) for blood-based microRNAs tests and 3.36 (95%CI: 2.39-4.73) for feces-based microRNAs tests. Neither of them was high enough for clinical purposes. The NLR values of blood-based tests and feces-based tests were 0.21 (95%CI: 0.16-0.26) and 0.34 (95%CI: 0.28-0.41), respectively, which were also not low enough to rule out CRC.

Because of the limited studies, we did not summarize the diagnostic performance of tissue-based microRNAs tests. Although the tissue-based microRNAs test might have higher diagnosis accuracy for CRC than blood-based microRNAs test, its invasive and inconvenient nature may still hinder its wide application.

There were 23 microRNAs used as diagnostic biomarkers involved in our meta-analysis. These microRNAs biomarkers may provide new insight into the early detection of CRC. Among them, miR-92a was found to be used as the biomarker in five studies (Huang et al., 2010; Koga et al., 2010; Wang et al., 2012; Wu et al., 2012; Wang et al., 2013a). Zhou et al. demonstrated that the high expression of miR-92a was closely associated with advanced clinical stage, lymph node metastases, distant metastasis, and poor overall survival in CRC (Zhou et al., 2013). MiR-21 was used as the biomarker in three studies (Koga et al., 2010; Kanaan et al., 2012; Wang et al., 2012). And it was also the most frequent microRNA to be studied in CRC. Multiple studies showed that high expression of miR-21 was associated with advanced disease and worse outcome (Schetter et al., 2008; Schetter et al., 2009; Liu et al., 2011). However, most reported microRNAs for the diagnosis of CRC had also been reported in other human malignancies. It is important to identify the microRNAs patterns that are specific to CRC in the future.

The overall results of this meta-analysis showed high statistical heterogeneity among studies. In order to explore the possible sources of heterogeneity, we performed subgroup analysis and meta-regression. The outcomes of the regression did not show any statistical significance. However, the subgroup analysis result showed that the heterogeneity across blood-based studies was still apparent in specificity, PLR and DOR.

There were also some limitations in our meta-analysis. First, the presence of spectrum bias might lead to the overestimation of diagnostic accuracy of microRNAs. Second, the Deeks’ test indicated a significant small publication bias. The potential reasons might be attributed to that small studies with positive results may be published easier than studies with negative results. Because of the relatively small number of relevant studies for the statistical analysis, it is still difficult to make a definitive conclusion about the diagnostic accuracy of microRNAs for CRC. In addition, most included studies were unclear about whether the index test results were interpreted without knowledge of the results of the standard test, which may cause review bias. But the review bias may not be important because the detection of microRNAs test is quantitative (Whiting et al., 2004).

In conclusion, our meta-analysis demonstrated that microRNAs may be potential novel biomarkers for detecting CRC. Moreover, the microRNAs in blood had higher diagnostic accuracy than in feces, which provides important evidence for the further development of noninvasive method for diagnosing CRC in the future. However, the microRNAs test may not be used alone as a screening tool for CRC. Combining microRNAs with other conventional tests such as FOBT may improve the diagnostic capability for detecting CRC.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 81271916) and Shandong Province Natural Science Foundation of China (No. ZR2010H004). The author(s) declare that they have no competing interests.

References

Collins JF, Lieberman DA, Durbin TE, et al (2005). Accuracy of screening for fecal occult blood on a single stool sample obtained by digital rectal examination: a comparison with recommended sampling practice. Ann Intern Med, 142, 81-5.

Corte H, Manceau G, Blons H, et al (2012). MicroRNA and colorectal cancer. Dig Liver Dis, 44, 195-200.
Xuan-Jun Zhou et al

Deeks JJ, Macaskill P, Irwig L (2005). The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. *J Clin Epidemiol*, 58, 882-93.

Deville WL, Buntinx F, Bouter LM, et al (2002). Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol*, 2, 9.

Gallagher EL (1998). Clinical utility of likelihood ratios. *Ann Emerg Med*, 31, 391-7.

Giraldez MD, Lozano JJ, Ramirez G, et al (2013). Circulating microRNAs as biomarkers of colorectal cancer: results from a genome-wide profiling and validation study. *Clin Gastroenterol Hepatol*, 11, 681-8 e3.

Glas AS, Lijmer JG, Prins MH, et al (2003). The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol*, 56, 1129-35.

Higgins JP, Thompson SG, Deeks JJ, et al (2003). Measuring inconsistency in meta-analyses. *BMJ*, 327, 557-60.

Huang Z, Huang D, Ni S, et al (2010). Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer*, 127, 118-26.

Jemal A, Siegel R, Xu J, et al (2010). Cancer statistics, 2010. *CA Cancer J Clin*, 60, 277-300.

Kalimutho M, Del Vecchio Blanco G, Di Cecilia S, et al (2011). Differential expression of miR-144* as a novel feto-oriented diagnostic marker for colorectal cancer. *J Gastroenterol*, 46, 1391-402.

Kanaan Z, Rai SN, Eichenberger MR, et al (2012). Plasma miR-21: a potential diagnostic marker of colorectal cancer. *Ann Surg*, 256, 544-51.

Koga Y, Yasunaga M, Takahashi A, et al (2010). MicroRNA expression profiling of exfoliated colonocytes isolated from feces for colorectal cancer screening. *Cancer Prev Res (Phila)*, 3, 1435-42.

Landsdorp-Vogelaar I, Kuntz KM, Knudsen AB, et al (2010). Stool DNA testing to screen for colorectal cancer in the Medicare population: a cost-effectiveness analysis. *Ann Intern Med*, 153, 368-77.

Levin B, Lieberman DA, McFarland B, et al (2008). Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin*, 58, 130-60.

Liu K, Li G, Fan C, et al (2011). Increased expression of microRNA-21 and its association with chemotherapeutic response in human colorectal cancer. *J Int Med Res*, 39, 2289-95.

Mandel JS (2008). Screening for colorectal cancer. *Gastroenterol Clin North Am*, 37, 97-115, vii.

Mitchell PS, Parkin RK, Kroh EM, et al. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A*, 105, 10513-8.

Ng EK, Chong WW, Jin H, et al (2009). Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut*, 58, 1375-81.

O’Connell JB, Maggard MA, Ko CY (2004). Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J Natl Cancer Inst*, 96, 1420-5.

Pu XX, Huang GL, Guo HQ, et al (2010). Circulating miR-221 directly amplified from plasma is a potential diagnostic and prognostic marker of colorectal cancer and is correlated with p53 expression. *J Gastroenterol Hepatol*, 25, 1674-80.

Reitsma JB, Glas AS, Rutjes AW, et al (2005). Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol*, 58, 982-90.

Schetter AJ, Leung SY, Sohn JJ, et al (2008). MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA*, 299, 425-36.

Schetter AJ, Nguyen GH, Bowman ED, et al (2009). Association of inflammation-related and microRNA gene expression with cancer-specific mortality of colon adenocarcinoma. *Clin Cancer Res*, 15, 5878-87.

Wang B, Zhang Q (2012). The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. *J Cancer Res Clin Oncol*, 138, 1659-66.

Wang Q, Huang Z, Ni S, et al (2012). Plasma miR-601 and miR-760 are novel biomarkers for the early detection of colorectal cancer. *PLoS One*, 7, e44398.

Wang S, Wang L, Bayaxi N, et al (2013a). A microRNA panel to discriminate carcinomas from high-grade intraepithelial neoplasms in colonoscopy biopsy tissue. *Gut*, 62, 280-9.

Wang S, Xiang J, Li Z, et al (2013b). A plasma microRNA panel for early detection of colorectal cancer. *Int J Cancer*.

Whiting P, Rutjes AW, Reitsma JB, et al (2003). The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMJ Med Res Methodol*, 3, 25.

Whiting P, Rutjes AW, Reitsma JB, et al (2004). Sources of variation and bias in studies of diagnostic accuracy: a systematic review. *Ann Intern Med*, 140, 189-202.

Wu CW, Ng SS, Dong YJ, et al (2012). Detection of miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. *Gut*, 61, 739-45.

Zhang B, Pan X, Cobb GP, et al (2007). MicroRNAs as oncogenes and tumor suppressors. *Dev Biol*, 302, 1-12.

Zhou T, Zhang G, Liu Z, et al (2013). Overexpression of miR-92a correlates with tumor metastasis and poor prognosis in patients with colorectal cancer. *Int J Colorectal Dis*, 28, 19-24.