Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Diagnostic and Prognostic Value of microRNA-21 in Colorectal Cancer: An Original Study and Individual Participant Data Meta-analysis

Honghe Zhang[1,2], Peiwei Li[1,3], Haixing Ju[4], Martin Pesta[5], Vlastimil Kulda[5], Wenjun Jin[6], Mao Cai[7], Changbao Liu[7], Han Wu[1], Jinming Xu[1,8], Yao Ye[1,8], Guanglin Zhang[1,2], Enping Xu[1,2], Jianting Cai[3], Maode Lai[1,2], Dajing Xia[1,8], Jun Yang[9,10], and Yihua Wu[1,8]

Abstract

Background: We aimed to systematically summarize the diagnostic and prognostic value of circulating/tissue miR21 in patients with colorectal cancer.

Methods: An original study was conducted to explore the potential value of circulating miR21 in colorectal cancer diagnosis and tissue miR21 in colorectal cancer prognosis. PUBMED and EMBASE were searched (to August, 2013) to identify eligible studies. To explore the diagnostic performance of circulating miR21, meta-analysis methods were used to pool sensitivity, specificity, positive and negative likelihood ratio, diagnostic OR and to construct a summary ROC curve. For prognostic meta-analysis, study-specific HRs of tissue miR21 for survival were summarized. Subgroup and sensitivity analyses were applied to explore heterogeneity.

Results: Finally, 14 studies (including our study) were included in the meta-analyses. The pooled sensitivity, specificity, and AUC of circulating miR21 were 0.76 [95% confidence interval (CI), 0.59–0.88], 0.81 (95% CI, 0.76–0.85), and 0.81 (95% CI, 0.78–0.85) in diagnosing colorectal cancer. Patients with higher expression of tissue miR21 had significant inferior overall survival (OS; pooled HR, 1.56; 95% CI, 1.16–2.11) and disease-free survival (DFS; pooled HR, 1.35; 95% CI, 1.08–1.69). The individual participant data (IPD) meta-analysis demonstrated that tissue miR21 level was independently associated with worse colorectal cancer OS (HR, 1.69; 95% CI, 1.07–2.67; $P = 0.023$), whereas this association seems to be confined to males ($P = 0.007$) but not for females ($P = 0.845$).

Conclusions: Circulating miR21 level has potential value for colorectal cancer early detection, whereas high tissue miR21 level is associated with adverse colorectal cancer prognosis.

Impact: miR21 is a promising biomarker for early detection and prognosis of colorectal cancer. *Cancer Epidemiol Biomarkers Prev; 23(12); 2783–92. ©2014 AACR.*

[1]Zhejiang University School of Medicine, Hangzhou, China. [2]Department of Pathology, Zhejiang University School of Medicine, Hangzhou, China. [3]Department of Gastroenterology, Second Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China. [4]Department of Surgical Oncology, Zhejiang Cancer Hospital, Hangzhou, China. [5]Department of Biochemistry, Faculty of Medicine in Pilsen, Charles University in Prague, Czech Republic. [6]Department of Clinical Laboratory, Huzhou Central Hospital, Huzhou, China. [7]Anorectal Department, Second Affiliated Hospital, Wenzhou Medical University, WenZhou, China. [8]Department of Epidemiology and Health Statistics, Zhejiang University School of Public Health, Hangzhou, China. [9]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China. [10]Department of Biomedicine, College of Biotechnology, Zhejiang Agriculture and Forestry University, Hangzhou, China.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

H. Zhang and P. Li contributed equally to this article.

Corresponding Authors: Yihua Wu, Department of Epidemiology and Health Statistics, Zhejiang University School of Public Health, Hangzhou 310058, China. Phone/Fax: 86-0571-88208140; E-mail: georgewuer@126.com, and Jun Yang, Department of Biomedicine, College of Biotechnology, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, China. Phone/Fax: 86-0571-88208141; E-mail: gastate@zju.edu.cn

doi: 10.1158/1055-9965.EPI-14-0598

Introduction

Colorectal cancer is one of the most common type of cancers and a leading cause of death worldwide (1). Screening tests for colorectal cancer, including colonoscopy and fecal occult-blood testing, have been frequently used in recent years (2). However, these screening tests are far from adequate because of their invasiveness, high cost, or low sensitivity (3). New biomarkers, especially noninvasive biomarkers are in urgent need for colorectal cancer early detection. In addition, new prognostic biomarkers for colorectal cancer are also in need to improve treatment strategies.

microRNAs (miRNA) are small noncoding RNAs that play important roles in the regulation of cell differentiation, cell-cycle progression, apoptosis, and tumorigenesis (4, 5). They regulate protein-coding mRNAs at the post-transcriptional level by binding to target mRNA, preventing its translation or targeting it for destruction, or by transcriptional gene silencing at the chromatin level (6, 7). About half of the human miRNAs are located at cancer-related regions of the genome, and miRNAs are reported

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

to act as oncogenes or tumor-suppressor genes (8, 9), implying their important roles in the progression and prognosis of cancers, including colorectal cancer (10, 11). miR21 is an oncogenic miRNA that can modulate the expression of cancer-related genes, including PTEN, TPM1, and PDCD (12–14). Interestingly, miR21 expression is elevated during tumor progression, for example, its level is upregulated in colorectal cancer tissues. Furthermore, it can influence patients survival and response to chemotherapy (10, 15, 16). Many studies have evaluated the application of miR21 in the diagnosis and prognosis of colorectal cancer; however, the results were contradictive (10, 17–19). As a consequence, the aim of this study was to comprehensively explore the potential value of miR21 in colorectal cancer diagnosis and prognosis.

Materials and Methods

Original study

We conducted an original study to explore the diagnostic and prognostic value of miR21 expression in colorectal cancer. Briefly, colorectal cancer tissues and corresponding normal tissues were obtained from 79 patients by surgical resection and blood samples were collected in EDTA tubes from 41 patients with colorectal cancer before surgical operation and from 30 healthy controls. Total RNA was extracted from tissue and plasma samples, followed with DNase I digestion to exclude genomic DNA contamination. Mature miR21 and internal control U6 were detected by stem-loop real-time RT-PCR methods. ROC analysis was performed and the AUC value was calculated to determine the diagnostic performance of serum miR21 level in colorectal cancer. Survival analyses were conducted using the Kaplan–Meier method. Univariate and multivariate Cox's proportional hazard regression analyses were applied to estimate HRs of death according to tissue miR21 expression levels. The detailed methods were described in the Supplementary File (Supplementary Methods for Original Study). The diagnostic and prognostic data calculated from the original study were pooled with studies identified from literature search in the meta-analysis process.

Meta-analysis

This meta-analysis was designed, conducted, and reported according to the PRISMA statement (20). The meta-analyses process, including the individual participant data (IPD) meta-analysis, was carried out in accordance with the Cochrane Handbook for Systematic Reviews of Intervention (21). The review has been registered in an international registry of systematic reviews PROSPERO (CRD42013005119).

Literature search and study selection

Comprehensive literature searches were conducted (to August, 2013) in PUBMED and EMBASE to identify eligible studies. The detailed selection process was presented in Supplementary File (Supplementary Methods for Literature search and Study selection).

Data extraction

Two reviewers (Drs. P. Li and H. Zhang), independently collected data using standardized forms and discrepancies were resolved by a third investigator. The following information from each study was extracted: first author, year of publication, origin of the study population, patient characteristics (age, sex, cancer type, and stage), source of samples, number of participants, miR21 assay method, follow-up time, and variables adjusted for in the analysis. For diagnostic studies, the numbers of true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) results were extracted. For prognostic studies, HR estimates with 95% confidence intervals (CI) for overall survival (OS), disease-free survival (DFS), recurrence-free survival, recurrence-free cancer–specific survival (RF-CSS), or progression-free survival were extracted. If the HRs and their 95% CIs were not provided, the numbers of deaths or recurrences and total samples in each study were extracted to calculate these numbers (22). An IPD meta-analysis approach was conducted to assess the prognostic performance of tissue miR21 expression in patients with colorectal cancer. The principal investigators of relevant studies were contacted and asked to provide the raw data. The quality of the diagnostic study was assessed using the quality assessment of diagnostic accuracy studies 2 (QUADAS-2; ref. 23). For prognostic studies, a modified version of the QUADAS-2 assessment tool (Supplementary Table S1) was applied to assess the risk of bias and the criteria for reporting observational studies proposed in the STROBE statement was used to complete the methodologic evaluation (24).

Statistical analysis

For the diagnostic meta-analysis, numbers of TP, FP, TN, and FN were analyzed and a bivariate model was constructed to summarize the sensitivity, specificity, positive and negative likelihood ratios (LR), diagnostic odds ratio (DOR), and generate the summary receiver operator characteristic curve (25). Confidence intervals were calculated, assuming asymptotic normality after a log transformation for variance parameters and for LRs and a logit transformation for proportions. The formula for a positive LR is sensitivity/(1-specificity), and the formula for a negative LR is (1-sensitivity)/specificity. The combined LRs provide the DOR (positive LR/negative LR). A clinically useful test was defined as having a positive LR greater than 5.0 and a negative LR less than 0.2 (26). Heterogeneity was assessed using the I^2 test (27), and the Deek funnel plot method was applied to test publication bias (28).

HR was adopted for prognostic evaluation in the current meta-analysis, because all the included studies used HR to measure the prognostic performance of miR21.

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

Study-specific HR estimates were pooled using a fixed-effects model, if there was no significant heterogeneity. Otherwise, a random-effects model was applied. The extent of heterogeneity across studies was checked using the χ^2 and I^2 tests; $P \leq 0.10$ and/or $I^2 > 50\%$ indicates significant heterogeneity. Subgroup analyses and sensitivity analysis were performed to dissect the heterogeneity. Begg funnel plots and Egger linear regression test were used to assess publication bias. In the Hong Kong Validation Cohort of Schetter and colleagues (10), tissue miR21 expression values were dichotomized with the highest tertile classified as high and the lower two tertiles defined as low, and this high–low cutoff was applied universally in the IPD analysis. Survival analyses were conducted using the Kaplan–Meier method. Then univariate Cox's proportional hazard regression analyses were applied to estimate HR of death according to tissue miR21 expression levels. And multivariate models were used to adjust potential confounding factors for death, including age, sex, TNM stage, pathologic differentiation, and side of the tumor (left or right).

All analyses were conducted using the Stata software (version 11.0; StataCorp.). A P value of <0.05 was considered statistically significant.

Results

The original study

A total of 71 individuals were included (41 patients with colorectal cancer and 30 normal controls) to evaluate the diagnostic value of miR21. Serum miR21 level was suggested to be a potential biomarker in discriminating patients with colorectal cancer from control subjects, with an AUC value of 0.657 (95% CI, 0.530–0.783; Supplementary Fig. S1), a sensitivity of 51.2% and specificity of 79.0%, respectively. To assess the prognostic value of tissue miR21 for patients with colorectal cancer, 79 patients with colorectal cancer were included. The mean follow-up time was 65.9 (95% CI, 61.5–70.3) months, and 22 patients died of colorectal cancer during the follow-up period. As Kaplan–Meier survival analysis indicated, patients with higher levels of miR21 in the tumor tissues had a nonsignificant worse OS (61.5 months vs. 68.4 months; $P = 0.280$;

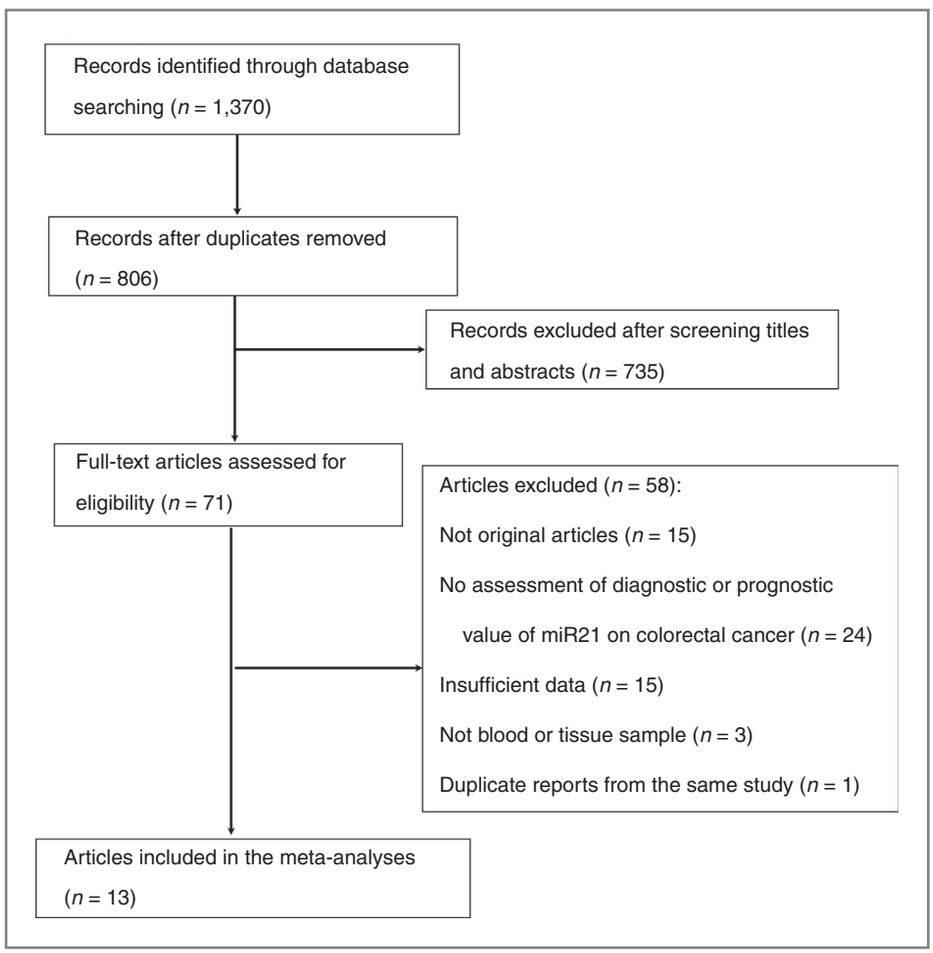

Figure 1. Flow diagram of the study selection process.

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

log-rank test; Supplementary Fig. S2). Furthermore, univariate Cox proportional hazard regression analysis revealed an HR of 1.58 (95% CI, 0.68–3.64; $P = 0.285$) for tissue miR21 in colorectal cancer prognosis. In the multivariable analysis, which included miR21 level, age, gender, side of the tumor, TNM stage, and differentiation, the HR for tissue miR21 in colorectal cancer prognosis was 1.92 (95% CI, 0.74–4.97; $P = 0.177$).

Study selection and characteristics

Searching PUBMED and EMBASE resulted in the inclusion of 1,307 articles. Finally, a total of 13 articles were identified as eligible studies (10, 16–19, 29–36). With our original study, 14 studies were finally included in the meta-analyses. The selection process was shown in Fig. 1, and the characteristics of the included studies were presented in Supplementary Tables S2 and S3. Among the included articles, 11 articles reported the prognostic value of miR21 (including our study; refs. 10, 16–19, 29–32, 36), whereas 6 examined diagnostic value of miR21 (including our study; refs. 17, 18, 33–35; 3 articles reported both prognostic and diagnostic value).

Diagnostic value of blood miR21 for colorectal cancer

Six studies with 1,071 patients assessed the diagnostic value of blood miR21 level for colorectal cancer. The included studies were conducted in Europe ($n = 1$), East Asian ($n = 4$), and the United States ($n = 1$). Sample size of each study ranged from 40 to 374. The types of specimen contain serum ($n = 3$) and plasma ($n = 3$). All the studies adopted the quantitative reverse transcription PCR (qRT-PCR) method to measure the expression of miR21. The quality assessments were shown in Supplementary Table S4.

The pooled sensitivity and specificity were 0.76 (95% CI, 0.59–0.88) and 0.81 (95% CI, 0.76–0.85), respectively (Fig. 2). And the area under the ROC curve was 0.81 (95% CI, 0.78–0.85; Fig. 3), indicating miR21 has a relatively high diagnostic performance in colorectal cancer. There was significant heterogeneity in sensitivity ($Q = 82.58$, df $= 5$, $I^2 = 93.94$, $P < 0.005$) but not in specificity ($Q = 6.57$, df $= 5$, $I^2 = 23.86$, $P = 0.25$). Sensitivity analyses indicated that country of origin, type of specimen, sample size, and study quality were not the source of heterogeneity. The pooled positive LR, negative LR, and DOR were 3.80 (95% CI, 3.00–4.81), 0.29 (95% CI, 0.17–0.50), and 13.68 (95% CI, 5.35–35.02), respectively.

The prognostic meta-analyses

A total of 11 studies were included in the prognostic analyses. All the studies were published in English and conducted in Europe ($n = 4$), East Asian ($n = 6$), and the United States ($n = 2$; one study included both American

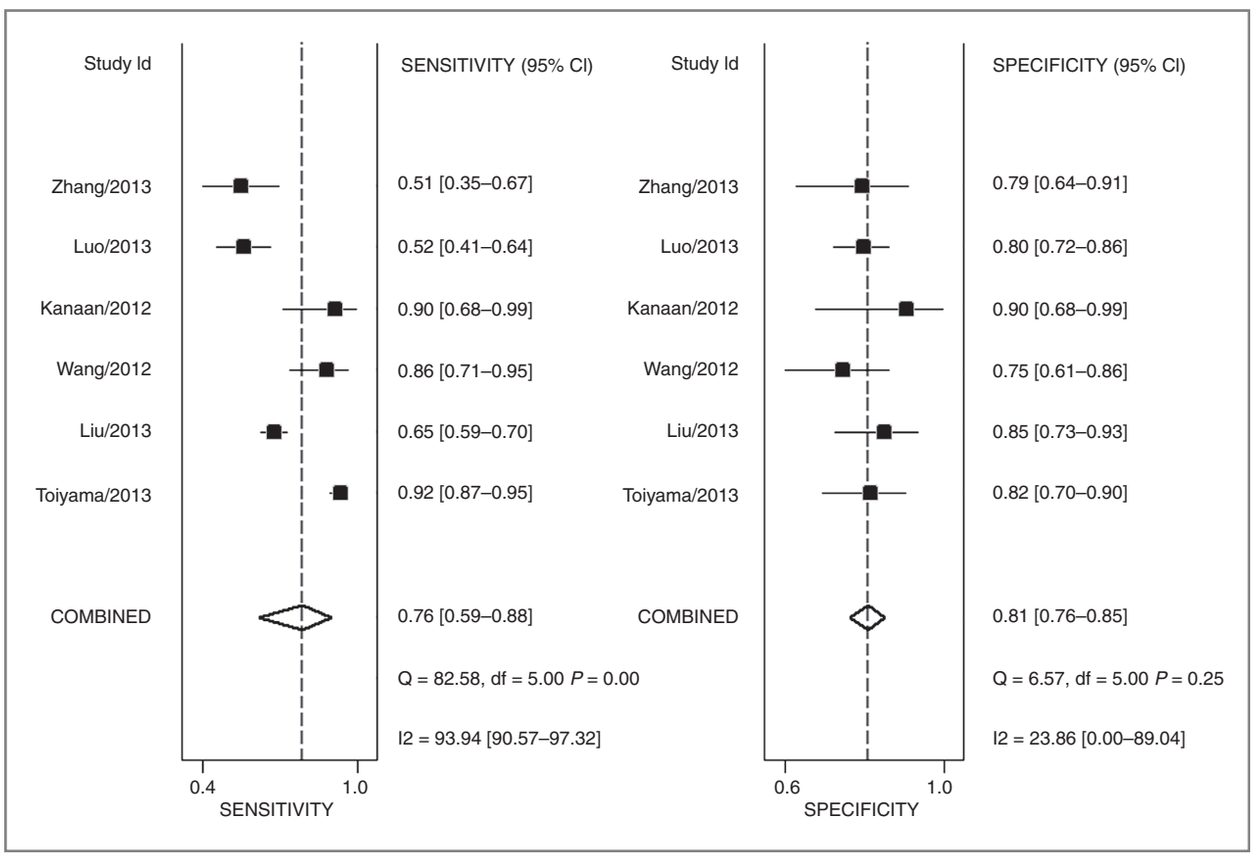

Figure 2. Forest plots of sensitivities and specificities of circulating miR21 in the diagnosis of colorectal cancer.

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

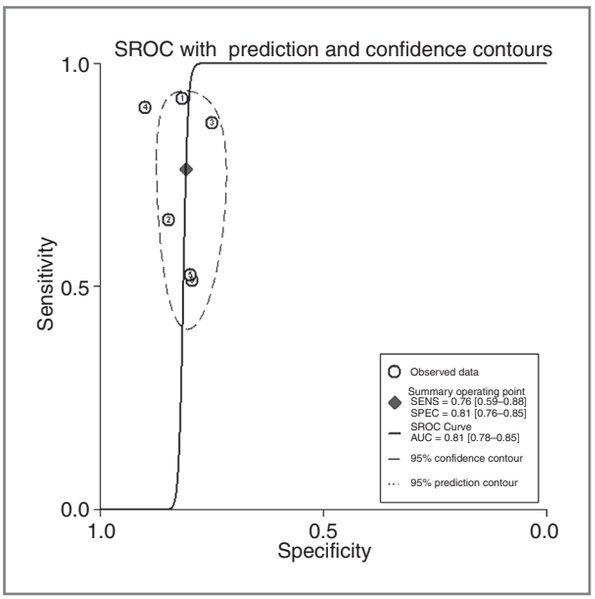

Figure 3. Summary ROC curves for miR21 in the diagnosis of colorectal cancer.

and Chinese population). Each study assessed 46 to 520 patients with colorectal cancer. The types of specimen contain tissue ($n = 9$), serum ($n = 2$), and tissue stroma ($n = 1$; one study evaluated both tissue and serum sample). Nine studies adopted the qRT-PCR method to measure the expression of miR21, whereas two studies applied the *in situ* hybridization (ISH) assay method. Three studies (including our study) presented IPD (10, 36). The quality assessments were shown in Supplementary Tables S5 and S6.

Tissue miR21 level and prognostic outcome

A total of nine studies (including our study) with 1,598 patients assessed the impact of tissue miR21 expression on colorectal cancer OS. The pooled HR was 1.56 (95% CI, 1.16–2.11) for all the studies, indicating that higher tissue miR21 expression level predicate poorer OS for patients with colorectal cancer (Fig. 4A). Significant heterogeneity across studies was observed ($I^2 = 85.1\%$, $P < 0.001$; Fig. 4A). Subgroup and sensitivity analyses suggested that methods to measure miR21 expression, country of origin might contribute to heterogeneity across studies (Table 1).

We then applied a meta-analysis of IPD to further explore the potential value of miR21 in colorectal cancer prognosis. Three studies (including our study) presented IPD data (10, 36), and Schetter and colleagues (10) only provided IPD of the Hong Kong Validation Cohort because the Maryland Test Cohort was not tested by the qRT-PCR method. A total of 236 patients with colorectal cancer were included. There was no significant association between miR21 expression and age, gender, tumor location, colorectal cancer stage, and differentiation (all $P > 0.05$). Kaplan–Meier survival analysis demonstrated that patients with higher miR21 levels in the tumor tissues had statistically worse OS (84.1 months vs. 110.6 months; $P = 0.001$; log-rank test; Fig. 5). As univariate analysis indicated, higher tissue miR21 level was associated with shorter OS (HR, 2.06; 95% CI, 1.35–3.15; $P = 0.001$). In the multivariable analysis, which included miR21 level, age, gender, side of the tumor, TNM stage, and differentiation, higher miR21 level served as an independent prognostic marker for indicating poorer OS in patients with colorectal cancer (HR, 1.69; 95% CI, 1.07–2.67; $P = 0.023$). Interestingly, the association between tissue miR21 expression and colorectal cancer survival appeared to be confined to male patients (multivariable HR, 2.47; 95% CI, 1.28–4.77; $P = 0.007$) but not for females (multivariable HR, 1.07; 95% CI, 0.53–2.16; $P = 0.845$).

Five studies comprising 953 patients evaluated colorectal cancer DFS for miR21. We found a significant association between higher miR21 expression level and poorer colorectal cancer DFS (pooled HR, 1.35; 95% CI, 1.08–1.69; Fig. 4B). There was significant heterogeneity in the analysis ($I^2 = 66.4\%$, $P = 0.018$; Fig. 4B). Through subgroup and sensitivity analyses, we found that methods to measure miR21 expression, country of origin, and sample size of the study might be the source of heterogeneity (Table 1).

Circulating miR21 level and prognostic outcome

Two studies explored the performance of circulating miR21 levels in the prognosis of colorectal cancer (17, 18). The HR of these two studies for colorectal cancer OS was 4.12 (95% CI, 1.10–15.4) and 1.58 (95% CI, 0.77–3.21), respectively.

Publication bias

The funnel plots of the diagnostic and prognostic meta-analyses were shown in Supplementary Fig. S3. Funnel plot tests (Begg and Egger tests) indicated no significant publication bias in this study. However, because of the limited number of the included studies, it is still difficult to ascertain whether publication bias exists or not.

Discussion

Although significant progress has been achieved in the diagnosis and prognosis of colorectal cancer over the years, development of better biomarkers is still important for colorectal cancer early detection and for predicting patients' outcome. The application of miRNAs as biomarkers for cancer diagnosis and prognosis has gained much attention in recent years. miR21 is one of the most studied miRNAs as a potential biomarker of colorectal cancer diagnosis and prognosis. To examine the reported diagnostic and prognostic accuracies and evaluate whether miR21 can be a useful biomarker for colorectal cancer, we performed this systematic review on 14 diagnostic or prognostic studies.

As the present meta-analysis showed, circulating miR21 achieved a pooled sensitivity of 0.76, specificity of 0.81, and AUC of 0.81. These results suggested that measuring blood miR21 level is a promising noninvasive method for colorectal cancer diagnosis. The DOR

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

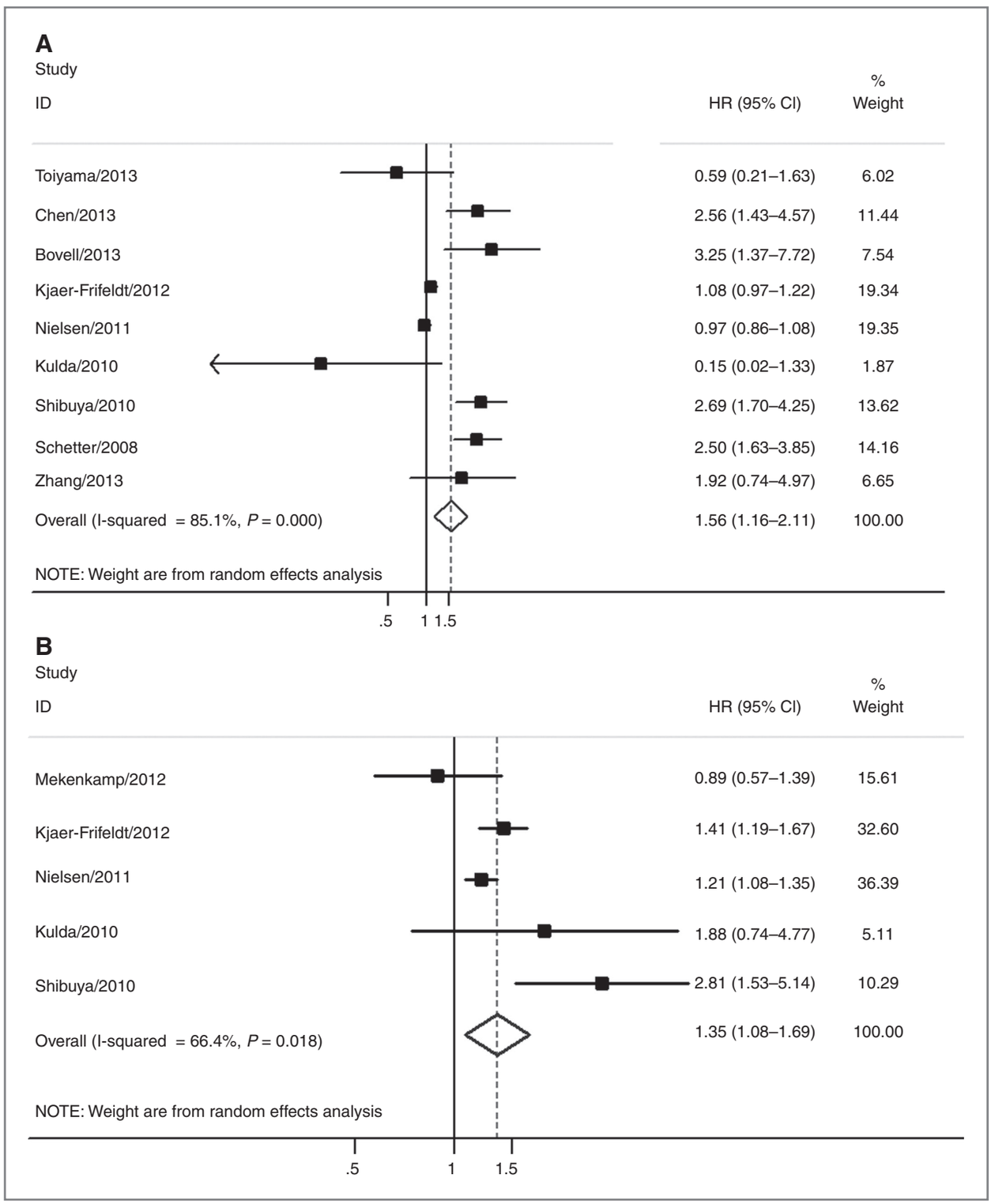

Figure 4. Forrest plots of studies evaluating tissue miR21 expression level and colorectal cancer prognosis. A, forrest plots of OS; B, forrest plots of DFS.

combines the strengths of both sensitivity and specificity, and was reported to be a useful indicator for evaluation of the diagnostic method (37). The DOR value of miR21 was 13.68, indicating a moderate diagnostic accuracy. However, the positive LR (3.8) and negative LR (0.29) suggested that miR21 may be not adequate enough to

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

Table 1. Subgroup analyses for association of tissue miR21 expression level with OS and DFS in colorectal cancer

	OS			DFS		
	No. of studies	Pooled HR	I^2 (%)	No. of studies	Pooled HR	I^2 (%)
Biochemical method						
qRT-PCR	7	1.98 (1.28–3.04)	59.3	3	1.62 (0.73–3.60)	78.8
ISH	2	1.02 (0.94–1.11)	41.1	2	1.27 (1.15–1.39)	54.2
Country of origin						
Asia	4	1.91 (1.10–3.31)	59.6	1	2.81 (1.53–5.14)	—
Europe	3	1.01 (0.86–1.19)	59.2	4	1.25 (1.14–1.37)	42.7
USA	2	2.63 (1.79–3.87)	0	—	—	—
Sample size						
Large (>100)	7	1.61 (1.18–2.20)	87.8	3	1.44 (1.12–1.85)	76.9
Small (< = 100)	2	0.64 (0.05–7.61)	78.7	2	1.15 (0.57–2.31)	50.3

discriminate and distinguish patients with colorectal cancer. We found a significant heterogeneity in sensitivity, whereas sensitivity analyses indicated that country of origin, type of specimen, sample size, and study quality were not the source. Different cutoff values of miR21 expression across studies may be one source of heterogeneity.

Measuring circulating miR21 might also be a useful screening method for colorectal advanced adenoma. In a study conducted by Toiyama and colleagues (17), in which 43 patients with Japanese advanced adenoma and 53 control subjects were enrolled, miR21 had a relatively high diagnostic performance for advanced adenoma (AUC value of 0.813, sensitivity of 0.811, and a specificity of 0.767). In another study, which included 50 Chinese advanced adenoma patients and 80 healthy controls, miR21 yielded an AUC value of 0.709 for discriminating advanced adenomas from controls (18). However, it should be noted that these two studies were both conducted in East Asia and the sample size was not large; thus, more studies are warranted to clarify this issue.

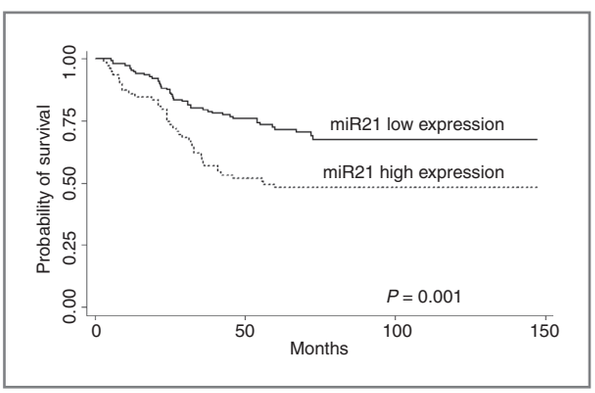

Figure 5. Kaplan–Meier curve of tissue miR21 expression in relation to OS of patients with colorectal cancer using IPD.

The results of the meta-analyses indicated that tissue miR21 expression level was a promising biomarker to predict survival in patients with colorectal cancer. Compared with patients with low tissue miR21 expression level, patients with an increased level of miR21 expression had a 1.56-fold higher risk of poor OS and 1.35-fold higher risk of poor DFS. There was significant heterogeneity in the meta-analyses of the data for OS and DFS. To decipher the reason for the heterogeneity, we applied subgroup and sensitivity analyses and found that methods used to measure miR21 expression, country of origin and sample size of the study could partially explain the heterogeneity. Besides, different cutoff values among the included studies may also be a potential source of heterogeneity. An IPD meta-analysis approach was conducted to further explore the prognostic potential of miR21 in patients with colorectal cancer. As the results demonstrated, higher miR21 level was an independent marker for predicting OS in patients with colorectal cancer. And our results showed that the effect of miR21 in predicting colorectal cancer survival was observed only in male participants, suggesting that gender may modify the observed effect. More well-designed studies with large sample size are warranted to clarify this issue and explore the relevant mechanisms. Besides, whether the prognostic value of other miRNAs is differed by gender may also need further study. Circulating miR21 was also developed as a noninvasive prognostic biomarker for colorectal cancer, and studies indicated that higher circulating miR21 level might be associated with poor OS for colorectal cancer (17, 18).

Though sensitivity and subgroup analyses were applied, heterogeneity in both diagnostic and prognostic meta-analyses was not fully explained. The heterogeneity across studies was probably due to the different methodology of evaluating miR21 expression. Different kinds of samples are used in assessing miR21 expression, including frozen tissues, formalin-fixed paraffin-embedded (FFPE) tissues, serum, and plasma. One previous study found that the results of frozen tissues differed from FFPE

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

tissues in evaluating the prognostic performance of miR21 expression for non–small cell lung cancer, suggesting that type of samples may influence the outcomes (38). Normalization is another problem for quantitative estimation of miRNAs. For the included studies, RNU6B, miR16, and total RNA were used by different studies. However, there is no conclusion about the performance of these normalization controls in the estimation of miRNAs and no optimal approach is generally recommended. To draw a convincing conclusion on the value of miR21 for the diagnosis and prognosis of colorectal cancer, an appropriate and unified method should be established and applied.

It is hypothesized that miRNAs enter the circulation directly secreted by cells, released by cells via exosomes, and via shedding of microvesicles (39). miRNAs have unusually high stability in tissues, and previous study has indicated that tumor-derived miRNAs are also present in plasma and serum in a remarkably stable form (40). Mostly, miRNAs demonstrate the same change in expression in blood (plasma or serum) and tumor tissues of patients with various types of cancer (39, 40). And thus, circulating miRNAs may serve as ideal biomarkers for cancer detection and prognosis. miR21 was reported to be one of the most relevant oncogene-like factors among various miRNA. Colon cancer cell lines with higher miR21 expression levels showed an enhanced ability of motility and invasion (41). Furthermore, suppression of miR21 inhibits cell growth *in vitro* and inhibits tumor growth in animal models by indirectly downregulating the antiapoptotic factor, B-cell lymphoma 2 (42). Studies in human cell lines further investigated the physiologic targets of miR21, and showed that miR21 could target tumor-suppressor genes, such as phosphatase, tensin homolog (PTEN), tropomyosin 1 (TPM1), and programmed cell death 4 (PDCD4; refs. 13, 43, 44). Besides, miR21 has been reported to play an important role in suppressing proapoptotic genes and modulating the vital components of the Ras/MEK/ERK pathway (45). Therefore, miR21 may be involved in the critical steps in carcinogenesis the genesis and progression of human cancer by promoting tumor growth, proliferation, antiapotosis, and migration (42, 43, 46, 47). It has been further demonstrated that tissue miR21 expression is associated with lymph node positivity and the development of distant metastases for colorectal cancer, and therefore miR21 expression serves as a marker clinicopathologic feature of the disease (15). These findings support a vital role for altered miR21 expression in tumorigenesis.

This systematic review had several important strengths. First, we conducted a relatively thorough systematic search and applied a comprehensive analytic approach to evaluate the diagnostic and prognostic value of miR21 in patients with colorectal cancer. Second, an original study was also conducted to explore the diagnostic and prognostic potential of miR21 in colorectal cancer, and an IPD meta-analysis was then used, which further supported the conclusions of the study. The methods of this study were rigorous and followed the guidelines for conducting and reporting systematic reviews.

There were also some limitations in our analysis. First, most of the diagnostic studies enrolled healthy people as controls and were not blind designed. This may affect the diagnostic performance. Second, there was considerable heterogeneity for both the diagnostic and prognostic meta-analyses. Subgroup and sensitivity analyses were applied, whereas the results could not fully explain the observed heterogeneity. Third, the different chemical assays used in the included studies might result in systematic errors among studies. Finally, only Asians and Caucasians were enrolled in this study, and thus the conclusions should be taken cautiously for other ethnic populations.

Taken together, in this study, it is concluded that circulating miR21 level is a useful biomarker for colorectal cancer detection, and tissue miR21 is a promising marker for colorectal cancer prognosis. Further research is needed to explore the combination of other variables associated with colorectal cancer diagnosis and prognosis, in an effort to develop better diagnostic and prognostic models with higher discriminative capacity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Authors' Contributions

Conception and design: P. Li, D. Xia, J. Yang, Y. Wu
Development of methodology: H. Zhang, M. Pesta
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Zhang, H. Ju, M. Pesta, V. Kulda, M. Cai, Y. Wu
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): W. Jin, C. Liu, H. Wu, J. Xu, Y. Ye, G. Zhang, J. Cai, Y. Wu
Writing, review, and/or revision of the manuscript: H. Zhang, P. Li, Y. Wu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Ju, M. Pesta, V. Kulda, G. Zhang, E. Xu, M. Lai
Study supervision: D. Xia

Acknowledgments

The authors thank Drs. Curtis C. Harris and Aaron J. Schetter from the Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, for their kind help with providing raw data adopted in the IPD meta-analysis process and revising the article.

Grant Support

The work was funded by the National Natural Science Foundation of China (grant numbers 81302455, 81201557, and 81373036).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 26, 2014; revised August 7, 2014; accepted August 22, 2014; published OnlineFirst September 1, 2014.

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
2. Weizman AV, Nguyen GC. Colon cancer screening in 2010: an update. Minerva Gastroenterol Dietol 2010;56:181–8.
3. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al. 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 2008;58:130–60.
4. Aslam MI, Taylor K, Pringle JH, Jameson JS. microRNAs are novel biomarkers of colorectal cancer. Br J Surg 2009;96:702–10.
5. Croce CM, Calin GA. miRNAs, cancer, and stem cell division. Cell 2005;122:6–7.
6. He L, Hannon GJ. microRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004;5:522–31.
7. Hede K. Studies define role of microRNA in cancer. J Natl Cancer Inst 2005;97:1114–5.
8. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002;99:15524–9.
9. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. Nature 2005;435:828–33.
10. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA 2008;299:425–36.
11. Ng EK, Chong WW, Jin H, Lam EK, Shin VY, Yu J, et al. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. Gut 2009;58:1375–81.
12. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133:647–58.
13. Zhu S, Si ML, Wu H, Mo YY. microRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). J Biol Chem 2007;282:14328–36.
14. Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S, et al. microRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 2008;27:2128–36.
15. Slaby O, Svoboda M, Fabian P, Smerdova T, Knoflickova D, Bednarikova M, et al. Altered expression of miR-21, miR-31, miR-143, and miR-145 is related to clinicopathologic features of colorectal cancer. Oncology 2007;72:397–402.
16. Shibuya H, Iinuma H, Shimada R, Horiuchi A, Watanabe T. Clinicopathological and prognostic value of microRNA-21 and microRNA-155 in colorectal cancer. Oncology 2010;79:313–20.
17. Toiyama Y, Takahashi M, Hur K, Nagasaka T, Tanaka K, Inoue Y, et al. Serum miR-21 as a diagnostic and prognostic biomarker in colorectal cancer. J Natl Cancer Inst 2013;105:849–59.
18. Liu GH, Zhou ZG, Chen R, Wang MJ, Zhou B, Li Y, et al. Serum miR-21 and miR-92a as biomarkers in the diagnosis and prognosis of colorectal cancer. Tumour Biol 2013;34:2175–81.
19. Chen TH, Chang SW, Huang CC, Wang KL, Yeh KT, Liu CN, et al. The prognostic significance of APC gene mutation and miR-21 expression in advanced stage colorectal cancer. Colorectal Dis 2013;15:1367–74.
20. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151:264–9.
21. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions (version 5.1.0). Accessed 31 March 2013. Available: http://www.cochrane.org/training/cochrane-handbook.
22. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med 1998;17:2815–34.
23. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 2011;155:529–36.
24. Mercier E, Boutin A, Lauzier F, Fergusson DA, Simard JF, Zarychanski R, et al. Predictive value of S-100beta protein for prognosis in patients with moderate and severe traumatic brain injury: systematic review and meta-analysis. BMJ 2013;346:f1757.
25. Harbord RM, Deeks JJ, Egger M, Whiting P, Sterne JA. A unification of models for meta-analysis of diagnostic accuracy studies. Biostatistics 2007;8:239–51.
26. Fischer JE, Bachmann LM, Jaeschke R. A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis. Intensive Care Med 2003;29:1043–51.
27. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
28. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58:882–93.
29. Bovell LC, Shanmugam C, Putcha BD, Katkoori VR, Zhang B, Bae S, et al. The prognostic value of microRNAs varies with patient race/ethnicity and stage of colorectal cancer. Clin Cancer Res 2013;19:3955–65.
30. Mekenkamp LJ, Tol J, Dijkstra JR, de Krijger I, Vink-Borger ME, van Vliet S, et al. Beyond KRAS mutation status: influence of KRAS copy number status and microRNAs on clinical outcome to cetuximab in metastatic colorectal cancer patients. BMC Cancer 2012;12:292.
31. Kjaer-Frifeldt S, Hansen TF, Nielsen BS, Joergensen S, Lindebjerg J, Soerensen FB, et al. The prognostic importance of miR-21 in stage II colon cancer: a population-based study. Br J Cancer 2012;107:1169–74.
32. Nielsen BS, Jorgensen S, Fog JU, Sokilde R, Christensen IJ, Hansen U, et al. High levels of microRNA-21 in the stroma of colorectal cancers predict short disease-free survival in stage II colon cancer patients. Clin Exp Metastasis 2011;28:27–38.
33. Luo X, Stock C, Burwinkel B, Brenner H. Identification and evaluation of plasma microRNAs for early detection of colorectal cancer. PLoS ONE 2013;8:e62880.
34. Wang B, Zhang Q. The expression and clinical significance of circulating microRNA-21 in serum of five solid tumors. J Cancer Res Clin Oncol 2012;138:1659–66.
35. Kanaan Z, Rai SN, Eichenberger MR, Roberts H, Keskey B, Pan J, et al. Plasma miR-21: a potential diagnostic marker of colorectal cancer. Ann Surg 2012;256:544–51.
36. Kulda V, Pesta M, Topolcan O, Liska V, Treska V, Sutnar A, et al. Relevance of miR-21 and miR-143 expression in tissue samples of colorectal carcinoma and its liver metastases. Cancer Genet Cytogenet 2010;200:154–60.
37. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol 2003;56:1129–35.
38. Saito M, Schetter AJ, Mollerup S, Kohno T, Skaug V, Bowman ED, et al. The association of microRNA expression with prognosis and progression in early-stage, non–small cell lung adenocarcinoma: a retrospective analysis of three cohorts. Clin Cancer Res 2011;17:1875–82.
39. Zen K, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev 2012;32:326–48.
40. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513–8.
41. Cottonham CL, Kaneko S, Xu L. miR-21 and miR-31 converge on TIAM1 to regulate migration and invasion of colon carcinoma cells. J Biol Chem 2010;285:35293–302.
42. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. Oncogene 2007;26:2799–803.
43. Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, et al. Involvement of human micro-RNA in growth and response to

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology 2006;130:2113–29.
44. Chen Y, Knosel T, Kristiansen G, Pietas A, Garber ME, Matsuhashi S, et al. Loss of PDCD4 expression in human lung cancer correlates with tumour progression and prognosis. J Pathol 2003;200:640–6.
45. Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E, et al. Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. Cancer Cell 2010;18:282–93.
46. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;65:6029–33.
47. Roldo C, Missiaglia E, Hagan JP, Falconi M, Capelli P, Bersani S, et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol 2006;24:4677–84.

Published OnlineFirst September 1, 2014; DOI: 10.1158/1055-9965.EPI-14-0598

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Diagnostic and Prognostic Value of microRNA-21 in Colorectal Cancer: An Original Study and Individual Participant Data Meta-analysis

Honghe Zhang, Peiwei Li, Haixing Ju, et al.

Cancer Epidemiol Biomarkers Prev 2014;23:2783-2792. Published OnlineFirst September 1, 2014.