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ORIGINAL RESEARCH

Tissue microRNA-21 expression predicted recurrence and poor survival in patients with colorectal cancer – a meta-analysis

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Objective: MicroRNA-21 (miR-21) has been shown to play an important role in cancer prognosis. We performed a meta-analysis to evaluate the prognostic effect of miR-21 from tissues and serum on survival of the patients with colorectal cancer (CRC).

Methods: Relevant studies were identified by searching PubMed, Embase, and Cochrane Library. Pooled hazard ratios (HRs) with 95% confidence intervals (CIs) of total and subgroup analyses, for overall survival (OS) and disease-free survival (DFS), were calculated to investigate the association between miR-21 expression and CRC prognosis.

Results: Our analysis included eleven studies (3,669 subjects). In addition, four studies explored the association between miR-21 and DFS, and ten studies focused on the prognostic value of miR-21 for OS. Our results indicated that increased miR-21 expression of tissues predicted both poor DFS and OS in patients with CRC (DFS: HR =1.59, 95% CI =1.20–2.10; OS: HR =1.53, 95% CI =1.23–1.90). Consistent results were observed among colon cancer and quantitative real-time polymerase chain reaction subgroups.

Conclusion: Meta-analysis indicated that miR-21 predicted recurrence and poor survival in patients with CRC. miR-21 may be more suitable to predict cancer prognosis in colon cancer patients.

Keywords: miR-21, prognostic value, colorectal cancer

Introduction

Colorectal cancer (CRC) is one of the most common types of cancers and the leading cause of cancer-related death worldwide. In the US, CRC is the third most common cancer, with >143,000 new cases and >52,000 deaths each year.[1] Moreover, the incidence and mortality of CRC in Asia have also increased rapidly in the past decades.[2,3] There are three frequently used screening modalities, namely fecal occult blood testing, flexible sigmoidoscopy, and total colonoscopy, which have aided in reducing the mortality associated with this disease.[3] However, none of these tests has been established as a well-accepted screening tool due to invasiveness, high cost, or low sensitivity. Since treatment of CRC in its nonmetastatic phase increases the survival rate, an insight into molecular pathways of CRC metastasis may be important for developing new prognostic molecular markers facilitating the reduction of CRC metastasis, particularly noninvasive biomarkers in serum or tissue.[4]

MicroRNAs (miRNAs) are small (21- to 25-nucleotide long) RNAs that participate in the regulation of cell differentiation, cell cycle progression, apoptosis, and tumorigenesis.[5] MicroRNA-21 (miR-21) is an oncogenic miRNA that modulates the expression of multiple cancer-related target genes such as *PTEN*, *TPM1*, and *PDCD* and has

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been shown to be overexpressed in CRC.[6–10] Several studies have investigated the prognostic effect of miR-21 expression on CRC.[11–20] Most of the research revealed an elevated expression of miR-21 in CRC tissues compared with normal tissues, which is related to a poor survival outcome.[4,12,16,20,21] However, there were reports showing the insignificant or opposite results.[14,18,19] Therefore, it is essentially necessary to carry out a systematic review and meta-analysis to summarize the published global findings, and get a better understanding on the significance of miR-21 expression in the prognosis of CRC patients.

In the current study, global-related literature were collected to conduct a systematic review and meta-analysis, and the risk of increased miR-21 expression to the survival of CRC patients was successfully assessed.

Materials and methods

Search strategy

The literature published from January 1, 2001 to March 20, 2014 were searched in PubMed, Embase, and Cochrane Library, using key words "microRNA-21 OR miR-21" AND "colon OR colorectal OR rectum" AND "cancer OR carcinoma OR tumor OR tumour OR neoplasm". A manual review of the references of relevant publications was also performed to obtain additional studies.

Study selection

First, two reviewers primarily examined the titles and abstracts of all literature. Then, full text of the articles was screened separately by two reviewers to determine whether they met the inclusion criteria. We contacted the corresponding authors when the crucial data were not reported in the original papers. Articles were independently read and selected according to the inclusion criteria. Disagreements were resolved through the consensus with a third reviewer.

Inclusion criteria

The primary literature contained expression profiles of miR-21 and the following: (i) survival analysis of CRC patients with overall survival (OS) or disease-free survival (DFS) or recurrence-free survival (RFS); (ii) hazard analysis with hazard ratio (HR), 95% confidence intervals (CIs), and *P*-value, or relevant data that could be used to calculate the HR and 95% CI.

Exclusion criteria

The following studies were excluded: (i) those that were not published in English and (ii) those not involving the association between miR-21 expression level and CRC prognosis.

Quality assessment

Methodological quality of included articles was assessed using the Newcastle–Ottawa Scale.[22] All studies were assessed for quality of selection (representativeness, selection of controls, ascertainment of exposure, absence of asthma at the start of study), comparability (confounding), and outcome (assessment of outcome, length and adequacy of follow-up). Studies could be awarded a maximum score of nine points. Studies with scores of five points or more were considered to be of moderate-to-good quality. Quality assessment was done by two authors using the Newcastle–Ottawa Scale independently. In case of disagreement, the third author was consulted. Quality assessment was completed before data extraction. The detailed assessments of each included article are shown in Table S1.

Data extraction

The extracted data elements included the following: (i) first author's name and publication year; (ii) characteristics of the studied population including sample size, population, ethnicity, the numbers of death and survival during follow-up, follow-up time, and stage and histological type; (iii) measurement and cut-off values of miRNA expression; and (iv) HRs of miR-21 for OS, or DFS or RFS, along with the 95% CIs. We selected adjusted HR if crude HR and adjusted HR were both provided. The HR and 95% CIs of the study by Shibuya et al[13] were reciprocals of the reported results of multivariate Cox regression due to inverse comparison.

Statistical methods

The effect size was summarized as HR. Analysis was done for OS and DFS (or RFS). The values were reported by a Forest plot, and uncertainty about the pooled estimates was quantified by 95% CI. Statistical heterogeneity was assessed by means of Cochran's Q test (significant at $P<0.10$) and I^2 test (ranging from 0% to 100%).[23] The degree of heterogeneity with values of 25%, 50%, and 75% was considered low, moderate, and high, respectively.[24] Meta-regression was performed to explore the source of heterogeneity. The variables included in the meta-regression were defined as follows:

1. Type of carcinoma: colon cancer, rectal cancer, or CRC
2. Methods of measurement: fluorescence in situ hybridization (FISH) and quantitative real-time polymerase chain reaction (qRT-PCR)
3. Location of study: Asia, Europe, or America.

We used a random-effect model to summarize the overall results and results within subgroups and based statistically significant heterogeneity on a *P*-value of <0.05.[25] We also used Begg's funnel plots[26] and Egger's test[27] to detect

possible publication bias. The statistical analyses were performed using STATA 12.0 (StataCorp LP, College Station, TX, USA). A two-tailed $P<0.05$ was considered statistically significant.

Results

Eligible studies

As shown in Figure 1, totally 107 studies of miR-21 and CRC prognosis were identified. Seventy-six studies were excluded based on manual screening of the title and abstract. Full text of the remaining 31 studies was identified. Of the 31 studies, 12 studies were not directly related to specific outcomes, seven studies lacked sufficient survival data or HR, and one study was found with an overlapping data set and not related to OS or DFS. Finally, eleven studies were considered eligible for inclusion in this review (Figure 1).

Characteristics of included studies

The characteristics, including the first author, publication year, origin of population, the numbers of patients, source of samples, survival results, types of outcomes, time of follow-up, assay type, and miR-21 expression, are listed in Table 1. As shown in Table 1, our analysis included eleven studies, containing 3,669 subjects. Nine studies[10,12,13,16–21] provided results of tissue samples, and three studies[14,15,18] provided results of serum samples. Moreover, four studies[13,16,17,19] involved the data of the association between miR-21 and DFS, and ten studies[10,12–16,18–21] focused on the prognostic value of miR-21 for OS. However, Zhang et al[17] reported three independent results of miR-21 with DFS in one study, while Nielsen et al[16] and Schetter et al[12] reported two independent results of miR-21 with OS in one study each. Each independent result was included in the present meta-analysis.

Tissue miR-21 expression and DFS or RFS in patients with CRC

The association of tissue miR-21 expression with DFS in patients with CRC is shown in Figure 2. In the meta-analysis of four studies (seven independent results, 1,567 subjects), high level of tissue miR-21 expression was significantly associated with poor DFS in patients with CRC (HR =1.59, 95% CI =1.20–2.10), with significant heterogeneity (I^2=74.2%, P=0.001). Then, meta-regression was performed to explore the source of heterogeneity, and the results showed that different types of carcinomas among different studies may be the major reason of heterogeneity (Table S2). Therefore, stratified analysis was performed. In the sub-analysis of tumor types (Table 2), high level of tissue miR-21 expression significantly predicted poor DFS in patients with colon carcinoma (HR =1.54, 95% CI =1.33–1.79), without significant heterogeneity (I^2=1.0%, P=0.40).

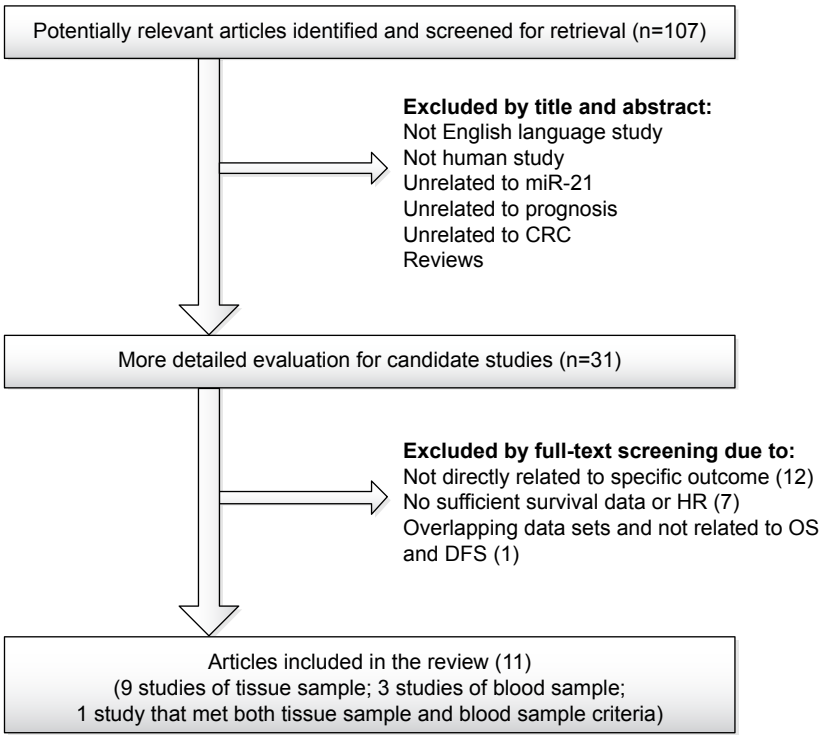

Figure 1 Methodological flow chart of the review.
Abbreviations: miR-21, microRNA-21; CRC, colorectal cancer; HR, hazard ratio; OS, overall survival; DFS, disease-free survival.

Table 1 Main characteristics of included studies

Authors	Year	Origin of population	N	Source of samples	Survival results	HR (95% CI)	Types of outcomes	Follow-up (month), n (range)	miRNAs assay
Zhang et al[17] (group 1)	2013	People's Republic of China	138	Tissue	DFS	1.98 (0.95, 4.15)	CC (stage II)	66 (50–86)	qRT-PCR
Zhang et al[17] (group 2)	2013	People's Republic of China	137	Tissue	DFS	1.88 (0.95, 3.75)	CC (stage II)	66 (50–86)	qRT-PCR
Zhang et al[17] (group 3)	2013	People's Republic of China	460	Tissue	DFS	1.79 (1.22, 2.62)	CC (stage II)	66 (50–86)	qRT-PCR
Kjaer-Frifeldt et al[19]	2012	Denmark	520	Tissue	DFS	1.41 (1.19, 1.67)	CC (stage II)	84	FISH
Nielsen et al[16] (group 1)	2011	Denmark	129	Tissue	DFS	2.39 (1.22, 4.69)	CC (stage II)	60	FISH
Nielsen et al[16] (group 2)	2011	Denmark	67	Tissue	DFS	0.96 (0.81, 1.15)	RC (stage II)	60	FISH
Shibuya et al[13]	2010	Japan	116	Tissue	DFS	2.53 (1.11, 5.38)	CRC	44 (2–84)	qRT-PCR
Oue et al[20] (Japan)	2014	Japan	87	Tissue	OS	3.13 (1.20, 8.17)	CC (stage II, III)	60	qRT-PCR
Oue et al[20] (Germany)	2014	Germany	145	Tissue	OS	2.65 (1.06, 6.66)	CC (stage II)	72	qRT-PCR
Bovell et al[10]	2013	USA	55	Tissue	OS	3.25 (1.37, 7.72)	CRC (stage IV)	Black: 228 White: 180	qRT-PCR
Toiyama et al[18]	2013	Japan	153	Tissue	OS	0.59 (0.21, 1.63)	CRC	60	qRT-PCR
Chen et al[21]	2013	Taiwan	195	Tissue	OS	2.56 (1.43, 4.57)	CRC (stage I–IV)	60	qRT-PCR
Kjaer-Frifeldt et al[19]	2012	Denmark	520	Tissue	OS	1.08 (0.97, 1.22)	CC (stage II)	84	FISH
Nielsen et al[16] (group 1)	2011	Denmark	129	Tissue	OS	1.17 (1.02, 1.34)	CC (stage II)	60	FISH
Nielsen et al[16] (group 2)	2011	Denmark	67	Tissue	OS	0.97 (0.83, 1.13)	RC (stage II)	60	FISH
Shibuya et al[13]	2010	Japan	156	Tissue	OS	1.95 (1.05, 3.57)	CRC	44 (2–84)	qRT-PCR
Schetter et al[12] (Maryland, USA)	2008	Maryland, USA	71	Tissue	OS	2.70 (1.30, 5.50)	CC (stage I–IV)	68	qRT-PCR
Schetter et al[12] (Hong Kong)	2008	Hong Kong	103	Tissue	OS	2.40 (1.40, 4.10)	CC (stage I–IV)	84.6	qRT-PCR
Toiyama et al[18]	2013	Japan	153	Serum	OS	4.12 (1.10, 15.40)	CRC	60	qRT-PCR
Menendez et al[15]	2013	Spain	102	Serum	OS	0.50 (0.25, 1.02)	CRC (stage I–IV)	23 (0–36)	qRT-PCR
Liu et al[14]	2013	People's Republic of China	166	Serum	OS	1.58 (0.77, 3.21)	CRC (stage I–IV)	36.4 (4–53)	qRT-PCR

Abbreviations: HR, hazard ratio; miRNAs, microRNAs; DFS, disease-free survival; CC, colon cancer; qRT-PCR, quantitative real-time polymerase chain reaction; FISH, fluorescence in situ hybridization; RC, rectal cancer; CRC, colorectal cancer; OS, overall survival.

Tissue miR-21 expression and OS in patients with CRC

As shown in Figure 3, meta-analysis of eleven studies (14 independent results, 2,102 subjects) showed that high level of tissue miR-21 expression was significantly associated with poor OS in CRC patients (HR =1.53, 95% CI =1.23–1.90, I^2=76.8%); however, significant heterogeneity existed. The meta-regression showed that measurement method of miR-21 expression across studies might have contributed to the heterogeneity (Table S2). Hence, the stratified analysis

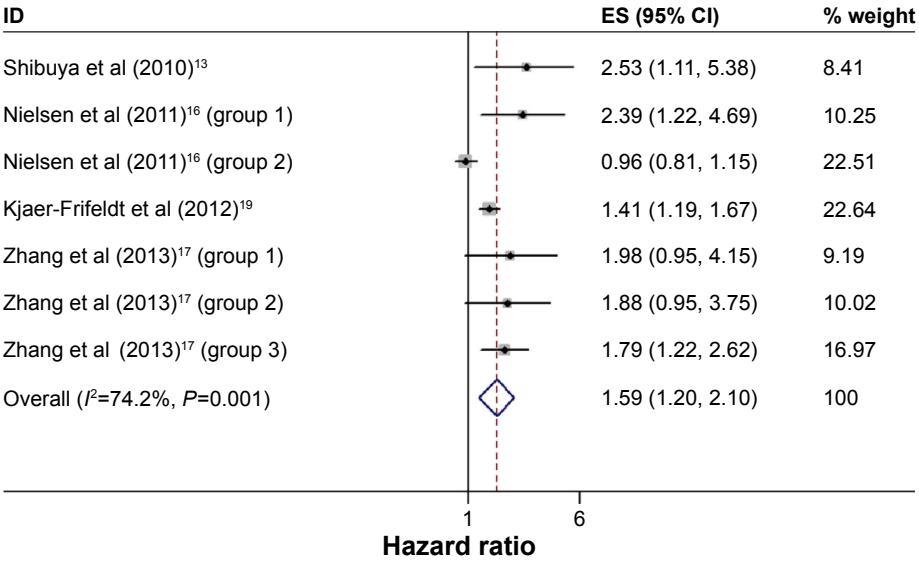

Figure 2 Forest plots of studies evaluating HRs of miR-21 with DFS in all studies (tissue).
Note: Weights are from random effects analysis.
Abbreviations: HRs, hazard ratios; miR-21, microRNA-21; DFS, disease-free survival; ES, effect size; CI, confidence interval.

Table 2 Stratified analysis of pooled HRs of CRC patients with elevated miR-21 expression from tissue

Subgroup	Number of studies	Pooled HR (95% CI)	Heterogeneity (I^2, *P*-value)
DFS			
Type of carcinoma			
Colon carcinoma	5	1.54 (1.33, 1.79)	1.0%, 0.400
RC or CRC	2	1.44 (0.56, 3.67)	81.9%, 0.019
OS			
Method of measurement			
qRT-PCR	8	2.29 (1.72, 3.04)	17.6%, 0.291
FISH	3	1.08 (0.98, 1.19)	37.2%, 0.204

Abbreviations: HRs, hazard ratios; CRC, colorectal cancer; miR-21, microRNA-21; CI, confidence interval; DFS, disease-free survival; RC, rectal cancer; OS, overall survival; qRT-PCR, quantitative real-time polymerase chain reaction; FISH, fluorescence in situ hybridization.

showed that (Table 2) high level of tissue miR-21 expression was significantly related to poor OS among studies that used qRT-PCR for measurement (HR =2.29, 95% CI =1.72–3.04, I^2=17.6%), while the association was not significant among studies that used FISH for measurement (HR =1.08, 95% CI =0.98–1.19, I^2=37.2%).

Serum miR-21 expression and OS in patients with CRC

Only three studies reported the association of serum miR-21 expression with prognosis in CRC patients (Figure 4). All three studies focused on serum miR-21 and OS. High level of serum miR-21 expression was not significantly associated with poor OS in patients with CRC (HR =1.34, 95% CI =0.45–4.01, I^2=79.3%).

Publication bias

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of the studies. *P*-value of Egger's regression intercepts for DFS was 0.573 in all studies of tissue samples, and the *P*-value for OS was 0.776 and 0.544 among studies that used FISH and qRT-PCR, respectively. Egger's test showed that there was no significant publication bias in studies of serum samples (*P*=0.500).

Discussion

Recently, the prognostic effect of miR-21 has been observed in the meta-analysis of other cancers, such as lung cancer, especially non-small-cell lung cancer.[28] Emerging studies have demonstrated that aberrant expression of miRNAs is associated with prognosis of CRC. Here, we searched more databases and late literature and pooled the prognostic value. In this meta-analysis, elevated miR-21 expression in tumor tissue was found to be prognostic of recurrence, especially among colon cancer patients. qRT-PCR measurement showed that high expression of miR-21 in tumor tissue was significantly associated with poor OS among CRC patients.

DFS is a clinical end point that is a surrogate for the true end point and requires less time to evaluate and may be less costly to assess. Sargent et al[29] evaluated DFS with 3 years

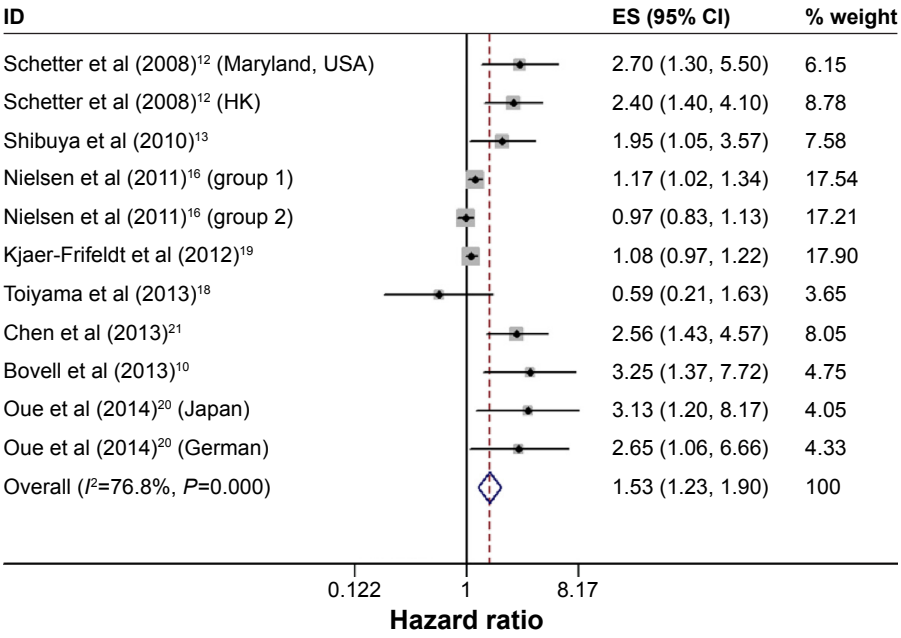

Figure 3 Forest plots of studies evaluating HRs of miR-21 with OS in all studies (tissue).
Note: Weights are from random effects analysis.
Abbreviations: HRs, hazard ratios; miR-21, microRNA-21; OS, overall survival; ES, effect size; CI, confidence interval.

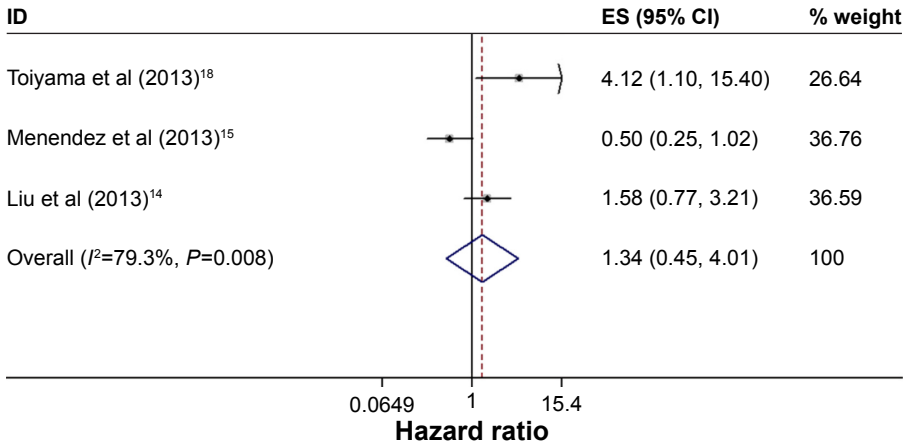

Figure 4 Forest plots of studies evaluating HRs of miR-21 with OS in all studies (serum).
Note: Weights are from random effects analysis.
Abbreviations: HRs, hazard ratios; miR-21, microRNA-21; OS, overall survival; ES, effect size; CI, confidence interval.

of follow-up ($DFS_{3\ years}$) as a surrogate for OS with 5 years of follow-up ($OS_{5\ years}$) and concluded that it was an appropriate primary end point to replace OS. Our results indicated that high expression of miR-21 in tumor tissue was a prognostic marker of CRC, especially among colon cancer patients. The reported surgically resected cases of CRC were known to have a 40%–60% recurrence rate in the first 3 years after surgery with the majority in the second year.[30] Recurrence of colon cancer still remains a major issue which affects nearly 50% of patients treated by conventional therapeutics.[31] Detecting the level of miR-21 in tumor tissues of cancer patients, especially among colon cancer patients, might contribute to an apparent decrease in the chance of recurrence, which might have an important potential clinical significance for patient risk stratification, guidance of further follow-up, and additional therapy. Human colon cancer tissues were reported to be more sensitive than rectal cancer tissues to antitumor drugs in vitro.[32] This is a potential reason that we found the obvious association between DFS and miR-21 in colon cancer patients in a sub-analysis. Similarly, Hansen et al[33] appealed to introduce a risk index for stratifying patients with stage II colon cancer which may lead to the identification of a considerably smaller group that the traditional approach would have categorized as high risk, sparing a large fraction of the patients of adjuvant chemotherapy with doubtful efficacy. However, to overcome the limitation of the significant heterogeneity across studies of CRC, more multicenter clinical investigations with larger sample sizes are needed.

Similar to Xia et al,[34] we also found a significant association between miR-21 expression and OS in CRC patients from studies that used qRT-PCR measurement. However, we specially identified the studies of miR-21 expression in tissues, excluding the studies of miR-21 expression in serum, due to the potential bias between different biological specimens. Moreover, we calculated pooled adjusted HRs,[12,18] which are more reliable than crude HRs. Our results indicated that, regardless of the method of measurement used, high expression of miR-21 in tumor tissue was associated with poor OS among CRC patients but was not significant among studies that used FISH measurement. This may be due to the small numbers of studies that used FISH. Currently, numerous techniques are available for studying miRNA expression levels, in addition to qRT-PCR and FISH. With the recent introduction of locked nucleic acid oligonucleotides as hybridization probes, miRNA-FISH has become a powerful technique for imaging the spatial localization of miRNA at the tissue, cellular, and even subcellular level.[35,36] Further studies are needed to confirm the exact association of the miR-21 expression with CRC in the future.

miRNAs are key players in a wide array of pathological processes, which may partly explain the prognostic associations with CRC. miR-21 has been found to play important roles in suppressing proapoptotic genes and modulating the pivotal components of the Ras/MEK/ERK pathway.[37] Additionally, previous studies reported that many tumor suppressors have been experimentally verified as the targets of miR-21 in CRC and other cancers, such as PTEN,[38,39] ITGβ4, and PDCD4.[40,41] miR-21 targets PTEN at the post-transcriptional level and regulates cell proliferation and invasion in CRC. In vitro, miR-21 blockade with ASO inhibits growth and induces apoptosis in CRC cell lines, which is mediated by retrieving PTEN expression. In vivo, injection of miR-21 ASO into CRC xenografts implanted subcutaneously in nude mice suppressed tumor growth.[38] Also, miR-21 gene causes

downregulation of tumor suppressor genes (eg, *PDCD4*) and epithelial keygenes, such as *ITGβ4*, enhancing malignant transformation and epithelial–mesenchymal transition in CRC.[41] Moreover, miR-21 in the tumor microenvironment plays an important role in CRC progression. Deregulation of miR-21 is a stromal phenomenon and supports CRC progression through myofibroblast transdifferentiation, resistance to oxaliplatin cytotoxicity, and promotion of tumor cell invasion. Stromal miR-21-induced invasion is mediated by RECK downregulation and a reciprocal rise in MMP2 activity.[42,43] Hence, miR-21 related to prognosis in CRC may be used as a potential target to identify patients for closer monitoring and therapy.

Limitation

There are several limitations in this paper: (1) It excluded non-English articles and the studies that lacked important survival data (eg, HR). (2) The proportion of cancers and TNM stage were not the same in all studies, and it might result in heterogeneity, especially OS analyses where several studies addressing stage IV patients were pooled with stage I–III. This may not be a safe way as OS in these two settings, the biology of the disease, and the function of miRNA-21 are not comparable. (3) The different cut-off values and other technical issues might also introduce heterogeneity, and it is impossible to identify the association of levels of miR-21 expression with OS or DFS in detail.

Conclusion

Our meta-analysis showed that elevated expression of miR-21 was associated with poor prognosis of CRC, especially among colon cancer patients. More large-scale and standard investigations should be conducted to confirm these findings.

Acknowledgments

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Authors' contributions

Zexin Chen, Hui Liu, and Wen Jin participated in the design of the study. Zheyuan Ding and Shuangshuang Zheng searched the articles and extracted data. Zexin Chen performed the statistical analysis and drafted the manuscript. Yunxian Yu conceived of the study, and participated in its design and coordination and helped to modify the manuscript. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest in this work.

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Supplementary materials

Table S1 The results of meta-regression

Variables	Coefficient	t	*P*-value
DFS			
Type of carcinoma	**−0.62**	**−3.91**	**0.017**
Methods of measurement[a]	−0.07	−0.33	0.760
Location of study[a]	−0.07	−0.33	0.760
OS			
Type of carcinoma	−0.07	−1.53	0.17
Methods of measurement	**−0.96**	**−3.67**	**0.008**
Location of study	0.27	0.90	0.399

Notes: [a]The distribution of two variables is same. The bold values means that this factor is significant ($P<0.05$).
Abbreviations: DFS, disease-free survival; OS, overall survival.

Table S2 The quality assessment of articles

Authors	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at the start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Scores
Zhang et al[1]	✻(b)	✻	✻	✻	×	✻	✻	✻	7
Kjaer-Frifeldt et al[2]	✻(a)	✻	✻	✻	✻	✻	✻	✻	9
Nielsen et al[3]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9
Shibuya et al[4]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9
Oue et al[5]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9
Toiyama et al[6]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9
Chen et al[7]	✻(b)	✻	✻	✻	✻	×	✻	✻	8
Schetter et al[8]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9
Menendez et al[9]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9
Liu et al[10]	✻(b)	✻	✻	✻	×	✻	✻	✻	7
Bovell et al[11]	✻(b)	✻	✻	✻	✻	✻	✻	✻	9

Notes: 'x' represents no score in corresponding items. "(a)" and "(b)" in this table represents the choice in first item according to the Newcastle–Ottawa Quality Assessment Scale-Cohort studies.

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