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MicroRNA-17 and the prognosis of human carcinomas: a systematic review and meta-analysis

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Abstract

Objectives MicroRNA-17 (miR-17) family has been thoroughly studied and reported to contribute to the progress of human carcinomas. However, the prognostic value of miR-17 in cancers remains unclear. Therefore, we put up with a systemic review and meta-analysis to summarize and analyze the relationship between the miR-17 status and clinical outcome in several kinds of human cancers.

Design Published articles associated with miR-17 and clinical outcome of cancers were screened by searching the7 online databases. The patients' survival results were pooled, and pooled hazard ratio (HR) with 95% confidential intervals (95% CI) were calculated and used for measuring the strength of association between miR-17 and the prognosis of cancers, including hepatocellular carcinoma, lung cancer, osteosarcoma, glioma, T-cell lymphoblastic lymphoma and colon cancer. Heterogeneity, publication bias and subgroup analysis were also conducted.

Results In all 12 articles, totally 1096 patients were included in this meta-analysis. The results indicated that the increased expression of miR-17 played an unfavorable role in overall survival (OS) in various human carcinomas with the HR of 1.342 (95% CI=1.238-1.456) concerning the publication bias. In subgroup analysis, HR of ethnicity

(Caucasian HR=1.48 and Asian HR=1.40), disease (digestive system HR=1.36 and non-digestive system HR=1.54), detection method (qRT-PCR HR=1.40 and in situ hybridization, ISH HR=2.59) and detection sample (tissue HR=1.45 and serum HR=1.32), all $p < 0.05$. On the analysis of disease-free survival (DFS) and recurrence-free survival (RFS), the unfavorable prognosis role was also found with the increased expression of miR-17 (HR=1.40, 95% CI=1.23-1.60).

Conclusions miR-17 might be a useful biomarker in predicting the clinical outcome of human cancers.

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Keywords microRNA-17; Cancer; Outcome; Prognosis; Meta-analysis.

Strength and limitation of this study

1. This is the first meta-analysis that summarized and reported the microRNA-17 as a novel cancer prognosis biomarker in medical filed
2. We used board search strategy in order to minimize any potential publication bias.
3. We conducted the subgroup analysis and we found out that the up-regulated expression of microRNA-17 may implies poor clinical outcome in digestive system cancers.
4. The major limitation of our research is our meta-analysis included limited studies in western countries, which may decrease the applicability of our result among various ethnicities.

Introduction

Despite great progresses have been made in the medical filed over the past few decades, cancer is still a key health burden problem all over the world. It has become the leading cause of death in worldwide. In the year 2017, it is estimated that 1,688,780 patients would be diagnosed with cancers, and 600,920 cancer deaths may occur in United States [1]. Since the implantations of the advanced methods of screening and adjuvant systemic therapies for newly diagnosed cases, the mortality rate of the cancers are declining in the developed countries [2], whereas the clinical outcome of human cancers in the developing countries are still poor [3 4].

Nowadays, there are several independent factors that identifying and evaluating the clinical outcome of human cancers, including tumor size, histological grade, age of the patients and metastasis of lymph node [5-8]. Tumor biomarkers based on the tissue and serum are widely used to predict the prognosis of neoplasm. However, those techniques are far from satisfactory owing to the low specificity and sensitivity [9-11]. Thus, a more less invasive and accurate biomarker is of great value and in need for predicting the prognosis of human tumors.

The discovery of microRNAs (miRNAs) provided an innovative method for the prognosis of cancers by a less-invasive detection [12]. miRNAs, a class of endogenous non-coding single-stranded RNAs with the length of 18-25nt nucleotide, act as regulators of gene expression regulators via pairing with the complementary section in the 3'-untranslated region (3'-UTR) of its target mRNAs MicroRNAs may act as a regulator in the metabolism process of cell growth, proliferation, differentiation and apoptosis [13].. As a tumor suppressors or oncogenes, microRNA potentially acts as a prognostic biomarker. Clinical studies have found that some miRNAs are differentially expressed between tumor and non-tumor tissues, and the abnormal expression of tumor-associated miRNAs can be detected in patient's blood, cancerous tissue and fecal samples [14 15]. Such as the microRNAs, miR-21, miR-203, and miR-206 are discovered aberrantly expressed in cancer patients [16-18]. Recent studies have demonstrated that aberrantly expressed miRNAs in kinds of cancers, especially those acting as suppressor or oncogene, are proved to be related to cancer development, progression and especially carcinogenic treatment [19-21]. Therefore, these miRNAs could be considered as useful prognostic biomarkers of various human cancers.

The miR-17 family, including six members, miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1, is one of the most thoroughly studied miRNA cluster with the critical role in the development of tumor [22]. These microRNAs are tightly located within an 800 base-pair region of human chromosome 13, play an essential role in the development of the heart, lung and human immune system [23]. Of the miR-17 family, recent studies are found that miR-17, functioning as a tumor suppressor, may act as an significant tumor indictor [24 25]. It is much more complicated in the development of cancer, and the increased expression of miR-17 may help to promote carcinogenesis and cancer

progression [26-28]. According miRBase (http://www.mirbase.org), miR-17 includes two members, miR-17-5p and miR-17-3p. Of the miR-17-3p and the miR-17-5p are located in the sequence of miR-17, with the structure of stem-loop. As a result, the detection of miR-17-5p, miR-17-3p has the same effect and result of detecting miR-17 [29-33].

After the systematic review of published documents and journals, we assumed that the higher expression of the miR-17 indicates poor prognosis of the cancer patients [34-45]. However, we must admit that different confounding factors, including race, detection method, tumor location, may cause inconsistent and different results. Generally, though the aberrantly expression of miR-17 may imply the clinical outcome of cancer patients, but the relationship is not consistent. Thus, we conducted a full-scale meta-analysis to further evaluate the clinical availability of miR-17 as novel prognosis indicator for cancer detection.

Material and Methods

Data Source and Search Strategy

All the relevant were searched articles in the following online electronic databases: PubMed, Web of Science, Embase, China Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure (CNKI), Technology of Chongqing (VIP) and Wan Fang databases up to May 15th, 2017. The year of publication and publishing status is without any restriction.

Keywords for searching included: (1) prognosis OR prognostic OR survival OR outcome OR mortality; (2) cancer OR tumor OR tumour OR carcinoma OR neoplasm; (3) miR-17 OR microRNA-17 OR hsa-mir-17. Additionally, we also searched the references in the included researches and relevant published articles via Google Scholar.

Inclusion and Exclusion Criteria

The inclusion criteria of the articles are: (1) The cancers was diagnosed by the histological examination or any other committed standard; (2) studied miR-17 in human cancers; (3) the expression of miR-17 and the clinical outcome of patients was included in the research; and (4) reports with survival outcome and the data was further explored

considering hazard ratio (HR) with 95% confidence interval (95% CI) and HR with a *P*-value.

The exclusion criteria are: (1) duplicate publications; (2) articles focus on other genes or other kind of cancer; (3) case report, reviews, letter, animals trail; (4) unqualified or insufficient data; (5) HR, 95% CI and *P*-value are not provided or cannot not be calculated and (6) articles concentrate on the polymorphisms or methylation patterns of a miRNA.

Any questions of suitability of the included articles was examined was discussed by the authors after the reviewing abstract and full text manuscript. The final decision was made by the academic committee.

Data extraction and quality assessment

All studies were decided by the two investigators (Huang and Yu) independently based on titles and abstracts. After the screening the studies, the full-text would require if the articles were potentially suitable for the research. Moreover, the literature search was performed again in the excluded articles by the investigators to avoid missing any potentially related to the study. We would turn to the original authors of the article if any supplementary data might be needed. Any disagreement was resolved by the two researchers. The extracted details of the articles are as follows: (1) publication information: the name of the authors, publication area, and publication year; (2) patient's characteristics: diseases, stage of the disease, RNA detection method, type of tissue sample and follow-up years; (3) the measurement of miR-17 measurement and it's cut-off value and (4) HR of miR-17 for overall survival (OS), disease-free survival (DFS) and recurrence-free survival (RFS), as well as their 95% CI and *P*-value. The HRs and their 95% were extracted from the original articles or the E-mails from the author. If not, we calculated HR and 95% CI using the data of observed deaths, cancer recurrences or the original data provided by the authors. All calculation mentioned above were based on the methods provided by Parmar, M. K. et al. [46]. The quality of the included articles were systematically assessed based on a systematic review checklist of the Dutch Cochrane Centre proposed by MOOSE [47].

Statistical analysis

The test of heterogeneity of pooled HRs was carried out by using Cochran's Q-test and Higgins I^2 statistic. A *P*-value of < 0.05 or $I^2 > 50\%$ was considered as statistical significance. If the heterogeneity exists, the random effects model was performed among the included studies; otherwise, the fixed effects model was selected. I^2 value ranges from 0% to 100%. All the *P*-values were two-sided.

HR >1 presents the up-regulated expression of miR-17 indicated poor prognosis in patients, and HR<1 suggested a better prognosis. Publication bias was evaluated by the Begg's test and Egger's test [48 49]. If the publication bias did exist, the Duval and the Tweedit's trim and fill method was used to adjust the results[50]. The STATA software Version 14.0 (StataCorp LP, College Station, TX, USA) was used in all of the statistical analyses.

Results

Literature selection

In total, 405 articles associated with miR-17 and cancer prognosis was identified from online database search. After removing the replicate records, 304 articles were left. 210 citations were removed from the analysis after the first screening base on the species, article type, and language. Then the remaining 104 studied were carefully reviewed and assessed the abstract and the full text. After that, 89 articles were excluded from the study because they were unrelated to miR-17 expression levels or because of lack of survival statistics such as HRs, 95% CI, or *P*-value. Finally, 15 studies, which investigated the potential relationship between miR-17 expression and prognosis of human digestive system cancers, remained for further detailed screening and data-extraction. Three of the study explains the relationship between miR-17 expression and the clinic outcome of cancer but the author did not provide the exact HR value, or the value cannot be calculated from the data. Thus, 12 articles (12 studies) [34-45] were included in this meta-analysis. (Figure 1)

Clinical characteristics and data of selected studies

After reviewing the articles, 12 studies published between 2010 and 2016 were considered for the meta-analysis. All of the published study included in the study were the retrospective study [34-45]. Of the 12 studies, all of them reported patient's OS, and three studies also focus on the DFS or RFS. The type of the cancers included gastrointestinal cancers (colorectal cancer, gastric cancer), lung cancer, pancreatic cancer, hepatocellular cancer, osteosarcoma, human glioma, T-cell lymphoblastic lymphoma and esophageal squamous cell carcinoma. A total of 1096 patients from People's Republic of China, Japan, Spain and Brazil were diagnosed with various types of cancers. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the expression of miR-17 in 12 studies, one of study used the in-situ hybridization (ISH). All of the authors used the tissue and serum samples as the source of the miR-17. The majority (10 of 12) of the HRs were reported in the present analysis, all of which were calculated in multivariate analysis. The rest HRs could be estimated by analyzing Kaplan-Meier analysis and relative risk (RR) value. Most of the studies have the follow-up research for at least 38 months. The clinical characteristics of the studies included in this article are summarized in the Table 1.

Association between miR-17 and overall survival (OS)

Due to low heterogeneity (I^2=38.2%, *P*-value=0.086), fixed effects model was used to calculate and analyze the pooled HR value (HR=1.42, 95% CI=1.30-1.55), suggesting that the higher expression level of miR-17 significance implied the poor OS in patients with diverse kinds of cancers. Details of the meta-analysis are systematically summarized in the Figure 2.

In order to demonstrate the predictive role of miR-17, subgroups analysis was conducted based on the patient ethnicity, cancer type, the methods identifying microRNAs and type of tissue sample. Association was found in the Asian patients (HR 1.40, 95% CI=1.27-1.55, fixed effects model) and Caucasian patients (HR 1.48, 95% CI=1.21-1.81, random effects model). In addition, the association was also significant in other subgroups, including digestive system cancers (HR 1.36, 95% CI=1.22-1.51, fixed effects model) and non-digestive system cancers (HR 1.54, 95% CI=1.33-1.78, fixed effects model), qRT-PCR detection method (HR 1.40, 95% CI=1.28-1.53, fixed effects

model) and ISH (HR 2.59, 95% CI=1.39-4.81), tissue sample (HR 1.45, 95% CI=1.31-1.61, fixed effects model) and serum sample (HR 1.32, 95% CI=1.10-1.57, fixed effects model). Details of the subgroup analysis are listed in the Table 2 and Figure 3.

Correlation between miR-17 and disease-free survival (DFS) and recurrence-free survival (RFS)

A total of 3 studies [38 39 43] are included in the analysis of DFS or RFS, revealing a predicative role of increased expression of miR-17 and the prognosis of the cancer patients (pooled HR= 1.40, 95% CI=1.23-1.60, $p < 0.001$), which is determined by a fix-effect model (I^2=15.8%, *P*-value=0.305) (Figure 4).

Publication bias

We used Begg's funnel plot and Egger's test to access the possible publication bias of the included researches [48 49]. In the analysis of relationship between miR-17 and the OS, the *P*-values of Egger's test and Begg's test were 0.014 and 0.011, respectively. Both of the Begg's test and Egger's test implies the publication bias, thus trim and fill method was performed to make pooled HR more reliable [50]. The adjusted HR was 1.342, 95% CI=1.238-1.456. The funnel plot and Egger's plot is demonstrated in the Figure 5.

Discussion

Previous studies have showed that miRNAs appear to own a special expression profile in cancerous tissues, and they can be precisely detected by qRT-PCR in frozen, formalin-fixed, paraffin-embedded tissues and serum samples. Recently, miRNAs, serving as tumor suppressive or oncogenic genes, have been proved to play important roles in tumor genesis and progression of cancer, which are closely associated with many pathways such as cell cycle, angiogenesis, innate and adaptive immune responses, invasion, and metastasis.[12 19] Simultaneously, lots of studies have revealed the presence of miRNAs. And the potential use of microRNAs us a tumor biomarker in detecting tumor occurrence, development, and prognosis are reported in numerous researches. Unfortunately, effective diagnosis techniques and prognosis indicator of cancer have not been found. Considering the small survival chance of terminal stage of cancer,

discovering a novel less-invasive detection method with higher accuracy in prognosis in cancer prognosis is of great significance in evaluating the patient's survival status.

Over the decades, there are increasing studies that made great contribution to uncover the acquaintance of miRNAs as biomarkers and the pathogenesis of cancer, as miRNAs could be obtained from the serum, urine, fecal samples without or less invasive procedure. The miR-17, a popular-studied microRNA, is found aberrantly expressed in different kinds of cancer, such as glioma [51], esophageal or oral squamous cell carcinoma [37 52], pancreatic cancer [36], gastrointestinal cancers [40], osteosarcoma [53] and Burkitt lymphoma [39], and are significantly related to the clinic outcome of cancers. Researches are also found the detection of microRNA is even more accuracy than traditional cancer biomarkers in predicting the clinical outcomes of the human colon cancers [54]. However, there is still lack of adequate evidences that allow miRNAs as cancer biomarkers in clinical practice.

Our meta-analysis indicated that the elevated miR-17 expression is significantly associated with poor OS (HR=1.42) in patients with various types of carcinoma. Due to the publication bias implied by the Begg's test and the Egger's test, thus trim and fill method was performed to make pooled HR more reliable. The adjusted HR was 1.342. In subgroup analysis, based on the characteristics of the individual studies, significant HR was found in the Caucasian and Asian group, the digestive and non-digestive system group, the qRT-PCR and ISH detection method group, the tissue and serum sample group. Furthermore, in the analysis of DFS and RFS, we found that the increasing expression of miR-17 indicated the poor DFS and RFS in HCC and gastrointestinal cancers.

Yang et al. found that the miRNA-17 is overexpressed in the HCC tissue, and promotes the phosphorylation of heat shock protein 27 (HSP27). As a consequence, phosphorylated HSP27 enhanced the migration of the HCC cells, implying a significant simulative role of miRNA-17 in the progression of HCC [55]. Wang et al. found that the up-regulated expression of miRNA-17-5p promote cancer cells proliferation and inhibit apoptosis by post-transcript modulation of mRNA p21 and tumor protein p53-induced nuclear protein 1 (TP53INP1) [56]. Ma et al. reported that the overexpressed of miRNA-17 promote cancer cells progression by targeting gene P130 [57]. Yan et al. reported that the over-expressed of the miR-17-5p is detected in the tissue of pancreatic cancer. The miR-17-5p inhibitor promotes the expression of Bim protein by targeting its

3'-untranslated region and negatively regulates at the posttranscriptional level. Therefore, the authors suggested that the miR-17-5p inhibitor may be novel therapeutic approach for pancreatic cancer. [58]. Combined with our meta-analysis, these findings suggest that the detection of tissue or serum miR-17 expression may be a useful prognosis biomarker in the patients with HCC, pancreatic cancer, and gastrointestinal cancers.

There are various of limitations to consider. First, the power of the pooled HRs was not sufficient strong as the researches included in this study mainly focus on Asian people, lacking adequate concern on Caucasian or African population. Second, the statistical significance of the association result of miR-17 with various kinds of cancer was reduced with a relatively limited sample size of 1031 patients, as well as all of the studies are retrospective studies. As a result, further validations and clinical trials are crucially needed. Third, the lack of global consensus of miR-17 expression level makes it difficult to define a standard cut-off value. The definition of cut-off value varies as the studies. Some choose median value to define the expression level of miR-17, but some prefer the mean value. Therefore, the pooled outcome may be different from the actual value, causing the bias in the result of the effectiveness of miR-17 as a cancer prognostic biomarker. Forth, no heterogeneity was found in the meta-analysis except for the sub-group of Caucasian. The reason of heterogeneity may likely due to the different cancer type, races, and microRNAs detection method. Robaina et al. [39] reported higher prognostic value of miR-17 in Burkitt lymphoma by using ISH method in detecting miRNAs. For instance, when we stratified the OS studies according to the detection method, the lower heterogeneity was found in the qRT-PCR group (I^2=28.0, P=0.086). Fifth, the publication bias was found in the meta-analysis. The quality of the researches, the sample size, and the actual effectiveness of miR-17 as a tumor biomarker are the reasons of the publication bias.

Conclusions

In summary, our research suggested that miR-17 is a potential biomarker in various types of cancers. Moreover, under the limitation of our present study, more clinical studies with larger sample size, multi-center and prospective studies should be carried out

before miR-17 could be applied to a prognostic biomarker in the routine clinical guidance of cancers.

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Disclosure of conflicts

The authors report no conflicts of interest in this work.

Ethics approval

The article does not contain any studies with human participants or animals performed by any of the authors.

Contributors

All authors conceived the study. Chengzhi Huang and Mengya Yu performed the search and reviewed the studeis. Chengzhi Huang performed the quality of evidence assessment. Chengzhi Huang and Mengya Yu performed the risk of bias assessment. The data were extracted and checked by Chengzhi Huang and Xueqing Yao, respectively. Chengzhi Huang and Xueqing Yao performed the statistical analysis. The manuscript was drafted by Chengzhi Huang and reviewed and amended by all authors. Xueqing Yao is guarantor.

Data sharing statement

No additional data are available.

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Figure legends

Figure 1 Flow diagram of the studies selection phase

Figure 2 Forest plots of meta-analysis of overall survival in association with miR-17 expression.

Figure 3 Forest plots of subgroup meta-analysis of OS in association with miR-17 expression.
(A) Forest plots of the merged analyses of OS in the different ethnic groups. Squarrs and lines correspond to the study-specific HRs and 95%Cis, respectively. The area of the squares represents the weight, and the diamond represent the summary of HRs and 95%CIs.
(B) Forest plots of the merged analyses of OS in the different diseases groups.
(C) Forest plots of the merged analyses of OS in the different RNA detection method groups.
(D) Forest plots of the merged analyses of OS in the different sample groups.

Figure 4 Forest plot of disease-free survival(DFS) and recurrence-free survival (RFS) in association with miR-17 expression.

Figure 5

(A) Funnel plot of merged analysis of OS comparing high or low expression of miR-17.

(B) Egger's plot of merged analysis of OS comparing high or low expression of miR-17.

Table 1. A summary table of the meta-analysis

Table 2 Subgroup analysis

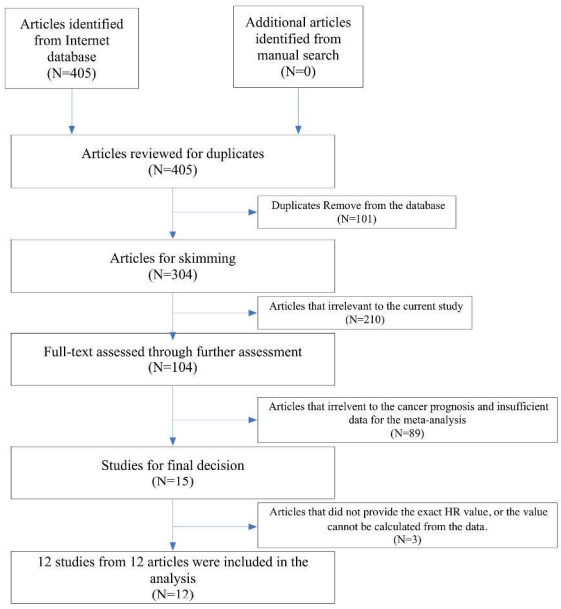

Figure 1 Flow diagram of the studies selection phase

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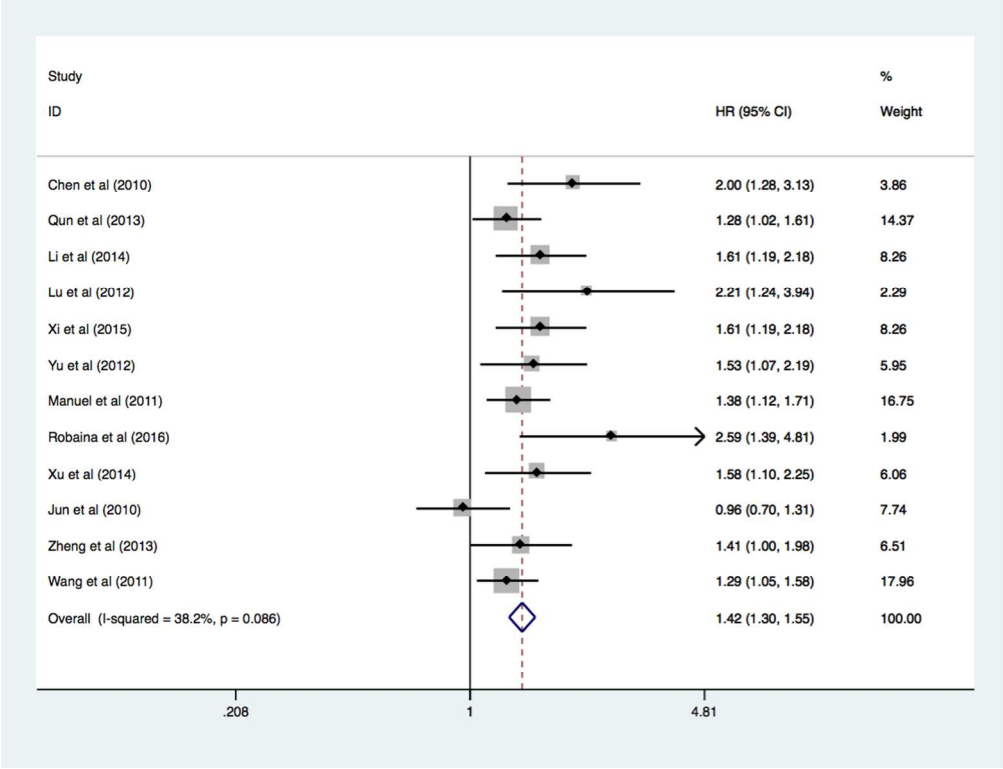

Figure 2 Forest plots of meta-analysis of overall survival in association with miR-17 expression.

210x161mm (144 x 144 DPI)

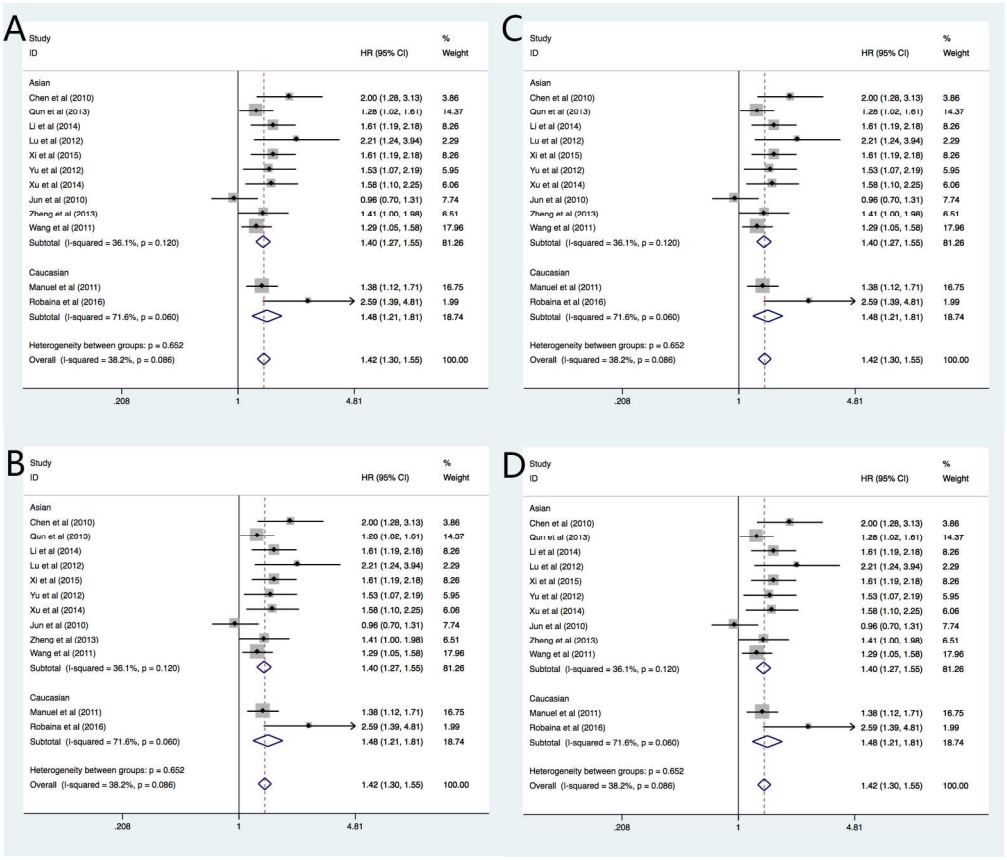

Figure 3 Forest plots of subgroup meta-analysis of OS in association with miR-17 expression.

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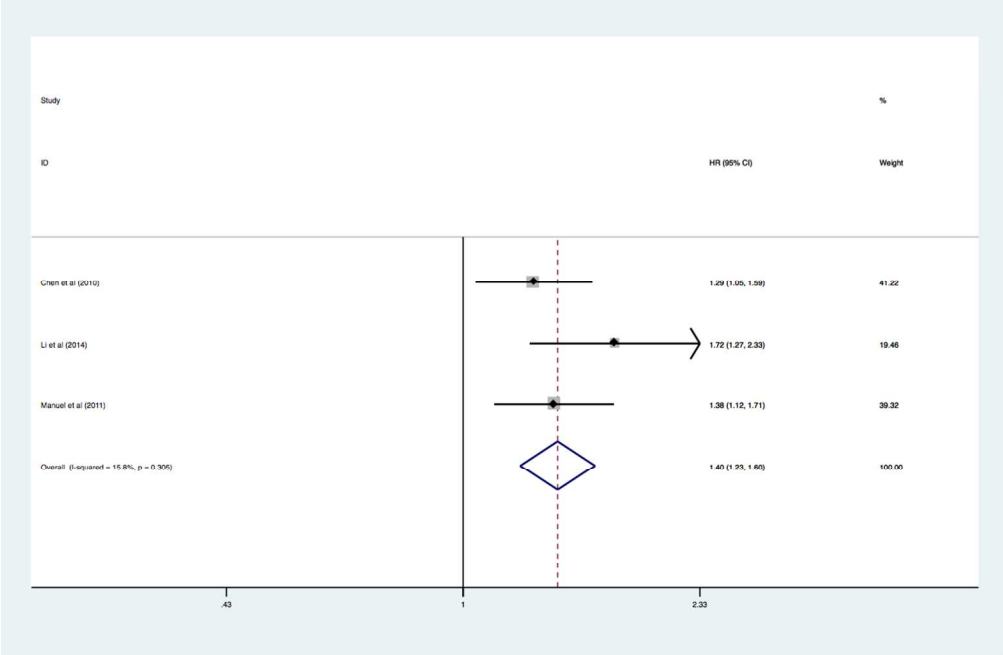

Figure 4 Forest plot of disease-free survival(DFS) and recurrence-free survival (RFS) in association with miR-17 expression.

232x152mm (144 x 144 DPI)

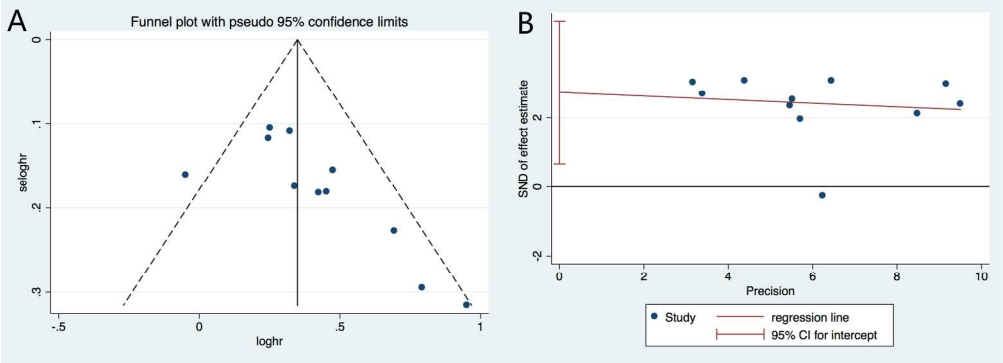

Figure 5 Funnel plot and Egger's plot of merged analysis of OS comparing high or low expression of miR-17.

444x161mm (144 x 144 DPI)

Table 1. A summary table of the meta-analysis

Study ID	Year	Country	Diseases	Case Number	Stage	Sample	Assay	Cut-off value	HR	Follow-up (months)
Chen et al	2012	China	HCC	120	I-IV	Tissue	qRT-PCR	Median	RR	46
Qun et al	2013	China	Lung Cancer	221	I-IV	Tissue	qRT-PCR	Median	Given	50
Li et al	2014	China	Osteosarcoma	117	I-III	Tissue	qRT-PCR	Median	Given	44
Lu et al	2012	China	Glioma	108	I-IV	Tissue	qRT-PCR	Mean	RR	60
Xi et al	2015	China	T-cell lymphoblastic lymphoma	57	III, IV	Tissue	qRT-PCR	Median	Given	Up to 13 years
Yu et al	2012	China	Colon Cancer	48	I-IV	Tissue	qRT-PCR	Median	Given	5-66
Manuel et al	2011	Spain	Gastrointestinal Cancer	38	I-IV	Tissue	qRT-PCR	Mean	Given	38
Robaina et al	2016	Brazil	Burkitt lymphoma	41	I-IV	Tissue	ISH	Median	Given	69
Xu et al	2014	China	Esophageal Squamous Cell Carcinoma	105	I-IV	Tissue	qRT-PCR	Mean	Given	52
Jun et al	2010	Japan	Pancreatic Cancer	80	I-IV	Tissue	qRT-PCR	Median	Given	60
Wang et al	2011	China	Gastric Cancer	65	I-IV	Serum	qRT-PCR	Median	Given	36
Zheng et al	2013	China	HCC	96	I-IV	Serum	qRT-PCR	Median	Given	NG

Abbreviations: miR-17, microRNA-17; HCC, Hepatocellular Carcinoma; ISH, in situ hybridization; qRT-PCR, quantitative real-time polymerase chain reaction; RR, risk ratio; OS, overall survival; DFS, disease-free survival; NG, not given.

Subgroup	N	Heterogeneity		pHR(95% CI)	*P*-value
		I^2	*P*-value		
Total	12	0.382	0.086	1.42(1.30-1.55)	<0.001
Ethnic subtotal					
Caucasian	2	0.716	0.060	1.48(1.21-1.81)	<0.001
Asian	10	0.361	0.120	1.40(1.27-1.55)	<0.001
Disease subtotal					
Digestive system cancers	7	0.348	0.163	1.36(1.22-1.51)	<0.001
Non-digestive system cancers	5	0.269	0.233	1.54(1.33-1.78)	<0.001
Detected method subtotal					
qRT-PCR	11	0.290	0.169	1.40(1.28-1.53)	<0.001
ISH	1			2.59(1.39-4.81)	0.003
Detected Sample subtotal					
Tissue	10	0.462	0.053	1.45(1.31-1.61)	<0.01
Serum	2	0	0.662	1.32(1.10-1.57)	0.002

Table 2 Subgroup analysis

Abbreviations: pHR, pooled hazard ratio; qRT-PCR, quantitative real-time polymerase chain reaction; ISH, in situ hybridization.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	9
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	11

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MicroRNA-17 and the prognosis of human carcinomas: a systematic review and meta-analysis

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Keywords:	microRNA-17, Cancer, Outcome, Prognosis, Meta-analysis

MicroRNA-17 and the prognosis of human carcinomas: a systematic review and meta-analysis

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Abstract

Objective The role of microRNA-17(miR-17) has been identified as a tumor biomarker in various studies, its prognostic value in cancers remains unclear. Therefore, we performed a systematic review and meta-analysis to analyze and summarize the relationship between the miR-17 status and clinical outcome in a variety of human cancers.

Design Systematic review and meta-analysis.

Data sources PubMed, Web of Science, and Embase from the first year of records through May 15th, 2017

Outcomes The patients' survival results were pooled, and pooled hazard ratio (HR) with 95% confidential intervals were calculated and used for measuring the strength of association between miR-17 and the prognosis of cancers, including hepatocellular carcinoma , lung cancer, osteosarcoma, glioma, T-cell lymphoblastic lymphoma, and colon cancer . Heterogeneity, publication bias, and subgroup analysis were also conducted.

Results A total of 1096 patients were included in this meta-analysis from 12 articles. The results indicated that the increased expression of miR-17 played an unfavorable role in overall survival (OS) in various human carcinomas with the HR of 1.342taking into account the publication bias. In subgroup analysis, HR of ethnicity (Caucasian HR=1.48 and Asian HR=1.40), disease (digestive system HR=1.36 and blood system cancer (HR=2.38), detection method (qRT-PCR HR=1.40 and in situ hybridization, ISH HR=2.59), and detection sample (tissue HR=1.45 and serum HR=1.32) were significant with $p < 0.05$. For the analysis of disease-free survival and recurrence-free survival , the increased expression of miR-17 was associated with unfavorable prognosis (HR=1.40).

Conclusions miR-17 may be a useful biomarker in predicting the clinical outcome of human cancers, but due to the limitations of the current studies, further verification of the role of miR-17 in human malignancies is urgently needed.

Keywords microRNA-17; Cancer; Outcome; Prognosis; Meta-analysis.

Strengths and limitations of the study

1. This is the first meta-analysis that summarized and reported the microRNA-17 as a novel potential cancer prognostic biomarker in the clinical field.
2. We used strict, broad search strategy of the internet databases to minimize any potential publication bias.
3. We conducted the subgroup analysis and found that the up-regulated expression of microRNA-17 may imply poor clinical outcome in digestive system cancers.
4. The major limitation of our meta-analysis is the inclusion of a limited number of studies carried out on Western populations decreasing the applicability of our results among other ethnicities. MicroRNA-17

detection is not routine clinical practice, and the prognostic value of microRNA-17 remains controversial. In the future, additional clinical trials are needed to verify the prognostic significance of microRNA 17.

Introduction

Despite significant advances in clinical research over the past few decades, cancer is still a key health burden and a leading cause of death worldwide. In the year 2017, it is estimated that 1,688,780 patients were diagnosed with cancers with 600,920 cancer deaths in the United States [1]. Due to the advanced screening methods and adjuvant systemic therapies for newly diagnosed cases, the mortality rate for cancers is declining in the developed countries [2], whereas the clinical outcome of cancers in the developing countries is still poor [3 4].

There are several independent factors for identifying and evaluating the clinical outcome of human cancers, including tumor size, histological grade, age of the patients, and metastasis to lymph nodes [5-8]. Tissue- and serum-based tumor biomarkers are widely used to predict the prognosis of neoplasms. However, these techniques are far from satisfactory due to the low specificity and sensitivity [9-11]. Thus, a less-invasive and more accurate biomarker would be of great value for the prognosis of human tumors.

The discovery of microRNAs (miRNAs) provided an innovative method for the prognosis of cancers by a less-invasive detection method [12]. miRNAs, a class of endogenous non-coding single-stranded RNAs with the length of 18-25 nucleotides, act as regulators of gene expression by pairing with the complementary nucleotides in the 3'-untranslated regions (3'-UTR) of their target mRNAs. miRNAs may act as regulators of cell growth, proliferation, differentiation and apoptosis [13]. Because of these fundamental activities, numerous studies have shown that miRNAs function as tumor suppressors or oncogenes. It has also been reported that some miRNAs are differentially expressed between tumor and non-tumor tissues, and the abnormal expression of tumor-associated miRNAs can be detected in patient's blood, cancerous tissue and fecal samples [14 15]. Recent studies have demonstrated that aberrantly expressed miRNAs, especially those acting as tumor suppressors or oncogenes, are related to cancer development, progression, and patients' response to therapy [16-18]. Therefore, miRNAs can be considered as useful prognostic biomarkers for various human cancers.

One such example is of miR-17 that is aberrantly expressed in cancer patients [19-21]. The miR-17 family, which includes six members, is one of the most extensively studied miRNA clusters [22]. These miRNAs are located within an 800 base-pair region of human chromosome 13, play an essential role in the development of the heart, lung, and human immune system [23]. Recent studies have found that miR-17 may play a critical role in the development of human cancers. [24 25] Increased expression of miR-17 promotes the metastasis of lung and pancreatic cancers, suggesting its role as an oncogene[26 27]. However, other studies have reported that miR-17 inhibits tumor cell invasion and metastasis in breast cancer[28]. In all, the role of miR-17 in cancer development as well as the exact mechanism are not yet clearly described. According to the miRBase (http://www.mirbase.org), miR-17 includes two members, miR-17-5p and miR-17-3p which are located in the sequence of miR-17 with a stem-loop structure. As a result, the detection of miR-17-5p, miR-17-3p has the same effect and result of detecting miR-17 [29-33].

Several published results indicate that the higher expression of the miR-17 is indicative of poor prognosis in cancer patients [26 27 34-43]. However, several confounding factors, including race, detection method, and tumor site, may affect the observations making the relationship between aberrant expression of miR-17 and the clinical outcome of cancer patients inconsistent. We, therefore, conducted a meta-analysis of available studies to evaluate the clinical utility of miR-17 as a novel cancer prognostic indicator.

Material and Methods

Data Source and Search Strategy

The following online electronic databases were used for the literature search: PubMed, Web of Science, and Embase. The search period was up to May 15th, 2017. Key search words used were: (1) prognosis OR prognostic OR survival OR outcome OR mortality; (2) cancer OR tumor OR tumour OR carcinoma OR neoplasm; (3) miR-17 OR microRNA-17 OR hsa-mir-17. Details are listed in the Supplementary Table 1. Additionally, we also searched the references and relevant published articles via Google Scholar.

Inclusion and Exclusion Criteria

The inclusion criteria of the articles were: (1) the cancers were diagnosed by the histological examination or any other accepted standard; (2) miR-17 was studied in human cancers; (3) the expression of miR-17 and the clinical outcome of patients were included in the research; and (4) reports with survival outcome and the data analyzed hazard ratio (HR) with 95% confidence interval (95% CI) and HR with a *P*-value.

The exclusion criteria were: (1) duplicate publications; (2) articles focused on other genes; (3) case reports, reviews, letters, and animal trails; (4) unqualified or insufficient data; (5) HR, 95% CI and *P*-value were not provided or could not be calculated and (6) articles concentrated on the polymorphisms or methylation patterns of miRNAs.

Questions of suitability of articles to be included were examined and discussed by the authors after reviewing the abstract and full text manuscript. The final decision was made by the academic committee.

Data extraction and quality assessment

All included studies were decided by the two investigators (Huang and Yao) independently based on titles and abstracts. Full-text of the articles was required if the articles were potentially suitable for the meta-analysis. Furthermore, the literature search was performed again in the excluded articles to avoid missing any article potentially relevant for the study. The original authors of the articles were contacted if any supplementary data were needed. Any disagreement was resolved by the two authors (Huang and Yao). The extracted details of the articles were as follows: (1) publication information: the name of the authors, publication area, and publication year; (2) patient's characteristics: diseases, stage of the disease, RNA detection method, type of tissue sample

and follow-up years; (3) the measurement of miR-17 measurement and its cut-off value and (4) HR of miR-17 for overall survival (OS), disease-free survival (DFS) and recurrence-free survival (RFS), as well as their 95% CI and *P*-values. The HRs and their 95% CI were extracted from the original articles or via e-mails from the authors. If not, we calculated HR and 95% CI using the data of observed deaths, cancer recurrences, or the original data provided by the authors. All calculations mentioned above were based on the methods provided by Parmar, M. K. et al. [44]. The quality of the included articles was assessed based on a systematic review checklist of the Dutch Cochrane Centre proposed by MOOSE [45].

Statistical Analysis

The test of heterogeneity of pooled HRs was carried out by using Cochran's *Q*-test and Higgins I^2 statistic. A *P*-value of < 0.05 or $I^2 > 50\%$ was considered as statistically significant. The 95%CI of I^2 was calculated by the method introduced by Hedges et al[46]. If heterogeneity existed, the random effects model was performed among the included studies; otherwise, the fixed effects model was selected. I^2 value ranged from 0% to 100%. All *P*-values were two-sided.

HR >1 presents of up-regulated expression of miR-17 indicated poor prognosis in patients, and HR<1 suggested a better prognosis. Publication bias was evaluated by the Begg's test and Egger's test [47 48]. If the publication bias did exist, the trim and fill method introduced by Duval and the Tweedit's was used to adjust the results[49]. The STATA software Version 14.0 (StataCorp LP, College Station, TX, USA) was used in all of the statistical analyses.

Study registration

The systematic review and meta-analysis is registered in PROSPERO (No. CRD42017065749).

Patients and Public Involvement Statement

The patients or public were not involved in the study.

Results

Literature selection

We started with 405 articles associated with miR-17 and cancer prognosis was identified from online database searches. After removing the replicate records, 304 miR-17-related articles were left. The first screening based on the species, article type, and language eliminated 210 citations from the analysis. Subsequently, the remaining 104 studies were carefully assessed by reviewing the abstract and full text of each article. After that, 89 articles were excluded from the study because they were unrelated to miR-17 expression levels or because of the lack of survival statistics such as HRs, 95% CI, or *P*-value. Finally, 15 studies, which

investigated the potential relationship between miR-17 expression and prognosis of human cancers, remained for further detailed screening and data-extraction. Three of the studies that explained the relationship between miR-17 expression and the clinical outcome of cancer had to be removed because the authors did not provide the exact HR value, or the value cannot be calculated from the data. Thus, 12 articles (12 studies) [26 27 34-43] were included in this meta-analysis. (Figure 1)

Characteristics of selected studies

All12 studies included in the meta-analysis were retrospective studies published between 2010 and 2016 [26 27 34-43]. Patient's OS was reported in all 12 studies, and three studies also examined the DFS or RFS. The type of the cancers included gastrointestinal cancers (colorectal cancer, gastric cancer), lung cancer, pancreatic cancer, hepatocellular cancer, osteosarcoma, glioma, T-cell lymphoblastic lymphoma and esophageal squamous cell carcinoma. Total of 1096 patients with various types of cancers were from People's Republic of China, Japan, Spain, and Brazil. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the expression of miR-17 in 12 studies, and one study used the in-situ hybridization (ISH). All studies used tissue and serum samples as the source of the miR-17. The majority (10 of 12) of the HRs reported in the present analysis were included in the multivariate analysis. The remaining two HRs could be estimated by Kaplan-Meier analysis and relative risk (RR) values. Most of the studies have the follow-up research for at least 38 months. The clinical characteristics of the studies included in this article are summarized in Table 1.

Study	Year	Country	Diseases	Case Number	Stage	Sample	Assay	Cut-off value	HR
en et al	2012	China	HCC	120	I-IV	Tissue	qRT-PCR	Median	RR
un et al	2013	China	Lung Cancer	221	I-IV	Tissue	qRT-PCR	Median	Given
i et al	2014	China	Osteosarcoma	117	I-III	Tissue	qRT-PCR	Median	Given
u et al	2012	China	Glioma	108	I-IV	Tissue	qRT-PCR	Mean	RR
i et al	2015	China	T-cell lymphoblastic lymphoma	57	III, IV	Tissue	qRT-PCR	Median	Given
u et al	2012	China	Colon Cancer	48	I-IV	Tissue	qRT-PCR	Median	Given
nuel et al	2011	Spain	Gastrointestinal Cancer	38	I-IV	Tissue	qRT-PCR	Mean	Given
aima et al	2016	Brazil	Burkitt lymphoma	41	I-IV	Tissue	ISH	Median	Given
u et al	2014	China	Esophageal Squamous Cell Carcinoma	105	I-IV	Tissue	qRT-PCR	Mean	Given
un et al	2010	Japan	Pancreatic Cancer	80	I-IV	Tissue	qRT-PCR	Median	Given
ang et al	2011	China	Gastric Cancer	65	I-IV	Serum	qRT-PCR	Median	Given
eng et al	2013	China	HCC	96	I-IV	Serum	qRT-PCR	Median	Given

Association between miR-17 and OS

Due to low heterogeneity, fixed effects model was used to calculate and analyze the pooled HR value. High expression level of miR-17 was associated with the poor OS in patients with diverse cancers. The statistical power of *Q*-test is low when there are limited studies included in the meta-analysis. We, therefore, conducted random effect analysis on the OS (HR=1.45, 95%CI=1.29-1.63, *P*<0.001), which was not significantly different compared to the analysis of fixed effect model. Details of the meta-analysis are systematically summarized in the Figure 2.

To demonstrate the predictive role of miR-17, subgroups analysis was conducted based on patients'

Subgroup	Number of studies	Heterogeneity		pooled HR (95% CI)	*P*-value
		I^2 (95%CI)	*P*-value		
Total	12	38.2% (0%-68.7%)	0.086	1.42(1.30-1.55)	<0.001
Ethnic subtotal					
Caucasian	2	71.6% (0%-93.6%)	0.06	1.48(1.21-1.81)	<0.001
Asian	10	36.1% (0%-69.5%)	0.12	1.40(1.27-1.55)	<0.001
Disease subtotal					
Digestive system	7	34.8% (0%-72.4%)	0.163	1.36(1.22-1.51)	<0.001
Respiratory system	1	NA	NA	1.28(1.02-1.61)	0.036
Blood system	2	0	0.713	2.38(1.56-3.63)	<0.001
Glioma	1	NA	NA	1.61(1.19-2.18)	0.002
Osteosarcoma	1	NA	NA	1.61(1.19-2.18)	<0.001
Detected method subtotal					
qRT-PCR	11	29.0% (0%-65.0%)	0.169	1.40(1.28-1.53)	<0.001
ISH	1	NA	NA	2.59(1.39-4.81)	0.003
Detected Sample subtotal					
Tissue	10	46.2% (0%-74.1%)	0.053	1.45(1.31-1.61)	<0.001
Serum	2	0	0.662	1.32(1.10-1.57)	0.002
Detection of miR-17 subtotal					
miR-17	8	60.1% (13.2%-81.7%)	0.057	1.29(1.11-1.49)	<0.001
miR-17-5p	4	7.5% (0%-43.4%)	0.372	1.50(1.34-1.67)	0.001

ethnicity, cancer type, methods identifying miRNAs and type of tissue samples. Clinical association between miR-17 and OS was found in the Asian and Caucasian patients. The association was also significant in other subgroups, including digestive system cancers and blood cancers, qRT-PCR detection method, and tissue and serum samples. miR-17 includes two members, miR-17-5p and miR-17-3p which are located in the sequence of miR-17 with a stem-loop structure. Therefore, analysis of miR-17-5p or miR-17-3p afforded the same effect (or result) as miR-17. To clarify the heterogeneity, we conducted a subgroup analysis concerning the detection method of miR-17 and found that the clinical value was also significant in miR-17 group and miR-17-5p group. There was no significant difference between the two groups (Figure 3E), implying that same effect existed when detecting miR-17 and miR-17-5p. Details of the subgroup analysis are listed in the Table 2

Correlation between miR-17 and DFS and RFS

A total of 3 studies [37 38 41] were included in the analysis of DFS and RFS. The analyses revealed a predictive role of increased expression of miR-17 for the prognosis of cancer patients (pooled HR= 1.40, 95% CI=1.23-1.60, P<0.001) as determined by the fix-effect model (I^2=15.8%, P =0.305) (Figure 4).

Publication bias

We used Begg's funnel plot and Egger's test to assess the possible publication bias of the included studies [47 48]. In the analysis of relationship between miR-17 and the OS, the P-values of Egger's test and Begg's test were 0.014 and 0.011, respectively. The funnel plot and Egger's plot are displayed in Figure 5. Both Begg's test and Egger's test implied a publication bias, thus the trim and fill method was performed to make pooled HR more reliable [49]. The altered HR was 1.34, 95% CI=1.24-1.46, P<0.001, which was not significantly different from the pooled HR (Supplementary Figure 1).

Discussion

Previous studies have shown that miRNAs have a distinct expression profile in cancerous tissues which can be detected by qRT-PCR in frozen, formalin-fixed, and paraffin-embedded tissues and in serum samples. Recently, miRNAs, serving as tumor suppressors or oncogenes, have been shown to play important roles in the evolution and progression of cancers. miRNAs are involved in a variety of crucial cellular pathways such as angiogenesis, innate and adaptive immune responses, cellular proliferation, invasion, and metastasis.[12 16] Several studies have reported the potential use of miRNAs as tumor biomarkers for detecting tumor occurrence, development, and prognosis. Unfortunately, effective diagnosis techniques and prognosis indicators of cancer have not been found. Developing a novel less-invasive detection method with higher accuracy for cancer prognosis is of great significance in evaluating cancer progression as well as monitoring patients' therapeutic response.

Over the last couple of decades, numerous studies have uncovered the involvement of miRNAs in the pathogenesis of cancer. Since miRNAs can be obtained noninvasively from the serum, urine, and fecal samples, their utility as diagnostic and prognostic biomarkers in cancer and other diseases has been extensively explored. It has been reported that miRNA could be detected with higher accuracy than traditional cancer biomarkers in predicting the clinical outcome of the human colon cancers [50]. However, adequate evidence is still lacking for the utility of miRNAs as cancer biomarkers in clinical practice.

miR-17, a widely-studied miRNA, is aberrantly expressed in different kinds of cancers, such as glioma [51], esophageal and oral squamous cell carcinomas [36 52], pancreatic cancer [26], gastrointestinal cancers [39], osteosarcoma [53] and Burkitt lymphoma [38], and is significantly related to the clinical outcome of cancers. Our meta-analysis indicated that the elevated miR-17 expression is significantly associated with poor OS (HR=1.42) in patients with various types of carcinomas. The analysis using the Cochran's Q-test and Higgins I^2 test

implied low heterogeneity. As limited number of studies were included in the meta-analysis, the *Q*-test had inadequate statistical power. We, therefore, applied the fixed effects model to calculate and analyze the pooled HR value. We also conducted random effect analysis on the OS, which was not significantly different when compared to analysis of fixed effect model (Figure 2). In the subgroup analysis, we found that the potential heterogeneity may have originated from the Caucasian group in the study conducted by Robaina et al.[38]. Unlike the commonly used RT-PCR, ISH technique was used to detect miR-17. Other factors contributing to the heterogeneity in the study may include absence of Hispanics in the Brazilian study and the limited number of patients (n=41) recruited in the study.

As the Begg's test and the Egger's test implied publication bias, we used the Trim and Fill method to obtain a more reliable pooled HR. We found that the adjusted HR was not significantly different from the pooled HR. In subgroup analysis, based on the characteristics of the individual studies, significant HR was found in the Caucasian and Asian groups, the qRT-PCR group and the tissue and serum sample groups. Furthermore, the increased expression of miR-17 indicated poor DFS and RFS in HCC and gastrointestinal cancers. Several investigators have explored the functional roles of miR-17 and its involvement in human cancers. Yang et al. found that the miRNA-17 was overexpressed in the HCC tissue, and promoted the phosphorylation of heat shock protein 27 (HSP27). The phosphorylated HSP27 then enhanced the migration of the HCC cells implying a significant role of miRNA-17 in the progression of HCC [54]. Wang et al. reported that the up-regulated expression of miRNA-17-5p promoted cancer cells proliferation and inhibited apoptosis by post-transcriptional modulation of mRNA p21 and tumor protein p53-induced nuclear protein 1 (TP53INP1) [55]. In the study by Ma et al. overexpression of miRNA-17 promoted cancer cells progression by targeting P130 [56]. Yan et al. found over-expression of the miR-17-5p in pancreatic cancer. The miR-17-5p inhibitor promoted the expression of Bim protein by targeting the 3'-untranslated regions of its mRNA and negatively regulating at the posttranscriptional level. Therefore, the authors suggested that the miR-17-5p inhibitor may be a novel therapeutic approach for pancreatic cancer[57]. Together with our meta-analysis, these findings suggest that the detection of tissue or serum miR-17 expression may be a useful prognostic biomarker in patients with HCC, pancreatic cancer, and gastrointestinal cancers.

There are potential limitations of this study. The literature searches using authentic and widely used data bases found studies performed predominantly on Asian populations not encompassing sufficient numbers of other populations such as Caucasians. Our results of miR-17 as a potential biomarker may, therefore, not be applicable to other populations. The pooled HR values were also not sufficiently strong. Furthermore, the relatively limited sample size of 1031 patients weakened the statistical significance of the prognostic potential of miR-17 expression levels.

Conclusions

In summary, our meta-analysis suggested that miR-17 is a potential biomarker in various types of cancers. However, further multi-center clinical trials with larger sample size and prospective studies including Caucasians and patients representing other ethnicities are needed to confirm the prognostic value of miR-17 and its subsequent application as a prognostic biomarker in the routine clinical guidance of cancers.

Acknowledgments

Not applicable.

Contributors

Chengzhi Huang and Mengya Yu conceived the study. Chengzhi Huang and Xueqing Yao performed the data extraction and analysed the data. Chengzhi Huang and Mengya Yu wrote the paper. All authors had full access to all of the data and approved the final version of manuscript.

Disclosure of conflicts

The authors report no conflicts of interest in this work.

Data Sharing Statement

No additional data are available

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Ethics approval

The study does not include human participants or animals.

Patients and Public Involvement Statement

The patients or public were not involved in the study.

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Figure and table legends

Figure 1 Flow diagram of the studies selection phase

Figure 2 Forest plot of meta-analysis of overall survival in association with miR-17 expression.

Figure 3 Forest plots of subgroup meta-analysis of OS in association with miR-17 expression.
(A) Forest plots of the merged analyses of OS in different ethnic groups. Squares and lines correspond to the study-specific HRs and 95% CIs, respectively. The area of the squares represents the weight, and the diamonds represent the summary of HRs and 95% CIs.
(B) Forest plots of the merged analyses of OS in different diseases groups.
(C) Forest plots of the merged analyses of OS in different RNA detection methods groups.
(D) Forest plots of the merged analyses of OS in different sample groups.
(E) Forest plots of the merged analyses of OS in the detection method of miR-17.

Figure 4 Forest plot of disease-free survival(DFS) and recurrence-free survival (RFS) in association with miR-17 expression.

Figure 5

(A) Funnel plot of merged analysis of OS comparing high or low expression of miR-17.

(B) Egger's plot of merged analysis of OS comparing high or low expression of miR-17.

Table 1. A summary table of the meta-analysis.
Abbreviations: miR-17, microRNA-17; HCC, hepatocellular carcinoma; ISH, in situ hybridization; qRT-PCR, quantitative real-time polymerase chain reaction; RR, risk ratio; OS, overall survival; DFS, disease-free survival; NG, not given.

Table 2 Subgroup analysis.
Abbreviations: miR-17, microRNA-17; miR-17-5p, microRNA-17-5p; qRT-PCR, quantitative real-time polymerase chain reaction; ISH, in situ hybridization; NA, not available.

Supplementary Table 1 The search strategy of online databases

Supplementary Figure 1 Funnel plot of adjusted pooled HRs after the analysis of the Trim and Fill method.

Supplementary Material 1 PRISMA 2009 checklist.

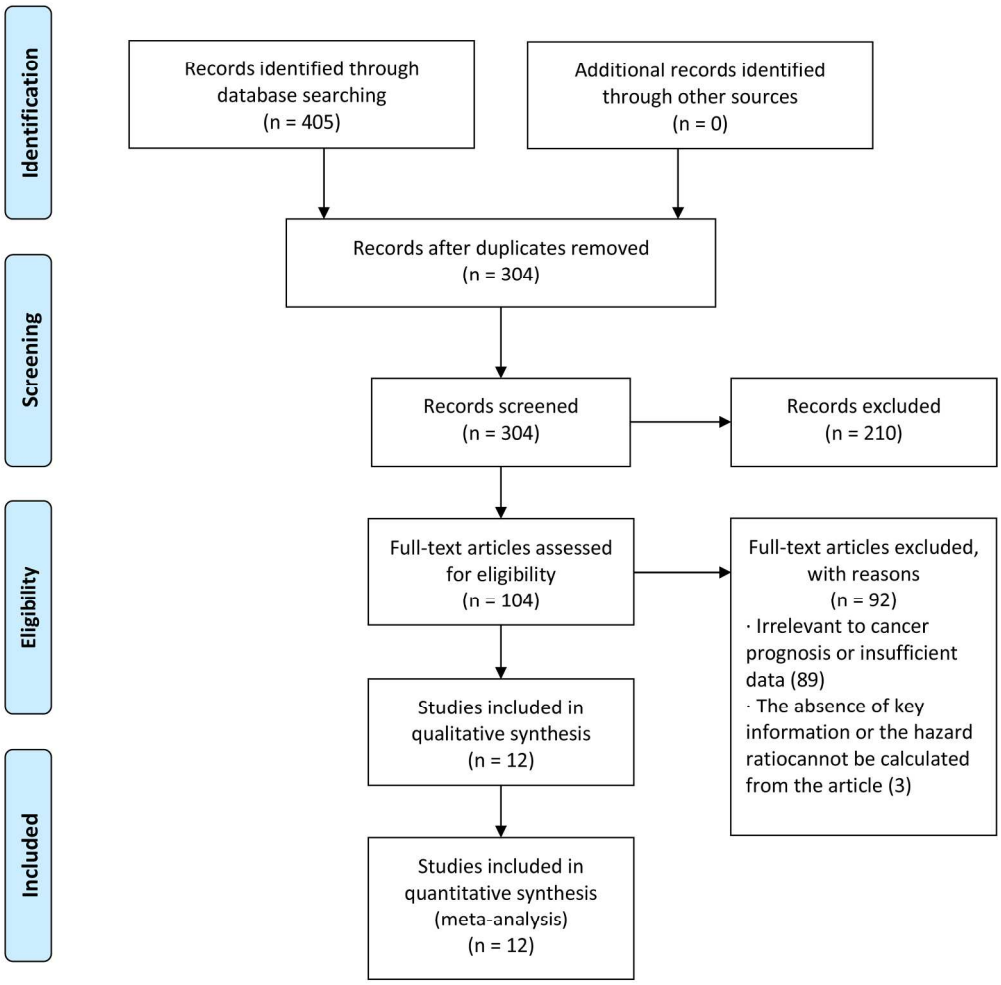

Figure 1 Flow diagram of the studies selection phase

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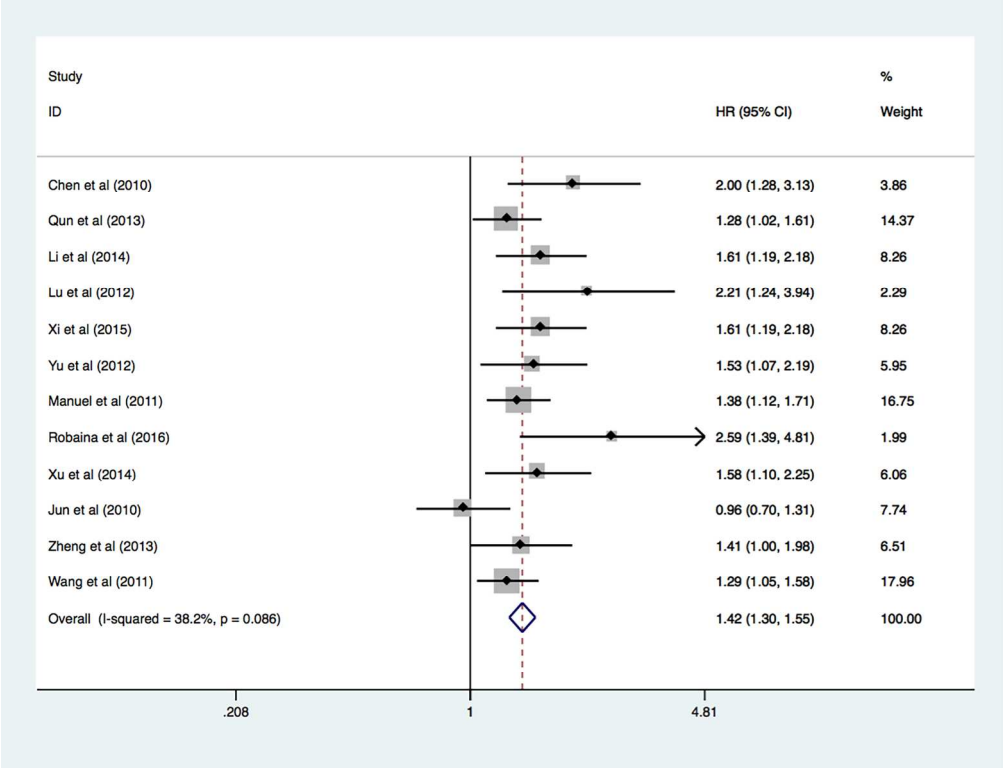

Figure 2 Forest plots of meta-analysis of overall survival in association with miR-17 expression.

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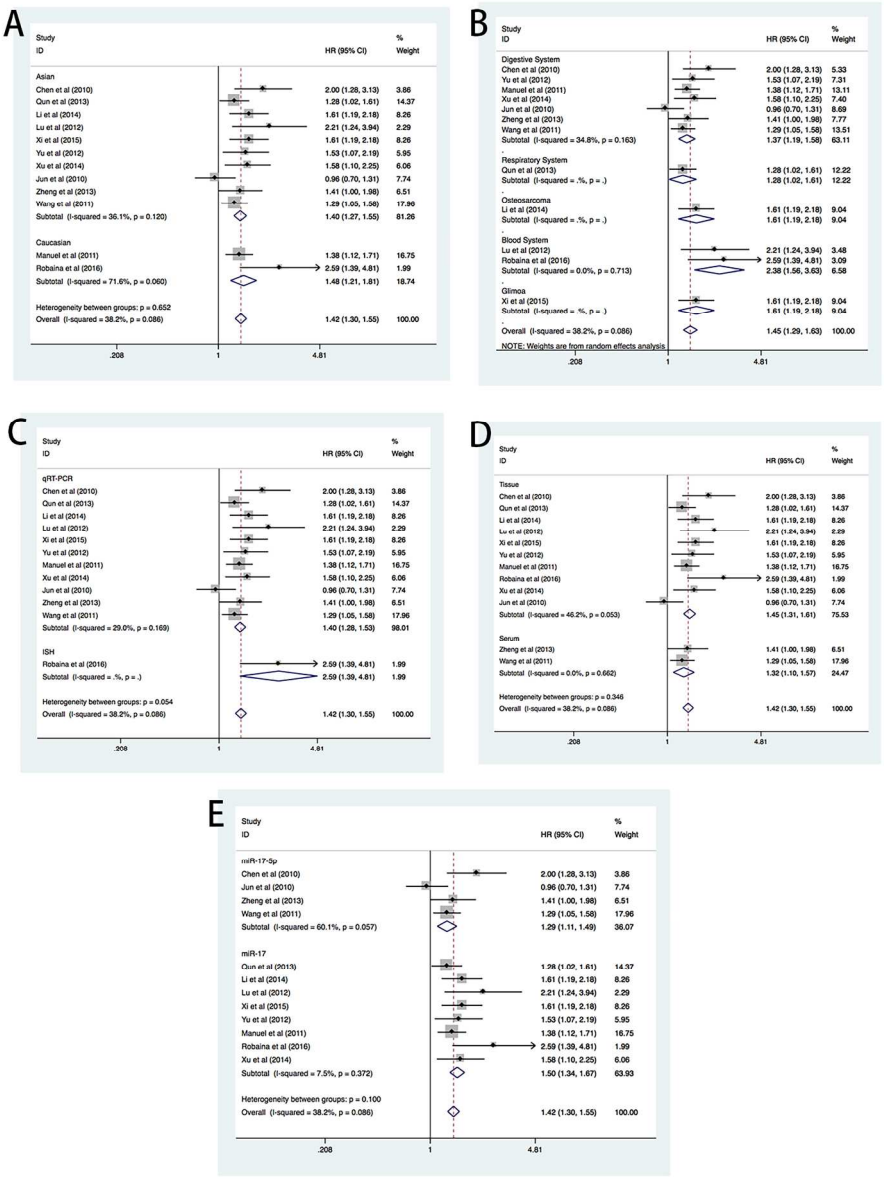

Figure 3 Forest plots of subgroup meta-analysis of OS in association with miR-17 expression.
(A) Forest plots of the merged analyses of OS in different ethnic groups. Squares and lines correspond to the study-specific HRs and 95% CIs, respectively. The area of the squares represents the weight, and the diamonds represent the summary of HRs and 95% CIs.
(B) Forest plots of the merged analyses of OS in different diseases groups.
(C) Forest plots of the merged analyses of OS in different RNA detection methods groups.
(D) Forest plots of the merged analyses of OS in different sample groups.
(E) Forest plots of the merged analyses of OS in the detection method of miR-17.

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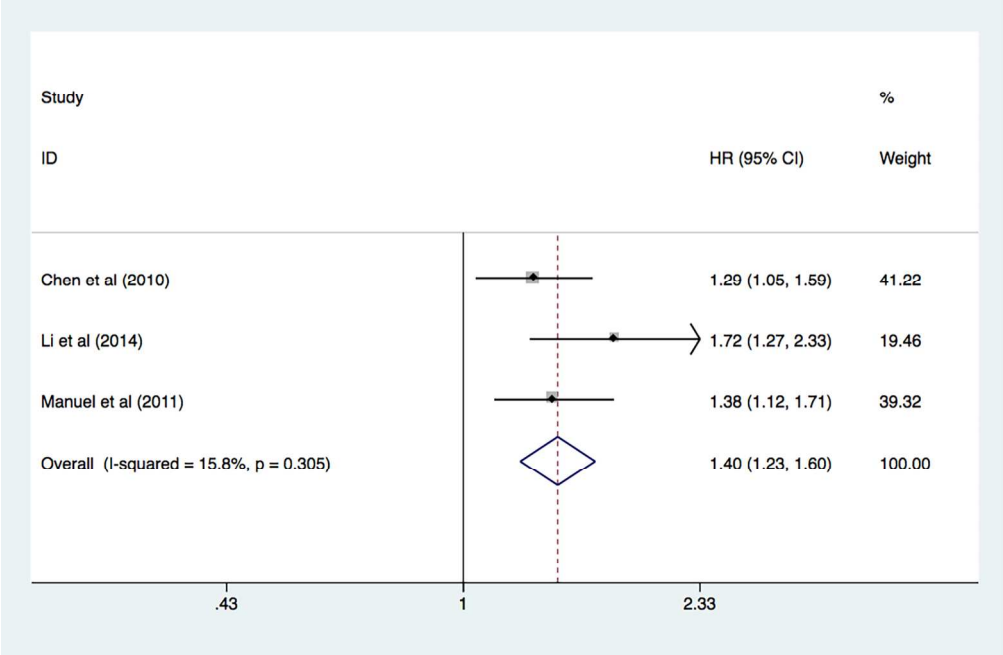

Figure 4 Forest plot of disease-free survival(DFS) and recurrence-free survival (RFS) in association with miR-17 expression.

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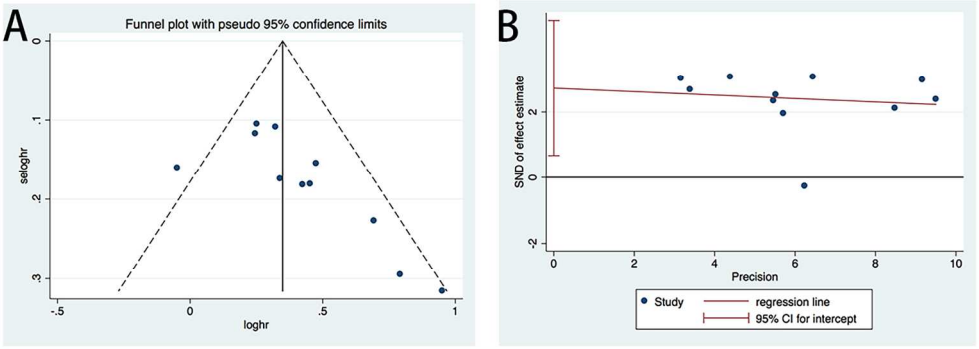

Figure 5
(A) Funnel plot of merged analysis of OS comparing high or low expression of miR-17.
(B) Egger's plot of merged analysis of OS comparing high or low expression of miR-17.

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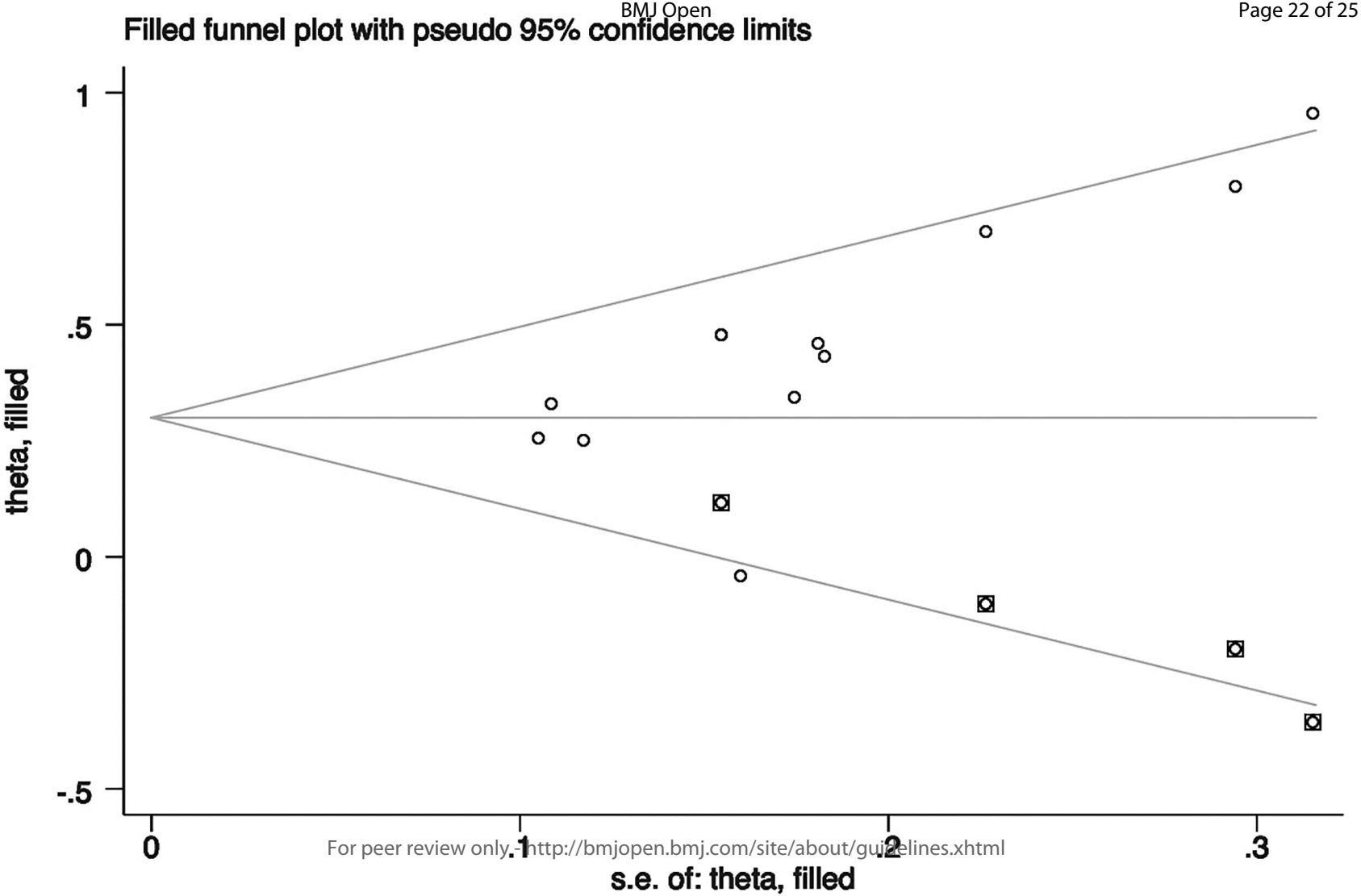

Supplementary Table 1 The search strategy of online databases

Databases	Search method
Pubmed	("MIRN17 microRNA, human" [Supplementary Concept] AND "Neoplasms"[Mesh] AND "Prognosis"[Mesh]
Web of Science	
1	TS=(cancer OR neoplas* OR carcinom* OR tumo*)
2	TS=(prognosis OR prognostic OR survival OR outcome OR mortality)
3	TS=(miR-17 OR microRNA-17 OR hsa-mir-17)
4	#1 AND #2 AND #3
Embase	
1	miR-17 OR microRNA-17 OR hsa-mir-17
2	cancer OR neoplas* OR carcinom* OR tumo*
3	prognosis OR prognostic OR survival OR outcome OR mortality
4	#1 AND #2 AND #3 AND ([embase]/lim AND 'human'/de)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

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MicroRNA-17 and the prognosis of human carcinomas: a systematic review and meta-analysis

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MicroRNA-17 and the prognosis of human carcinomas: a systematic review and meta-analysis

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Abstract

Objective Although the role of microRNA-17(miR-17) has been identified as a tumor biomarker in various studies, its prognostic value in cancers remains unclear. Therefore, we performed a systematic review and meta-analysis to analyze and summarize the relationship between the miR-17 status and clinical outcome in a variety of human cancers.

Design Systematic review and meta-analysis.

Data sources PubMed, Web of Science, and Embase from the first year of records through May 15th, 2017

Outcomes The patients' survival results were pooled, and pooled hazard ratio (HR) with 95% confidential intervals were calculated and used for measuring the strength of association between miR-17 and the prognosis of cancers, including hepatocellular carcinoma, lung cancer, osteosarcoma, glioma, T-cell lymphoblastic lymphoma, and colon cancer. Heterogeneity, publication bias, and subgroup analysis were also conducted.

Results A total of 1096 patients were included in this meta-analysis from 12 articles. The results indicated that the increased expression of miR-17 played an unfavorable role in overall survival (OS) in various human carcinomas with the HR of 1.342taking into account the publication bias. In subgroup analysis, HR of ethnicity (Caucasian HR=1.48 and Asian HR=1.40), disease (digestive system HR=1.36 and blood system cancer (HR=2.38), detection method (qRT-PCR HR=1.40 and in situ hybridization, ISH HR=2.59), and detection sample (tissue HR=1.45 and serum HR=1.32) were significant with $p < 0.05$. For the analysis of disease-free survival and recurrence-free survival, the increased expression of miR-17 was associated with unfavorable prognosis (HR=1.40).

Conclusions miR-17 may be a useful biomarker in predicting the clinical outcome of human cancers, but due to the limitations of the current studies, further verification of the role of miR-17 in human malignancies is urgently needed.

Keywords microRNA-17; Cancer; Outcome; Prognosis; Meta-analysis.

Strengths and limitations of the study

1. This is the first meta-analysis that summarized and reported the microRNA-17 as a novel potential cancer prognostic biomarker in the clinical field.
2. We used strict, broad search strategy of the internet databases to minimize any potential publication bias.
3. We conducted the subgroup analysis and found that the up-regulated expression of microRNA-17 may imply poor clinical outcome in digestive system cancers.
4. The major limitation of our meta-analysis is the inclusion of a limited number of studies carried out on Western populations decreasing the applicability of our results among other ethnicities. MicroRNA-17

detection is not routine clinical practice, and the prognostic value of microRNA-17 remains controversial. In the future, additional clinical trials are needed to verify the prognostic significance of microRNA 17.

Introduction

Despite significant advances in clinical research over the past few decades, cancer is still a key health burden and a leading cause of death worldwide. In the year 2017, it is estimated that 1,688,780 patients were diagnosed with cancers with 600,920 cancer deaths in the United States [1]. Due to the advanced screening methods and adjuvant systemic therapies for newly diagnosed cases, the mortality rate for cancers is declining in the developed countries [2], whereas the clinical outcome of cancers in the developing countries is still poor [3 4].

There are several independent factors for identifying and evaluating the clinical outcome of human cancers, including tumor size, histological grade, age of the patients, and metastasis to lymph nodes [5-8]. Tissue- and serum-based tumor biomarkers are widely used to predict the prognosis of neoplasms. However, these techniques are far from satisfactory due to the low specificity and sensitivity [9-11]. Thus, a less-invasive and more accurate biomarker would be of great value for the prognosis of human tumors.

The discovery of microRNAs (miRNAs) provided an innovative method for the prognosis of cancers by a less-invasive detection method [12]. miRNAs, a class of endogenous non-coding single-stranded RNAs with the length of 18-25 nucleotides, act as regulators of gene expression by pairing with the complementary nucleotides in the 3'-untranslated regions (3'-UTR) of their target mRNAs. miRNAs may act as regulators of cell growth, proliferation, differentiation and apoptosis [13]. Because of these fundamental activities, numerous studies have shown that miRNAs function as tumor suppressors or oncogenes. It has also been reported that some miRNAs are differentially expressed between tumor and non-tumor tissues, and the abnormal expression of tumor-associated miRNAs can be detected in patient's blood, cancerous tissue and fecal samples [14 15]. Recent studies have demonstrated that aberrantly expressed miRNAs, especially those acting as tumor suppressors or oncogenes, are related to cancer development, progression, and patients' response to therapy [16-18]. Therefore, miRNAs can be considered as useful prognostic biomarkers for various human cancers.

One such example is of miR-17 that is aberrantly expressed in cancer patients [19-21]. The miR-17 family, which includes six members, is one of the most extensively studied miRNA clusters [22]. These miRNAs are located within an 800 base-pair region of human chromosome 13, play an essential role in the development of the heart, lung, and human immune system [23]. Recent studies have found that miR-17 may play a critical role in the development of human cancers. [24 25] Increased expression of miR-17 promotes the metastasis of lung and pancreatic cancers, suggesting its role as an oncogene[26 27]. However, other studies have reported that miR-17 inhibits tumor cell invasion and metastasis in breast cancer[28]. In all, the role of miR-17 in cancer development as well as the exact mechanism are not yet clearly described. According to the miRBase (http://www.mirbase.org), miR-17 includes two members, miR-17-5p and miR-17-3p which are located in the sequence of miR-17 with a stem-loop structure. As a result, the detection of miR-17-5p, miR-17-3p has the same effect as detecting miR-17 [29-33].

Several published results indicate that the higher expression of the miR-17 is indicative of poor prognosis in cancer patients [26 27 34-43]. However, several confounding factors, including race, detection method, and tumor site, may affect the observations making the relationship between aberrant expression of miR-17 and the clinical outcome of cancer patients inconsistent. We, therefore, conducted a meta-analysis of available studies to evaluate the clinical utility of miR-17 as a novel cancer prognostic indicator.

Material and Methods

Data Source and Search Strategy

The following online electronic databases were used for the literature search: PubMed, Web of Science, and Embase. The search period was up to May 15th, 2017. Key search words used were: (1) prognosis OR prognostic OR survival OR outcome OR mortality; (2) cancer OR tumor OR tumour OR carcinoma OR neoplasm; (3) miR-17 OR microRNA-17 OR hsa-mir-17. Details are listed in the Supplementary Table 1. Additionally, we also searched the references and relevant published articles via Google Scholar.

Inclusion and Exclusion Criteria

The inclusion criteria of the articles were: (1) the cancers were diagnosed by the histological examination or any other accepted standard; (2) miR-17 was studied in human cancers; (3) the expression of miR-17 and the clinical outcome of patients were included in the research; and (4) reports with survival outcome and the data analyzed hazard ratio (HR) with 95% confidence interval (95% CI) and HR with a *P*-value.

The exclusion criteria were: (1) duplicate publications; (2) articles focused on other genes; (3) case reports, reviews, letters, and animal trails; (4) unqualified or insufficient data; (5) HR, 95% CI and *P*-value were not provided or could not be calculated and (6) articles concentrated on the polymorphisms or methylation patterns of miRNAs.

Questions of suitability of articles to be included were examined and discussed by the authors after reviewing the abstract and full text manuscript. The final decision was made by the academic committee.

Data extraction and quality assessment

All included studies were decided by the two investigators (Huang and Yao) independently based on titles and abstracts. Full-text of the articles was required if the articles were potentially suitable for the meta-analysis. Furthermore, the literature search was performed again in the excluded articles to avoid missing any article potentially relevant for the study. The original authors of the articles were contacted if any supplementary data were needed. Any disagreement was resolved by the two authors (Huang and Yao). The extracted details of the articles were as follows: (1) publication information: the name of the authors, publication area, and publication year; (2) patient's characteristics: diseases, stage of the disease, RNA detection method, type of tissue sample

and follow-up years; (3) the measurement of miR-17 measurement and its cut-off value and (4) HR of miR-17 for overall survival (OS), disease-free survival (DFS) and recurrence-free survival (RFS), as well as their 95% CI and *P*-values. The HRs and their 95% CI were extracted from the original articles or via e-mails from the authors. If not, we calculated HR and 95% CI using the data of observed deaths, cancer recurrences, or the original data provided by the authors. All calculations mentioned above were based on the methods provided by Parmar, M. K. et al. [44]. The quality of the included articles was assessed based on a systematic review checklist of the Dutch Cochrane Centre proposed by MOOSE [45].

Statistical Analysis

The test of heterogeneity of pooled HRs was carried out by using Cochran's *Q*-test and Higgins I^2 statistic. A *P*-value of < 0.05 or $I^2 > 50\%$ was considered as statistically significant. The 95%CI of I^2 was calculated by the method introduced by Hedges et al[46]. If heterogeneity existed, the random effects model was performed among the included studies; otherwise, the fixed effects model was selected. I^2 value ranged from 0% to 100%. All *P*-values were two-sided.

HR >1 presents of up-regulated expression of miR-17 indicated poor prognosis in patients, and HR<1 suggested a better prognosis. Publication bias was evaluated by the Begg's test and Egger's test [47 48]. If the publication bias did exist, the trim and fill method introduced by Duval and the Tweedit's was used to adjust the results[49]. The STATA software Version 14.0 (StataCorp LP, College Station, TX, USA) was used in all of the statistical analyses.

Study registration

The systematic review and meta-analysis is registered in PROSPERO (No. CRD42017065749).

Patients and Public Involvement Statement

The patients or public were not involved in the study.

Results

Literature selection

We started with 405 articles associated with miR-17 and cancer prognosis was identified from online database searches. After removing the replicate records, 304 miR-17-related articles were left. The first screening based on the species, article type, and language eliminated 210 citations from the analysis. Subsequently, the remaining 104 studies were carefully assessed by reviewing the abstract and full text of each article. After that, 89 articles were excluded from the study because they were unrelated to miR-17 expression levels or because of the lack of survival statistics such as HRs, 95% CI, or *P*-value. Finally, 15 studies, which

investigated the potential relationship between miR-17 expression and prognosis of human cancers, remained for further detailed screening and data-extraction. Three of the studies that explained the relationship between miR-17 expression and the clinical outcome of cancer had to be removed because the authors did not provide the exact HR value, or the value cannot be calculated from the data. Thus, 12 articles (12 studies) [26 27 34-43] were included in this meta-analysis. (Figure 1)

Characteristics of selected studies

All 12 studies included in the meta-analysis were retrospective studies published between 2010 and 2016 [26 27 34-43]. Patient's OS was reported in all 12 studies, and three studies also examined the DFS or RFS. The type of the cancers included gastrointestinal cancers (colorectal cancer, gastric cancer), lung cancer, pancreatic cancer, hepatocellular cancer, osteosarcoma, glioma, T-cell lymphoblastic lymphoma and esophageal squamous cell carcinoma. Total of 1096 patients with various types of cancers were from People's Republic of China, Japan, Spain, and Brazil. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to assess the expression of miR-17 in 12 studies, and one study used the in-situ hybridization (ISH). All studies used tissue and serum samples as the source of the miR-17. The majority (10 of 12) of the HRs reported in the present analysis were included in the multivariate analysis. The remaining two HRs could be estimated by Kaplan-Meier analysis and relative risk (RR) values. Most of the studies have the follow-up research for at least 38 months. The clinical characteristics of the studies included in this article are summarized in Table 1(A summary table of the meta-analysis).

Study	Year	Country	Diseases	Case Number	Stage	Sample	Assay	Cut-off value	HR	Follow-up (months)	Type of miR-17 detection
Chen et al	2012	China	HCC	120	I-IV	Tissue	qRT-PCR	Median	RR	46	miR-17-5p
Qun et al	2013	China	Lung Cancer	221	I-IV	Tissue	qRT-PCR	Median	Given	50	miR-17
Li et al	2014	China	Osteosarcoma	117	I-III	Tissue	qRT-PCR	Median	Given	44	miR-17
Lu et al	2012	China	Glioma	108	I-IV	Tissue	qRT-PCR	Mean	RR	60	miR-17
Xi et al	2015	China	T-cell lymphoblastic lymphoma	57	III, IV	Tissue	qRT-PCR	Median	Given	Up to 13 years	miR-17
Yu et al	2012	China	Colon Cancer	48	I-IV	Tissue	qRT-PCR	Median	Given	5-66	miR-17
Manuel et al	2011	Spain	Gastrointestinal Cancer	38	I-IV	Tissue	qRT-PCR	Mean	Given	38	miR-17
Robaina et al	2016	Brazil	Burkitt lymphoma	41	I-IV	Tissue	ISH	Median	Given	69	miR-17
Xu et al	2014	China	Esophageal Squamous Cell Carcinoma	105	I-IV	Tissue	qRT-PCR	Mean	Given	52	miR-17
Jun et al	2010	Japan	Pancreatic Cancer	80	I-IV	Tissue	qRT-PCR	Median	Given	60	miR-17-5p
Wang et al	2011	China	Gastric Cancer	65	I-IV	Serum	qRT-PCR	Median	Given	36	miR-17-5p
Zheng et al	2013	China	HCC	96	I-IV	Serum	qRT-PCR	Median	Given	NG	miR-17-5p

Table 1(A summary table of the meta-analysis).

Association between miR-17 and OS

Due to low heterogeneity, fixed effects model was used to calculate and analyze the pooled HR value. High expression level of miR-17 was associated with the poor OS in patients with diverse cancers. The statistical power of *Q*-test is low when there are limited studies included in the meta-analysis. We, therefore, conducted random effect analysis on the OS (HR=1.45, 95%CI=1.29-1.63, *P*<0.001), which was not significantly different compared to the analysis of fixed effect model. Details of the meta-analysis are systematically summarized in the Figure 2.

Subgroup	Number of studies	Heterogeneity		pooled HR (95% CI)	*P*-value
		I^2 (95%CI)	*P*-value		
Total	12	38.2% (0%-68.7%)	0.086	1.42(1.30-1.55)	<0.001
Ethnic subtotal					
Caucasian	2	71.6% (0%-93.6%)	0.06	1.48(1.21-1.81)	<0.001
Asian	10	36.1% (0%-69.5%)	0.12	1.40(1.27-1.55)	<0.001
Disease subtotal					
Digestive system	7	34.8% (0%-72.4%)	0.163	1.36(1.22-1.51)	<0.001
Respiratory system	1	NA	NA	1.28(1.02-1.61)	0.036
Blood system	2	0	0.713	2.38(1.56-3.63)	<0.001
Glioma	1	NA	NA	1.61(1.19-2.18)	0.002
Osteosarcoma	1	NA	NA	1.61(1.19-2.18)	<0.001
Detected method subtotal					
qRT-PCR	11	29.0% (0%-65.0%)	0.169	1.40(1.28-1.53)	<0.001
ISH	1	NA	NA	2.59(1.39-4.81)	0.003
Detected Sample subtotal					
Tissue	10	46.2% (0%-74.1%)	0.053	1.45(1.31-1.61)	<0.001
Serum	2	0	0.662	1.32(1.10-1.57)	0.002
Detection of miR-17 subtotal					
miR-17	8	60.1% (13.2%-81.7%)	0.057	1.29(1.11-1.49)	<0.001
miR-17-5p	4	7.5% (0%-43.4%)	0.372	1.50(1.34-1.67)	0.001

Table 2(Subgroup analysis).

To demonstrate the predictive role of miR-17, subgroups analysis was conducted based on patients' ethnicity, cancer type, methods identifying miRNAs and type of tissue samples. Clinical association between miR-17 and OS was found in the Asian and Caucasian patients(Figure 3A). The association was also significant in other subgroups, including digestive system cancers and blood cancers(Figure 3B), qRT-PCR detection method(Figure 3C), and tissue and serum samples(Figure 3D). miR-17 includes two members, miR-17-5p and miR-17-3p which are located in the sequence of miR-17 with a stem-loop structure. Therefore, analysis of miR-17-5p or miR-17-3p afforded the same effect (or result) as miR-17. To clarify the heterogeneity, we conducted a subgroup analysis concerning the detection method of miR-17 and found that the clinical value was also significant in miR-17 group and

miR-17-5p group. There was no significant difference between the two groups (Figure 3E), implying that same effect existed when detecting miR-17 and miR-17-5p. Details of the subgroup analysis are listed in the Table 2(Subgroup analysis).

Correlation between miR-17 and DFS and RFS

A total of 3 studies [37 38 41] were included in the analysis of DFS and RFS. The analyses revealed a predictive role of increased expression of miR-17 for the prognosis of cancer patients (pooled HR= 1.40, 95% CI=1.23-1.60, P<0.001) as determined by the fix-effect model (I^2=15.8%, P =0.305) (Figure 4).

Publication bias

We used Begg's funnel plot and Egger's test to assess the possible publication bias of the included studies [47 48]. In the analysis of relationship between miR-17 and the OS, the P-values of Egger's test and Begg's test were 0.014 and 0.011, respectively. The funnel plot and Egger's plot are displayed in Figure 5A and Figure 5B. Both Begg's test and Egger's test implied a publication bias, thus the trim and fill method was performed to make pooled HR more reliable [49]. The altered HR was 1.34, 95% CI=1.24-1.46, P<0.001, which was not significantly different from the pooled HR (Supplementary Figure 1).

Discussion

Previous studies have shown that miRNAs have a distinct expression profile in cancerous tissues which can be detected by qRT-PCR in frozen, formalin-fixed, and paraffin-embedded tissues and in serum samples. Recently, miRNAs, serving as tumor suppressors or oncogenes, have been shown to play important roles in the evolution and progression of cancers. miRNAs are involved in a variety of crucial cellular pathways such as angiogenesis, innate and adaptive immune responses, cellular proliferation, invasion, and metastasis.[12 16] Several studies have reported the potential use of miRNAs as tumor biomarkers for detecting tumor occurrence, development, and prognosis. Unfortunately, effective diagnosis techniques and prognosis indicators of cancer have not been found. Developing a novel less-invasive detection method with higher accuracy for cancer prognosis is of great significance in evaluating cancer progression as well as monitoring patients' therapeutic response.

Over the last couple of decades, numerous studies have uncovered the involvement of miRNAs in the pathogenesis of cancer. Since miRNAs can be obtained noninvasively from the serum, urine, and fecal samples, their utility as diagnostic and prognostic biomarkers in cancer and other diseases has been extensively explored. It has been reported that miRNA could be detected with higher accuracy than traditional cancer biomarkers in predicting the clinical outcome of the human colon cancers [50]. However, adequate evidence is still lacking for the utility of miRNAs as cancer biomarkers in clinical practice.

miR-17, a widely-studied miRNA, is aberrantly expressed in different kinds of cancers, such as glioma [51], esophageal and oral squamous cell carcinomas [36 52], pancreatic cancer [26], gastrointestinal cancers [39], osteosarcoma [53] and Burkitt lymphoma [38], and is significantly related to the clinical outcome of cancers. Our meta-analysis indicated that the elevated miR-17 expression is significantly associated with poor OS (HR=1.42) in patients with various types of carcinomas. The analysis using the Cochran's *Q*-test and Higgins I^2 test implied low heterogeneity. As limited number of studies were included in the meta-analysis, the *Q*-test had inadequate statistical power. We, therefore, applied the fixed effects model to calculate and analyze the pooled HR value. We also conducted random effect analysis on the OS, which was not significantly different when compared to analysis of fixed effect model (Figure 2). In the subgroup analysis, we found that the potential heterogeneity may have originated from the Caucasian group in the study conducted by Robaina et al.[38]. Unlike the commonly used RT-PCR, ISH technique was used to detect miR-17. Other factors contributing to the heterogeneity may include the limited number of patients (n=41) recruited in the study. However, both studies from Spain and Brazil recruited population of Caucasians decreasing the heterogeneity.

As the Begg's test and the Egger's test implied publication bias, we used the Trim and Fill method to obtain a more reliable pooled HR. We found that the adjusted HR was not significantly different from the pooled HR. In subgroup analysis, based on the characteristics of the individual studies, significant HR was found in the Caucasian and Asian groups, the qRT-PCR group and the tissue and serum sample groups. Furthermore, the increased expression of miR-17 indicated poor DFS and RFS in HCC and gastrointestinal cancers. Several investigators have explored the functional roles of miR-17 and its involvement in human cancers. Yang et al. found that the miRNA-17 was overexpressed in the HCC tissue, and promoted the phosphorylation of heat shock protein 27 (HSP27). The phosphorylated HSP27 then enhanced the migration of the HCC cells implying a significant role of miRNA-17 in the progression of HCC [54]. Wang et al. reported that the up-regulated expression of miRNA-17-5p promoted cancer cells proliferation and inhibited apoptosis by post-transcriptional modulation of mRNA p21 and tumor protein p53-induced nuclear protein 1 (TP53INP1) [55]. In the study by Ma et al. overexpression of miRNA-17 promoted cancer cells progression by targeting P130 [56]. Yan et al. found over-expression of the miR-17-5p in pancreatic cancer. The miR-17-5p inhibitor promoted the expression of Bim protein by targeting the 3'-untranslated regions of its mRNA and negatively regulating at the posttranscriptional level. Therefore, the authors suggested that the miR-17-5p inhibitor may be a novel therapeutic approach for pancreatic cancer[57]. Together with our meta-analysis, these findings suggest that the detection of tissue or serum miR-17 expression may be a useful prognostic biomarker in patients with HCC, pancreatic cancer, and gastrointestinal cancers.

There are potential limitations of this study. The literature searches using authentic and widely used data bases found studies performed predominantly on Asian populations not encompassing sufficient numbers of other populations such as Caucasians. Our results of miR-17 as a potential biomarker may, therefore, not be applicable to other populations. The pooled HR values were also not sufficiently strong. Furthermore, the

relatively limited sample size of 1031 patients weakened the statistical significance of the prognostic potential of miR-17 expression levels.

Conclusions

In summary, our meta-analysis suggested that miR-17 is a potential biomarker in various types of cancers. However, further multi-center clinical trials with larger sample size and prospective studies including Caucasians and patients representing other ethnicities are needed to confirm the prognostic value of miR-17 and its subsequent application as a prognostic biomarker in the routine clinical guidance of cancers.

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Contributors

Chengzhi Huang and Mengya Yu conceived the study. Chengzhi Huang and Xueqing Yao performed the data extraction and analysed the data. Chengzhi Huang and Mengya Yu wrote the paper. All authors had full access to all of the data and approved the final version of manuscript.

Disclosure of conflicts

The authors report no conflicts of interest in this work.

Data Sharing Statement

No additional data are available

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Ethics approval

The study does not include human participants or animals.

Patients and Public Involvement Statement

The patients or public were not involved in the study.

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Figure and table legends

Figure 1 Flow diagram of the studies selection phase

Figure 2 Forest plot of meta-analysis of overall survival in association with miR-17 expression.

Figure 3 Forest plots of subgroup meta-analysis of OS in association with miR-17 expression.
(A) Forest plots of the merged analyses of OS in different ethnic groups. Squares and lines correspond to the study-specific HRs and 95% CIs, respectively. The area of the squares represents the weight, and the diamonds represent the summary of HRs and 95% CIs.
(B) Forest plots of the merged analyses of OS in different diseases groups.
(C) Forest plots of the merged analyses of OS in different RNA detection methods groups.
(D) Forest plots of the merged analyses of OS in different sample groups.
(E) Forest plots of the merged analyses of OS in the detection method of miR-17.

Figure 4 Forest plot of disease-free survival(DFS) and recurrence-free survival (RFS) in association with miR-17 expression.

Figure 5

(A) Funnel plot of merged analysis of OS comparing high or low expression of miR-17.

(B) Egger's plot of merged analysis of OS comparing high or low expression of miR-17.

Table 1. A summary table of the meta-analysis.
Abbreviations: miR-17, microRNA-17; HCC, hepatocellular carcinoma; ISH, in situ hybridization; qRT-PCR, quantitative real-time polymerase chain reaction; RR, risk ratio; OS, overall survival; DFS, disease-free survival; NG, not given.

Table 2 Subgroup analysis.
Abbreviations: miR-17, microRNA-17; miR-17-5p, microRNA-17-5p; qRT-PCR, quantitative real-time polymerase chain reaction; ISH, in situ hybridization; NA, not available.

Supplementary Table 1 The search strategy of online databases

Supplementary Figure 1 Funnel plot of adjusted pooled HRs after the analysis of the Trim and Fill method.

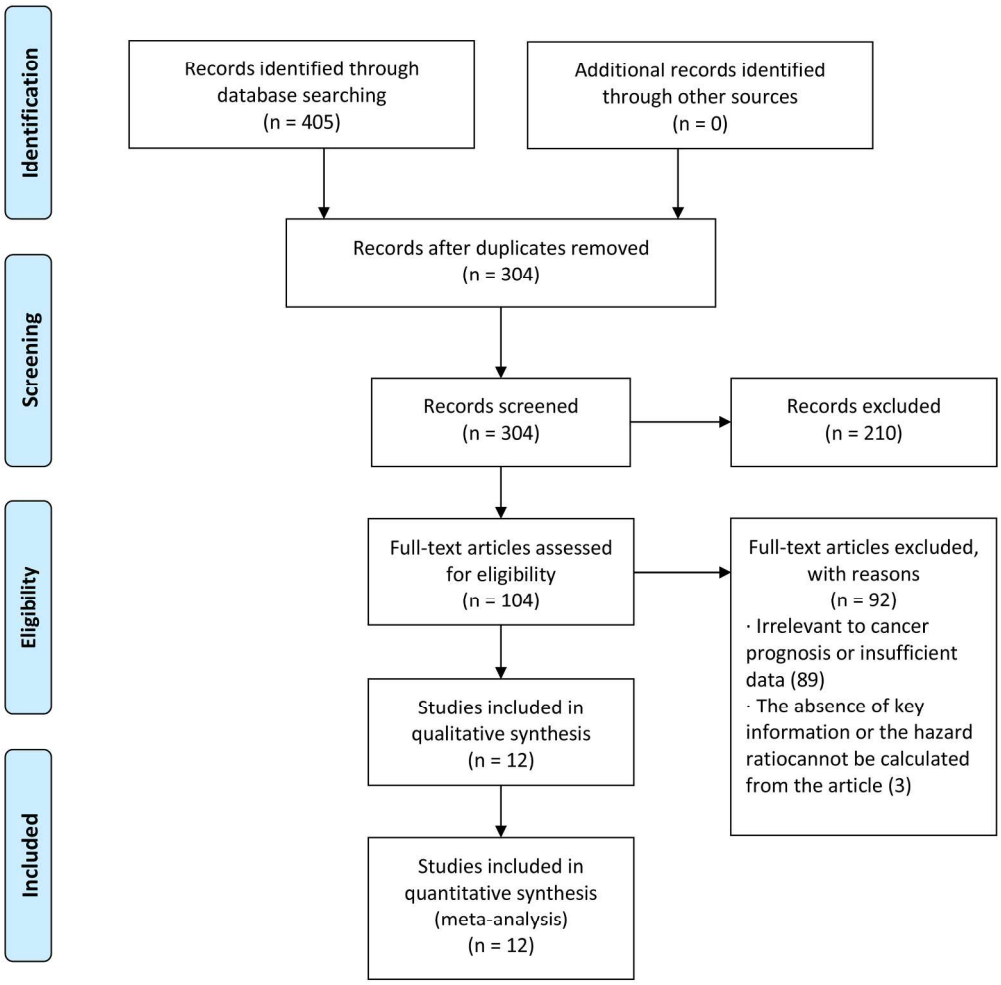

Figure 1 Flow diagram of the studies selection phase

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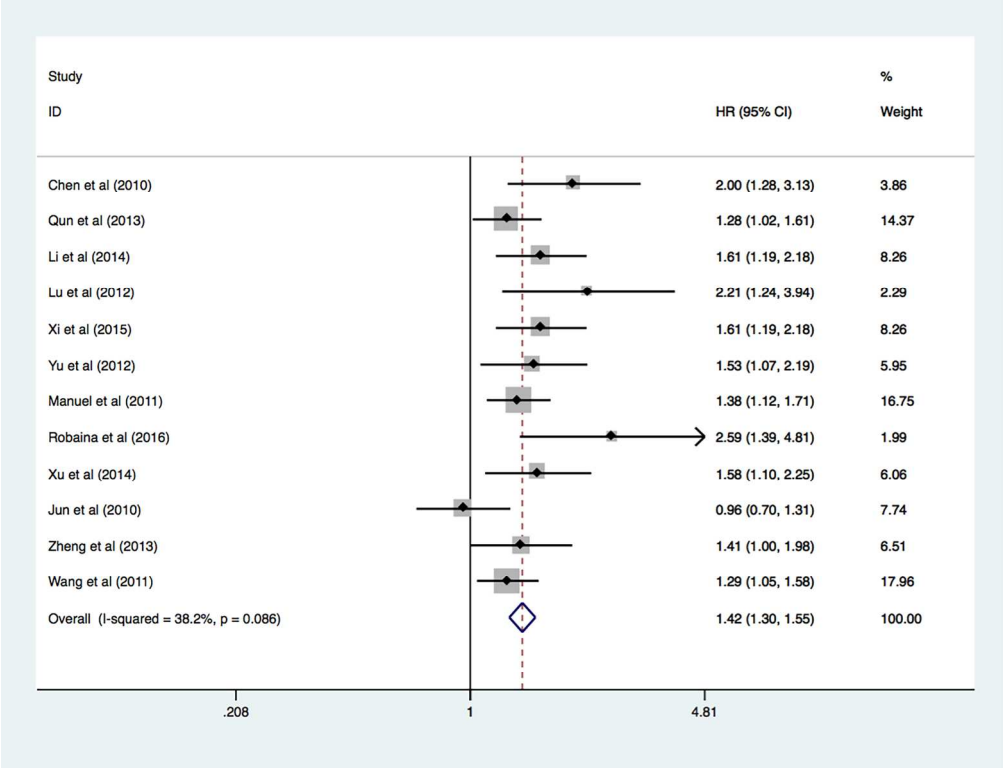


Figure 2 Forest plots of meta-analysis of overall survival in association with miR-17 expression.

210x161mm (300 x 300 DPI)

Figure 3 Forest plots of subgroup meta-analysis of OS in association with miR-17 expression.
(A) Forest plots of the merged analyses of OS in different ethnic groups. Squares and lines correspond to the study-specific HRs and 95% CIs, respectively. The area of the squares represents the weight, and the diamonds represent the summary of HRs and 95% CIs.
(B) Forest plots of the merged analyses of OS in different diseases groups.
(C) Forest plots of the merged analyses of OS in different RNA detection methods groups.
(D) Forest plots of the merged analyses of OS in different sample groups.
(E) Forest plots of the merged analyses of OS in the detection method of miR-17.

233x299mm (300 x 300 DPI)

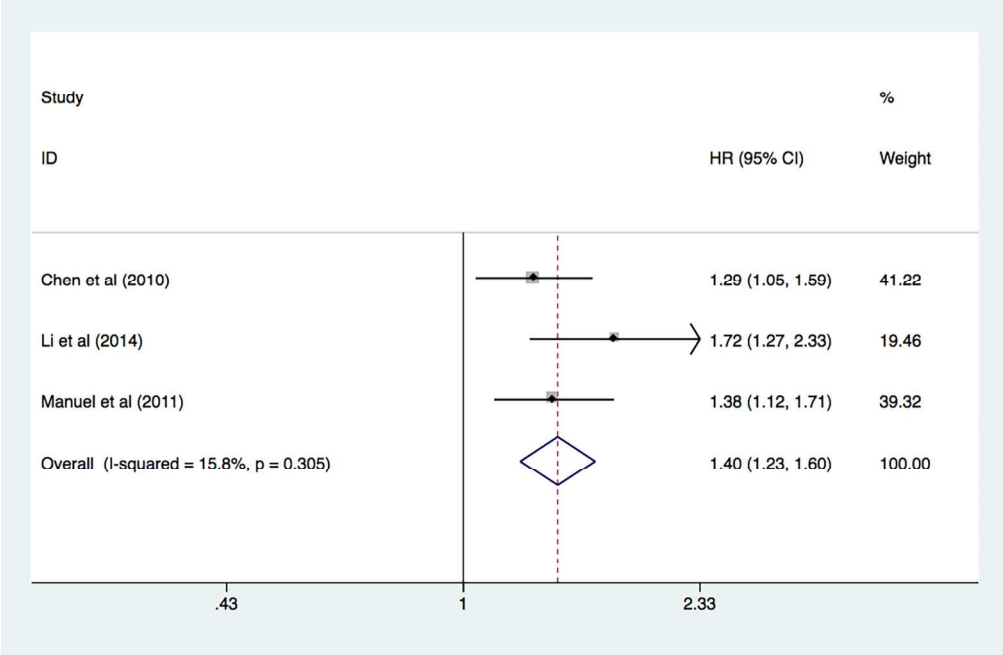


Figure 4 Forest plot of disease-free survival(DFS) and recurrence-free survival (RFS) in association with miR-17 expression.

190x124mm (300 x 300 DPI)

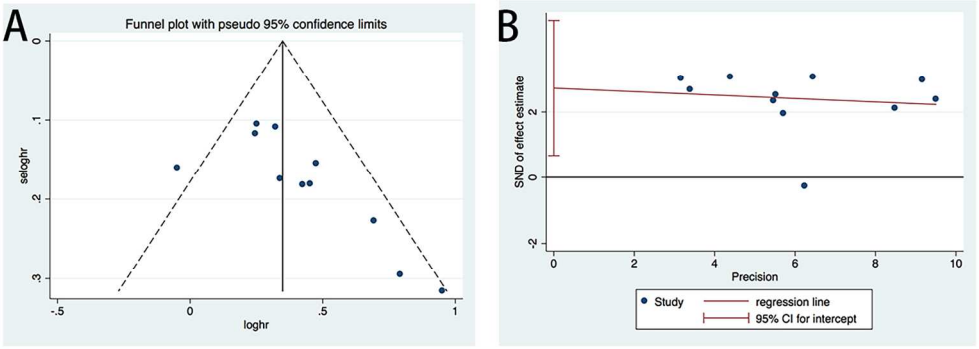
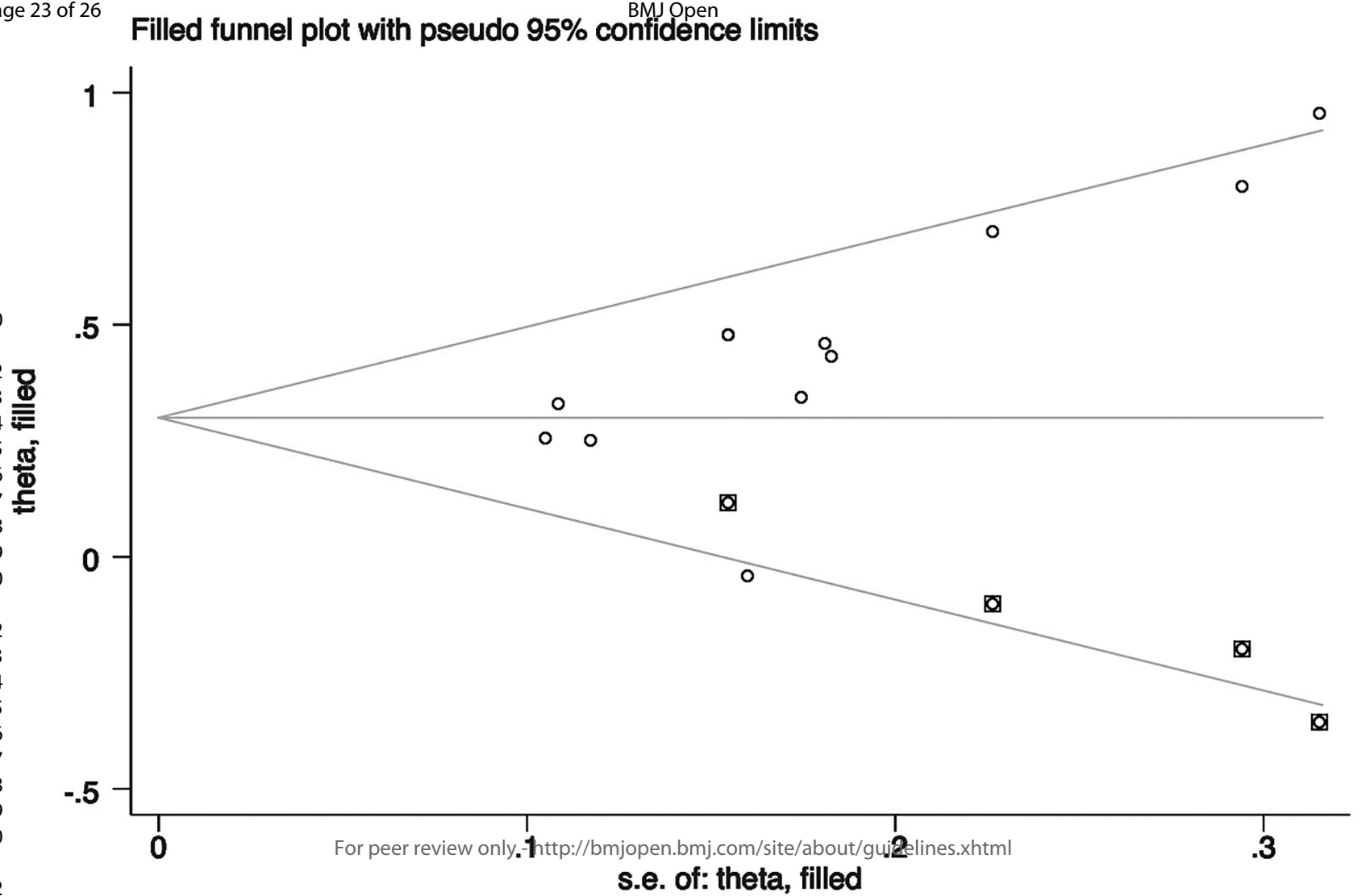


Figure 5
(A) Funnel plot of merged analysis of OS comparing high or low expression of miR-17.
(B) Egger's plot of merged analysis of OS comparing high or low expression of miR-17.

209x81mm (300 x 300 DPI)



Supplementary Table 1 The search strategy of online databases

Databases	Search method
Pubmed	("MIRN17 microRNA, human" [Supplementary Concept] AND "Neoplasms"[Mesh] AND "Prognosis"[Mesh]
Web of Science	
1	TS=(cancer OR neoplas* OR carcinom* OR tumo*)
2	TS=(prognosis OR prognostic OR survival OR outcome OR mortality)
3	TS=(miR-17 OR microRNA-17 OR hsa-mir-17)
4	#1 AND #2 AND #3
Embase	
1	miR-17 OR microRNA-17 OR hsa-mir-17
2	cancer OR neoplas* OR carcinom* OR tumo*
3	prognosis OR prognostic OR survival OR outcome OR mortality
4	#1 AND #2 AND #3 AND ([embase]/lim AND 'human'/de)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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