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Original Article

Prognostic significance of MiR-34a in solid tumors: a systemic review and meta-analysis with 4030 patients

Fanghui Ren[1], Xin Zhang[1], Haiwei Liang[1], Dianzhong Luo[1], Minhua Rong[2], Yiwu Dang[1], Gang Chen[1]

[1]Department of Pathology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, P. R. China; [2]Department of Research, Affiliated Cancer Hospital, Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, P. R. China

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Abstract: Purpose: The aim of the present meta-analysis and systematic review was to explore the association between the expression of miR-34a and prognosis in solid tumor. Methods: PubMed, Google Scholar, Web of Science and NCBI databases were used to search studies to evaluate the effect of miR-34a expression on clinical outcomes, including overall survival (OS), recurrence-free survival (RFS), disease-free survival (DFS), progression-free survival (PFS) and event-free survival (EFS) in solid tumor. The pooled random effect models were performed to calculate pooled hazard ratio (HR), 95% confidence interval (CI) to assess the association. Results: Twenty-three eligible studies with 4030 patients were included in this meta-analysis. It was confirmed that increased expression of miR-34a was in relevant with better DFS/RFS/PFS/EFS, which was identified with both univariate and multivariate models (univariate model: HR = 0.62, 95% CI: 0.42-0.92, P = 0.019; multivariate model: HR = 0.55, 95% CI: 0.34-0.88, P = 0.013). Furthermore, in the analysis of relationship between miR-34a and DFS/RFS/PFS/EFS, the results remained similar when excluding the studies contributed to the heterogeneity (univariate analysis: HR = 0.57, 95% CI: 0.46-0.70, P < 0.001; multivariate analysis: HR = 0.57, 95% CI: 0.43-0.75, P < 0.001). With univariate analysis, it was also demonstrated that miR-34a overexpression might be positively associated with a favorable OS in solid tumor (HR = 0.73, 95% CI: 0.54-1.00, P = 0.005) with considering an obvious heterogeneity. Conclusion: Our current study supports the notion that miR-34a may be a potential biomarker to predict OS and RFS/PFS/DFS/EFS in solid tumor.

Keywords: miR-34a, prognosis, cancer, meta-analysis

Introduction

Cancer is a leading cause of mortality in the world and the occurrence of cancer is experiencing an increased frequency. About 8.2 million deaths occurred among the 14.1 million new cancer cases in the world based on GLOBOCAN estimates in 2012 [1]. It is well-known that the imbalance between oncogenes and tumor suppressor genes plays a critical role in the development of cancer [2]. Therefore, identification of these genes is of importance in understanding the tumorigenesis and progression of cancer. At a further step, it will contribute to exploring potential biomarkers and specific targets for prevention and treatment of cancer.

MicroRNAs are a class of short non-coding RNAS, which are comprised of about 18-25 nucleotides and modulate the gene expression at the post-transcriptional level and miRNA dysregulation [3]. Several studies have identified that miRNAs expression is related to the oncogenesis and progression of the solid tumors [3-6]. However, the relationship between miRNAs and prognosis of solid tumors remains unclear. Although there exist studies demonstrating that decreased expression of miRNAs could predict poor prognosis [6-8], other articles conclude the opposite results [9-11].

MiR-34a is a member of the miR-34 family, and it is located in chromosome 1p36 encoded by its own transcript [12]. The miR-34 gene promoter region contains p53-binding sites and CpG island, and the expression of miR-34a is decreased due to the inactivation of the p53-binding sites and hypermethylation of the CpG island [13]. MiR-34a has been found to be

decreased in a variety of cancers, including Ewing sarcoma (EWS), gallbladder cancer (GBC), glioma, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic ductal adenocarcinoma (PDAC) and prostate cancer, thus, miR-34a has been regarded as a tumor suppressor gene [14-20]. Furthermore, it was also shown that high level of miR-34a expression could promote the sensitivity of tumors to chemotherapy in bladder cancer and gastric carcinoma [21, 22]. Although it is well recognized that miR-34a is a tumor suppressor gene, some researches demonstrate that decreased expression of miR-34a is strongly positive with favorable outcome in gastric cancer, colorectal cancer, lung cancer, glioblastoma [18, 23-25]. In addition, a series of studies have concluded that in vitro, ectopic overexpression of miR-34a could induce cell cycle arrest, differentiation, apoptosis and reduce migration in different classes of cancers, such as prostate cancer, colorectal cancer, colon cancer, hepatocellular carcinoma [12]. However, other studies in vitro have revealed that increased expression of miR-34a could promote the progression of tumors including glioblastoma cancer and breast cnacer [25-27]. Therefore, the function and prognostic value of miR-34a in solid tumors is still controversial. Herein, we conducted the present meta-analysis and systemic review to evaluate the prognostic value of miR-34a in solid tumors with the aim of providing insights into the clinical implication.

Methods

Search strategy

A systematic literature search was conducted to identify articles regarding miR-34a and prognosis of solid tumors. We searched PubMed, Google scholar, Web of Science and NCBI, with the combination of terms in Text Word "miR-34a OR microRNA-34a OR hsa-mir-34a" AND "neoplasm OR neplasia OR tumor OR tumour OR cancer OR sarcoma OR carcinoma OR cancer OR malignan* " AND "prognos* OR surviv* OR mortality OR outcome OR follow-up" (up to 15th May, 2015). We initially reviewed all articles browsing the titles and abstracts to select the relevant studies, and full texts were further screened if they were qualified as relevant reports. We also searched the references within the relevant review papers to identify additional studies.

Study selection criteria and data extraction

The selection criteria were as follows: 1) the expression of miR-34a was measured in tumor tissues; 2) the method to evaluate miR-34a expression was either reverse transcription-polymerase chain reaction (RT-PCR) or in situ hybridization (ISH); 3) the articles explored the relationship between miR-34a expression and the prognosis of solid tumor, such as overall survival (OS), disease free survival (DFS), relapse free survival (RFS), event free survival (EFS) or progression free survival (PFS); 4) there were clear, sufficient and available data to estimate or extract the individual HR and 95% CI; 5) studies were included if they were associated with the analysis of survival in humans not animal experiments; 6) No language restrictions were applied; 7) the latest and/or most complete one was included, when the same research group reported relative articles with the same cohort repetitively.

The exclusion criteria for this study were as follows: 1) Reviews, letters to the editors, and articles published in a book; 2) Articles without OS or DFS or RFS or EFS or PFS; 3) Studies with the evaluation of prognosis in cancer patients, but without clear, sufficient and available data to estimate or extract the individual HR and 95% CI; 4) Articles with only animal experiments.

Two authors independently reviewed eligible articles and extracted information according to predefined criteria. The following information was collected from each study: first author, publication year, country, cancer type, number and ages of patients, follow-up time, cut-off values for miR-34a expression, assessment method of miR-34a expression, outcome endpoint, HR and 95% CI for miR-34a up-regulation (exposed group) versus miR-34a down-regulation (unexposed group). Both univariate and multivariate analyses were taken into consideration, although they were separately analyzed.

Statistical analysis

The summary HR and 95% CI calculated by random effects models was used to evaluate the relation between miR-34a expression and solid tumor prognosis. A two-tailed $P < 0.05$ was considered statistically significant. In those studies without detailed information but with K-M sur-

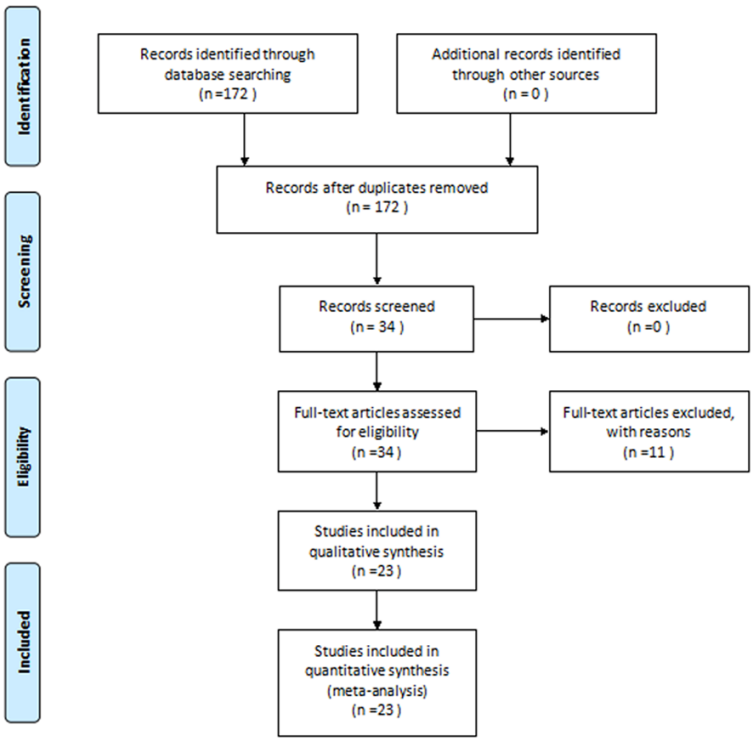

Figure 1. Flow diagram of the eligible studies selection process.

vival curve, HR and 95% CI were conducted according to the Parmar method by using Engage software. Heterogeneity between studies was assessed by the Chi-square test and I^2 measure inconsistency. It was considered as significant heterogeneity when a *P*-value < 0.05 by the Chi-square test, or an I^2 measure > 50%. We evaluated publication bias by estimating Begg's funnel plots analysis. Sensitivity analysis was carried out to investigate potential sources of heterogeneity by excluding one study at a time. Two-sided P < 0.05 was recognized as statistically significant. HR < 1 was considered a better prognostic factor for patients with miR-34a overexpression. All statistical analyses in this meta-analysis were conducted by Stata 12.0.

Results

The primary search identified 177 potentially relevant reports. Based on the initial screening of the title and abstract, 34 articles were retrieved for full-text screening. Eleven references were removed due to lack of available date to calculate HR and 95% CI, leaving 23 studies eligible for our meta-analysis. Eight of the 11 studies excluded at the first time extraction were included in the systemic review. The flow chart of the literature search was shown in **Figure 1**. Characteristics of eligible studies are summarized in **Tables 1** and **2**.

A total of 4030 patients from Spain, China, Austria, Italy, Germany, Denmark, Finland, Ireland, USA and Japan were identified to evaluate the relationship between miR-34a expression and tumor prognosis. All patients involved in this meta-analysis were diagnosed with various cancers, including EWS [14, 28], GBC [15], glioma [16], NSCLC [17, 29, 30], PDAC [19], prostate cancer [20], hepatocellular carcinoma (HCC) [31-33], colorectal cancer (CRC) [23, 34-36], breast cancer [27], gastric cancer [37], esophageal cancer [38], sinonasal squamous cell carcinoma [39], ovarian cancer [40], osteosarcoma [41] and bladder cancer [42]. The tissue samples available in this current study were obtained by the method of formalin-fixed paraffin-embedded (FFEP), snap frozen and GEO databases. The included 23 studies provided the information on prognosis of miR-34a overexpression in cancer patients (15 for OS, 6 for RFS and 2 for DFS/EFS). Twenty studies reported a reverse impact of miR-34a expression on tumor prognosis, while 2 studies reported opposite results and 1 study was controversial.

Quantitative analysis of relationship between miR-34a expression and OS

Sixteen cohort studies were included in the evaluation of the relationship between miR-34a expression and OS in cancer patients. The univariate analysis of 14 studies included in the 16 studies showed that increased expression of miR-34a was related to unfavorable OS in patients with solid cancers (HR: 0.73; 95% CI: 0.54-1.00, *P* = 0.050) (**Figure 2A**). However, there existed high heterogeneity between the

Table 1. main characteristics of the eligible studies in this meta-analysis

Cancer	Author	Year	Country	Tissue samples	Method	No.	High of miR-34a	Age (year)	Follow-up (median months)	Cut-off
NSCLC	Elena Gallardo	2009	Spain	FFPE	qRT-PCR	70	46 (65.7%)	64[a] (36-86)	38 (1-127)	Median expression of Normal tissue
NSCLC	Zhen Wang	2011	Chnia	FFPE	qRT-PCR	161	58 (36.0%)	62[a]	18 (1-40)	Median value ($2^{-\Delta\Delta Ct}$)
NSCLC	Johannes Voortman	2010	Austria	FFPE	qRT-PCR/ISH	636	318 (50%)	NR	96	Median
HCC	Benedek Gyongyosi	2014	Italy	FFPE	NR	20	NR	68[a] (52-82)	33.6	Median value 3.88
HCC	Xianping Gui	2015	China	FFPE	qRT-PCR	NR	52 (58.4%)	55.4 ± 12.9	31 (2-52)	Median value 0.87 (range 0.06-21.54)
HCC	Fan Yang	2014	China	snap frozen	qRT-PCR	30	15 (50%)	50[b]	NR	Median expression
Colorectal Cancer	Jing Gao	2014	China	snap frozen	qRT-PCR	268	135	55[b]	36	Median $2^{-\Delta\Delta Ct}$
Colon Cancer	Helge Siemens	2012	Germany	FFPE	qPCR	93	42 (45.2%)	NR	NR	Mean value ($2^{-\Delta\Delta Ct}$) Mean = -19.105
Colotectal cancer	Jakob V. Schou	2014	Danish	FFPE	qRT-PCR	138	NR	63[a] (36-87)	NR	NR
Colorectal cancer	Shan Li	2013	China	snap frozen	RT-PCR	80	60 (75%)	recurrence 61[a] (28-87) no recurrence 57.5[a] (36-79)	45 (12.5-77.5)	$2^{-\Delta\Delta CT}$
GBC	Ke Jin	2013	China	snap frozen	RT-PCR	77	21 (27.3%)	52.8 ± 9.7[a] (32-79)	24	$2^{-\Delta\Delta CT}$
EWS	Maria Teresa Marino	2014	Italy	snap frozen	qRT-PCR	109	55 (50.5%)	14[b]	EFS 57 (6-241) OS 67 (9-241)	Median value of $2^{-\Delta\Delta CT}$ Median = -2.740
EWS	Fumihiko Nakatani	2012	Italy	snap frozen	qRT-PCR	83	EFS 24 (49.0%) OS 17 (50%)	14[b]	88 (26-217)	$^{-\Delta\Delta CT}$
Glioma	HaiFei Gao	2013	China	snap frozen	qRT-PCR	146	77 (52.7%)	65[b]	23 (3-72)	Median $2^{-\Delta Ct}$
Breast cancer	Hanna Peurala	2011	Filand	GEO ID GSE24450	ISH	1172	NR	50[b]	NR	NR
Gastric cancer	Miaoxia He	2013	China	FFPE	qRT-PC	122	MALT-BCL16 (25%) DLBCL 5 (8.6%)	60[b]	63 (3-123)	NR
PDAC	Nigel B. Jamieson	2011	UK	snap frozen	RT-PCR	48	24 (50%)	65[b]	23.9	Gene median expression
Prostate cancer	Claire Corcoran	2014	Ireland	GEO ID GSE21036	qPCR	113	40 (35.4%)	NR	NR	NR
Esophageal cancer	YuXin Hu	2011	USA	FFPE	RT-PCR	99	41 (41.4%)	NR	16.25 (0.37-256.43)	NR
Ovarian cancer	Daniel Reimer	2011	Austria	FFPE	RT-PCR	130	NR	NR	DFS: 23.5 (10.0-91.0) OS: 45.0 (20.8-108.3)	$0.44^{\Delta Ct}$
Osteosarcoma	Yuan Wang	2014	China	snap frozen	RT-PCR	80	27 (33.8%)	56[a] (12-83)	33 (1-72)	$2^{-\Delta\Delta Ct}$
Bladder cancer	Angeline S. Andrew	2014	Lebanon	FFPE	ISH	229	63 (27.5%)	30-79	NR	Low (0-1+) High (2-3+)
Gastric cancer	Soshi Osawa	2011	Japan	FFPE	qRT-PCR	37	8 (21.6%)	65[b]	NR	Gene median expression

EWS: Ewing sarcoma; PDAC: Pancreatic Ductal Adenocarcinoma; FFPE: Formalin Fixed Paraffin Embedded; qRT-PCR: quantitative real-time polymerase chain reaction; ISH: In Situ Hybridization; RT-PCR: reverse transcription-polymerase chain reaction; qPCR: quantitative polymerase chain reaction; NR: not reported; a: median age; b: cut-off age.

Table 2. The association between miR-34a expression and prognosis of solid tumors in this meta-analysis

Cancer	Author	Country	Expression of miR-34a	Method	HR (95% CI)
NSCLC	Elena Gallardo	Spain	downregulate	K-M	RFS (U/M) 0.39 (0.043-3.55)/0.981 (0.549-1.754)
NSCLC	Zhen Wang	Chnia	downregulate	reported	OS (U/M) 0.783 (0.405-1.511)/1.538 (0.778-3.04) RFS (U) 0.864 (0.493 1.517)
NSCLC	Johannes Voortman	Italy	downregulate	reported	OS (U) 0.9 (0.72-1.14)
HCC	Benedek Gyongyosi	Italy	downregulate	reported	OS (U) 0.543 (0.181-1.639) PFS (U) 0.763 (0.275-2.128)
HCC	Xianping Gui	China	downregulate	reported	RFS (U) 1.52 (1.19-1.83)
HCC	Fan Yang	China	downregulate	K-M	OS (U) 0.6 (0.04-8.95)
Colorectal cancer	Jing Gao	China	downregulate	reported	RFS (U/M) 0.31 (0.18-0.53)/1.44 (1.13-1.72)
Colon Cancer	Helge Siemens	Germany	downregulate	reported	PFS (U) 0.5 (0.188-1.322)
Colorectal cancer	Jakob V. Schou	Danish	upregulate	reported	OS (U/M) 1.787 (1.349-2.369)/1.191 (0.843-1.683)
Colorectal cancer	Shan Li	China	downregulate	K-M	RFS (U/M) 0.47 (0.21-1.06)/0.262 (0.167 0.41)
Gallbladder cancer	Ke Jin	China	downregulate	K-M	OS (U) 0.33 (0.16-0.66)
EWS	Maria Teresa Marino	Italy	downregulate	K-M/reported	OS (U/M) 0.34 (0.15-0.77) 0.372 (0.159-0.873) EFS (U/M) 0.54 (0.24-1.2)/0.406 (0.244 1.033)
EWS	Fumihiko Nakatani	Italy	downregulate	K-M/reported	OS (U) 0.22 (0.053-0.927) EFS (U/M) 0.33 (0.12-0.93) 0.182 (0.033-0.526)
Glioma	HaiFei Gao	China	downregulate	reported	OS (U/M) 0.539 (0.336-0.856)/0.47 (0.273-0.809) PFS (U/M) 0.59 (0.37-0.942)/0.5 (0.295-0.845)
Gastric cancer	Miaoxia He	China	upregulate	reported	OS (M) 2.287 (1.108-4.754)
Breast cancer	Hanna Peurala	Filand	multivariate: downregulate univariate: upregulate	reported	OS (U/M) 1.09 (0.91-1.31)/0.63 (0.41-0.96)
PDAC	Nigel B. Jamieson	UK	downregulate	reported	OS (U/M) 0.31 (0.11-0.81)/0.15 (0.06-0.37)
Prostate Cancer	Claire Corcoran	Ireland	downregulate	K-M	OS (U) 0.54 (0.02-17.81)
Esophageal cancer	YuXin Hu	USA	downregulate	reported	OS (U/M) 0.81 (0.53-1.24)/0.71 (0.41-1.24) DFS (U/M) 0.74 (0.48-1.14)/0.72 (0.43-1.22)
Ovarian cancer	Daniel Reimer	Austria	downregulate	reported	OS (M) 0.808 (0.33-1.98) PFS (M) 0.744 (0.336-1.675)
Osteosarcoma	Yuan Wang	China	downregulate	reported	DFS (U/M) 0.67 (0.14-3.35)/0.291 (0.129-0.872)
Bladder cancer	Angeline S. Andrew	Lebanon	downregulate	reported	RFS (M) 0.57 (0.34-0.93)
Gastric cancer	Soshi Osawa	Japan	upregulate	K-M	OS (U) U 5.62 (1.12-28.08)

EWS: Ewing sarcoma; PDAC: Pancreatic Ductal Adenocarcinoma; K-M: Kaplan-Meier; U: univariate analysis; M: multivariate analysis.

studies (I^2 = 77.3%, $P_{heterogeneity}$ < 0.001). In a sensitivity analysis, the pooled HR ranged from 0.66 (95% CI: 0.50-0.90) to 0.80 (95% CI: 0.59-1.08) when excluding the 14 studies successively. However, the conclusions were not stable due to the existence of obvious heterogeneity. In addition, in the multivariate analysis of 9 studies included in the 15 studies, the summary HR was 0.73 (95% CI: 0.47-1.12, P = 0.15) and there existed heterogeneity (I^2 = 78.8%, $P_{heterogeneity}$ < 0.001, **Figure 2B**). The pooled HR changed from 0.64 (0.42-0.0.98) when excluding the study reported by He et al. [37] to 0.85 (0.58-1.24) when excluding the study of Jamieson et al. [19] in the sensitivity analysis and it was not statistically significant. Nevertheless, the heterogeneity was always present. Survival subgroup analyses were further performed to identify the sources of heterogeneity. As was shown in **Table 3**, the heterogeneity of survival analysis might due to the population of Italy, the HR (95% CI) reported in the text, the method of measuring expression of miR-34a and collecting tissues by FFPE.

Quantitative analysis of relationship between miR-34a expression and DFS/RFS/PFS

Thirteen studies were included in the analysis of the correlation between miR-34a expression and DFS/RFS/PFS in cancers. The univariate analysis of 12 studies demonstrated that high level of miR-34a expression was in association with better DFS/PFS/RFS (HR: 0.62, 95% CI: 0.42-0.92, *P* = 0.019) with existence of heterogeneity (I^2 = 78.7%, $P_{heterogeneity}$ < 0.001) (**Figure 3A**). The summary HR ranged from 0.57 (95% CI: 0.46-0.70, P < 0.001, I^2 = 2.7%, $P_{heterogeneity}$ = 0.417) to 0.68 (95% CI: 0.47-0.99, *P* = 0.047, I^2 = 71.3%, $P_{heterogeneity}$ < 0.001) after sequential

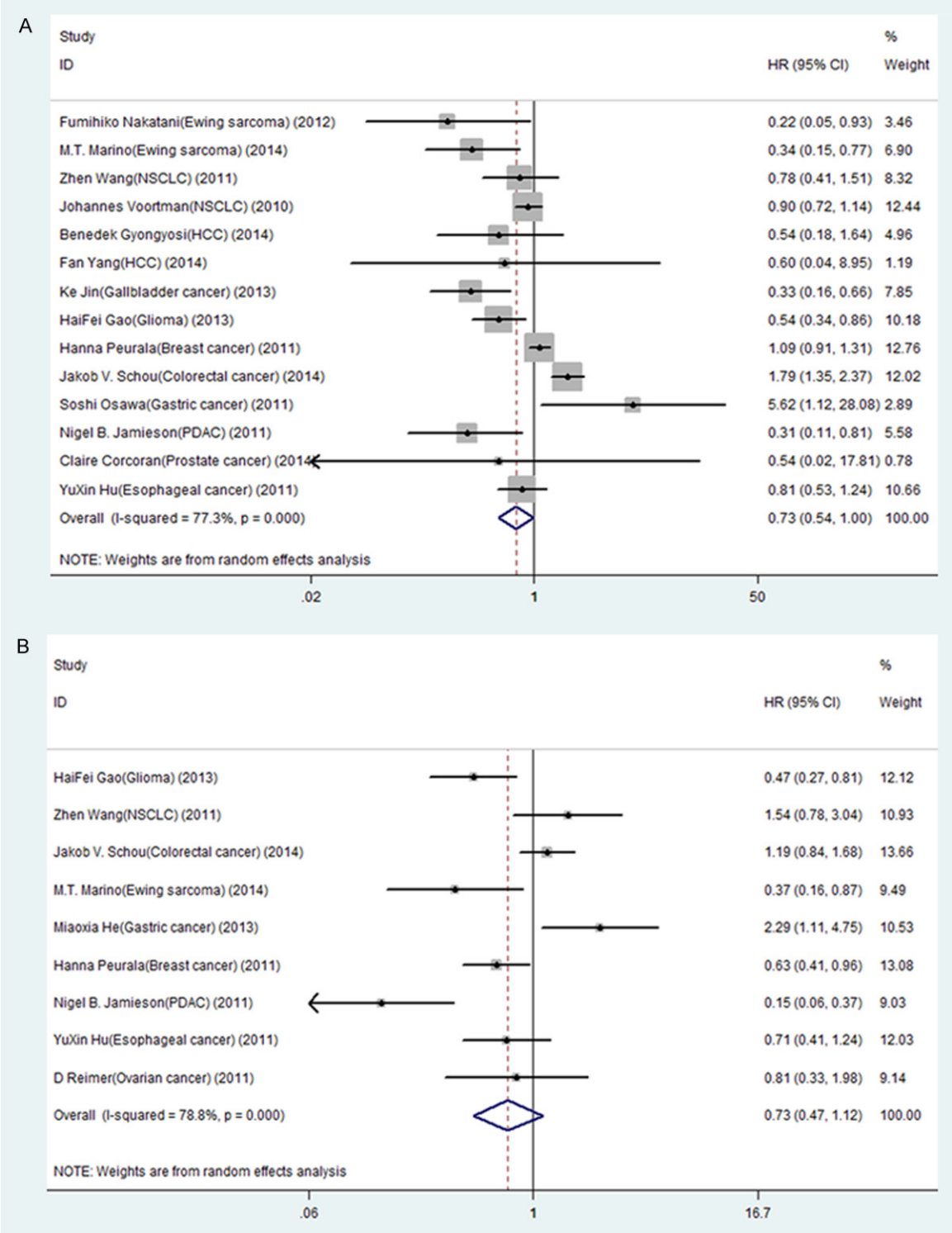

Figure 2. Forest Plot of the association between miR-34a overexpression and OS of solid tumor by univariate analysis (A) and multivariate analysis (B).

removal of individual studies in sensitivity analysis. The heterogeneity was gone after the study reported by Gui et al. [32] was excluded (I^2 = 2.7%, $P_{heterogeneity}$ = 0.417) and the correlation between miR-34a and DFS/RFS/PFS in solid tumors was statistically significant (HR:

Table 3. Summarized HRs of subgroup analyses for OS

Stratified analysis	Univariate analysis					Multivariate analysis				
	Study (N)	HR	P	Heterogeneity		Study (N)	HR	P	Heterogneity	
				I^2	P				I^2	P
Region										
China	4	0.53 (0.38-0.75)	0.000	2.6%	0.380	3	1.16 (0.43-3.10)	0.774	85.6%	0.001
Italy	4	0.51 (0.26-1.02)	0.057	66.0%	0.032					
Method (miR-34a)										
qRT-PCR	8	0.81 (0.50-1.33)	0.404	82.4%	0.000	5	0.95 (0.53-1.72)	0.872	80.1%	0.000
RT-PCR	3	0.47 (0.24-0.95)	0.039	68.3%	0.043	3	0.46 (0.17-1, 20)	0.133	78.5%	0.009
Tissue sources										
Snap frozen	6	0.41 (0.30-0.57)	0.000	0.0%	0.707	3	0.32 (0.17-0.61)	0.001	55.4%	0.606
FFPE	5	1.14 (0.74-1.78)	0.550	81.2%	0.000	5	1.18 (0.81-1.71)	0.387	47.8%	0.105
GEO	2	1.09 (0.91-1.31)	0.365	0.0%	0.686					
Tumor type										
Ewing sarcoma	2	0.31 (0.15-0.62)	0.001	0.0%	0.605					
NSCLC	2	0.89 (0.71-1.10)	0.276	0.0%	0.695					
HCC	2	0.55 (0.20-1.53)	0.252	0.0%	0.947					
Method (HR)										
K-M survival	7	0.45 (0.23-0.89)	0.022	49.2%	0.066					
Reported	7	0.93 (0.70-1.24)	0.632	77.6%	0.000					

0.57, 95% CI: 0.46-0.70, P < 0.001) (**Figure 3B**). The association between miR-34a overexpression and DFS/RFS/PFS was also clearly evident in multivariate analysis (HR: 0.55, 95% CI: 0.34-0.88, P = 0.013), which was in consistent with the results of the univariate analysis (**Figure 4A**). Although there existed heterogeneity (I^2 = 87.4, P < 0.001), in the sensitivity analysis, the pooled HR remained similar to the one calculated by the multivariate analysis with ranging from 0.49 (95% CI: 0.35-0.69, P < 0.001, I^2 = 59.6%, $P_{heterogeneity}$ = 0.011) to 0.62 (95% CI: 0.40-0.95, P = 0.029, I^2 = 81.3%, $P_{heterogeneity}$ < 0.001) when excluding every study successively. Besides, the sensitivity analysis showed that the source of the heterogeneity came from the study reported by Gao et al. [34] and Li et al. [36], and removal of the two studies changed HR to one in favor of miR-34a overexpression relevant with better prognosis of cancer patients (HR: 0.57, 95% CI: 0.43-0.75, P < 0.001, I^2 = 30.8%, $P_{heterogeneity}$= 0.182) (**Figure 4B**).

Univariate analysis data for RFS were available from 5 studies (2 for NSCLCs, 2 for CRCs and 1 for HCC), the pooled HR was 0.65 (95% CI: 0.30-1.42) with obvious heterogeneity (I^2 = 88.0%, P < 0.001) and no association was observed between expression of miR-34a and RFS in patients with solid tumors (P = 0.280). After removal of the study reported by Gui et al. [32] in the sensitivity analysis, the HR was statistically changed to 0.49 (95% CI: 0.28-0.89, P = 0.018) and no heterogeneity was observed (I^2 = 55.4%, $P_{heterogeneity}$ = 0.081). In the subgroup analysis of cancer type, the results showed that miR-34a overexpression had an inverse impact on RFS for patients with colorectal cancer (HR: 0.35, 95% CI: 0.23-0.55, P < 0.001, I^2 = 0.0%, $P_{heterogeneity}$ = 0.402). Multivariate analysis for RFS including 4 studies identified that cancer patients with miR-34a overexpression had no risk for RFS (0.68, 95% CI: 0.30-1.58, P =0.372, I^2 = 94.1%, $P_{heterogeneity}$ < 0.001) and sensitivity analysis with the individual study being excluded offered no significant change of the results (data not shown).

Both in the univariate and multivariate analyses, it was found that overexpression of miR-34a was correlated with a favorable PFS in cancer patients. The pooled HR for univariate and multivariate analysis were 0.62 (95% CI: 0.40-0.94, P = 0.026) and 0.56 (95% CI: 0.36- 0.88, P = 0.011), respectively. And no heterogeneity was observed in the univariate analysis (I^2 = 0%, $P_{heterogeneity}$ = 0.654) and multivariate analysis (I^2 = 0.0%. $P_{heterogeneity}$ = 0.417). The relationship between EFS and up-regulation of miR-34a in cancer patients was evaluated in the same two studies by both of univariate and multivariate analysis, and the HRs were 0.45 (95% CI: 0.24-0.84, P = 0.013) and 0.34 (95%

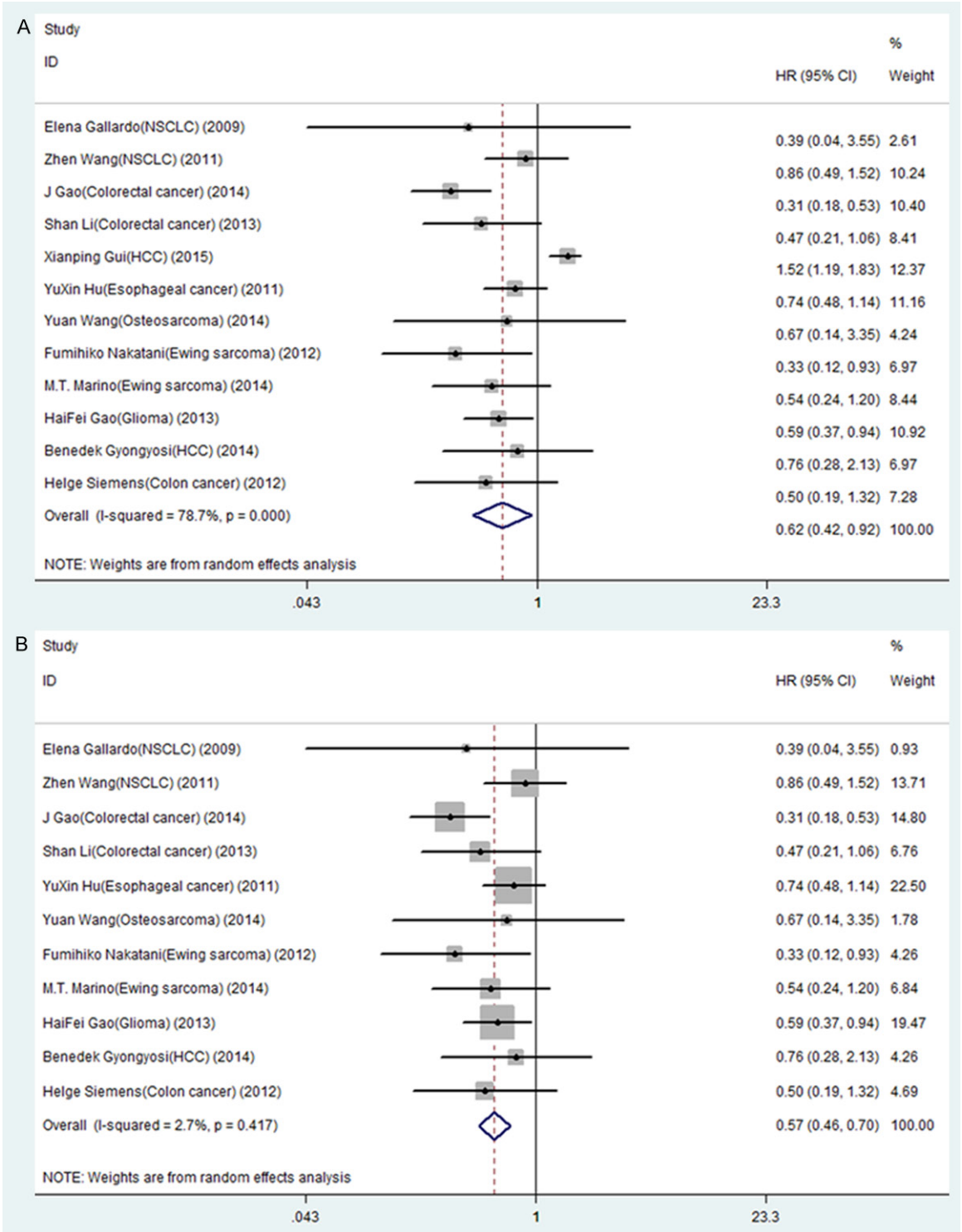

Figure 3. Forest Plot of the association between miR-34a overexpression and RFS/PFS/DFS/EFS of solid tumors by univariate analysis (A) and univariate analysis after sensitivity analysis (B).

CI: 0.18-0.65, P = 0.001), respectively. There existed no heterogeneity. The combined analysis of 2 datasets in relation to DFS showed that miR-34a up-regulation was not associated with better DFS, these associations were clearly identified both in univariate (HR: 0.74, 95% CI:

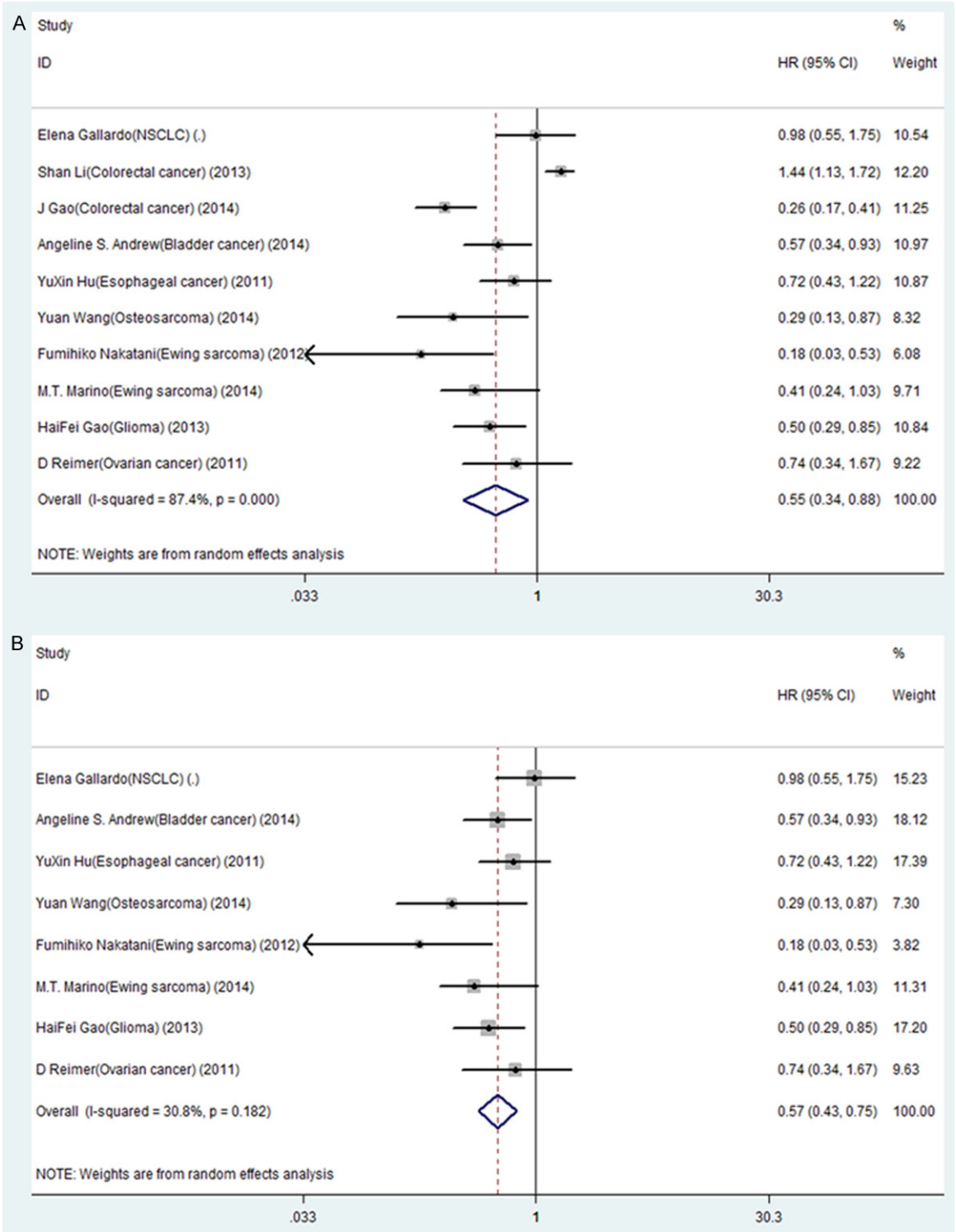

Figure 4. Forest Plot of the association between miR-34a overexpression and RFS/PFS/DFS/EFS of Solid tumors by multivariate analysis (A) and multivariate analysis after sensitivity analysis (B).

0.48-1.12, P = 0.148) and multivariate models (HR: 0.50, 95% CI: 0.21-1.19, P = 0.120). And heterogeneity was absent in the univariate and multivariate analysis ($P_{univariate}$ = 0.906, $P_{multivariate}$ = 0.103) of the relationship between miR-34a and DFS.

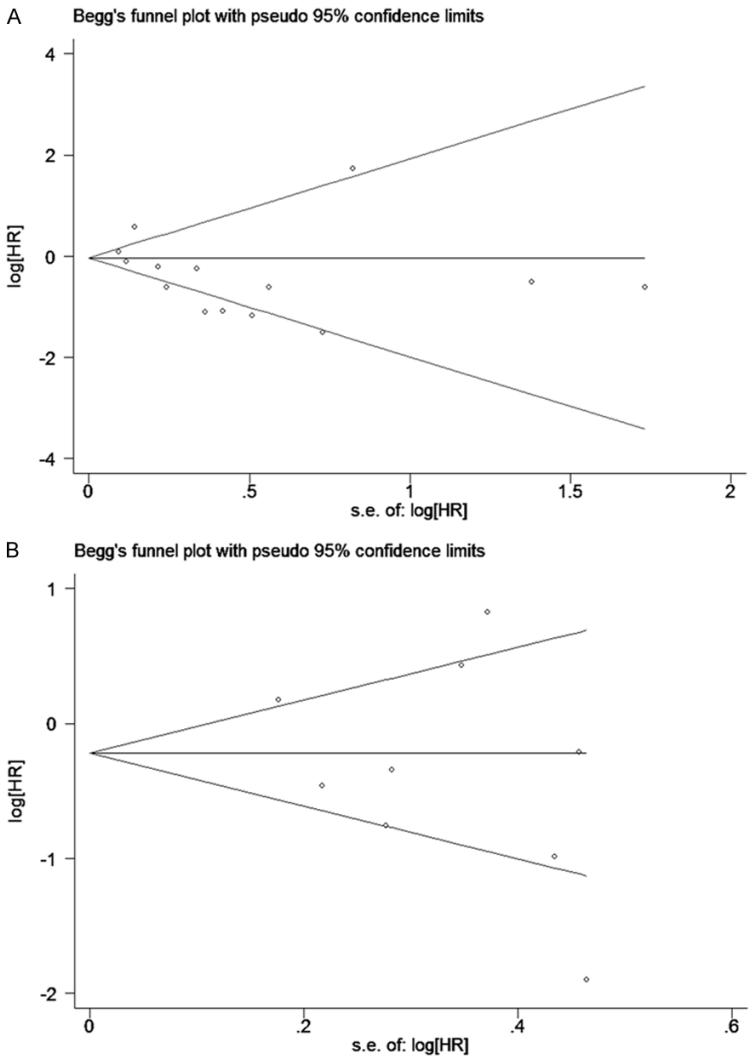

Figure 5. Begg's funnel plot for publication bias test on studies assessing miR-34a overexpression and OS of solid tumors by univariate analysis (A) and multivariate analysis (B).

Publication bias

There was no indication of publication bias for the analysis of OS in cancer patients with miR-34a expression with Begg's test in univariate (P = 0.956) and multivariate (P = 0.532) analysis, although the funnel plots revealed evidence of obvious asymmetry (**Figure 5**). The same results were shown in univariate (P = 0.891) and multivariate (P = 0.325) analysis of RFS/DFS/EFS/PFS in cancer patients with miR-34a overexpression (**Figure 6**).

Systemic review

Given to the insufficient information on calculating HR or 95% CI, eight studies were described in the systemic review. Three of the 8 studies analyzed the association between miR-34a expression and OS/DFS of gastric patients, while the results were controversial. Huang et al. [43] found that miR-34a was upregulated in gastric cancer tissues and predicted poor OS, which was opposite to the results of another two articles from Hu et al. [21, 22]. Mudduluru et al. [44] concluded that overexpression of miR-34a had a positive association with longer survival in patients with NSCLC, while Lee et al. [24] demonstrated that miR-34a expression was in negative relation to the PFS of SCLC patients. There was one study suggesting that decreased expression of miR-34a could be an independent prognostic biomarker in sinonasal squamous cell carcinoma patients treated with cis-diamminedichloroplatinum [39]. Another report showed that miR-34a played a role in promoting tumor aggressiveness in patients with glioblastoma (GBM), especially in proneural subtype [25]. However, it was also demonstrated that no significant association was observed between expression of miR-34a and OS/DFS in triple-negative breast cancer patients [45].

Discussion

To date, the role of miR-34a expression as a prognostic biomarker has been widely explored in a variety of solid tumors as shown in **Table 1**. Nevertheless, the prognostic value of miR-34a expression in solid tumors remains controversial. Therefore, we performed the current systemic review and meta-analysis to provide more evidence for sufficiently facilitating the functions of miR-34a on the progression of cancers.

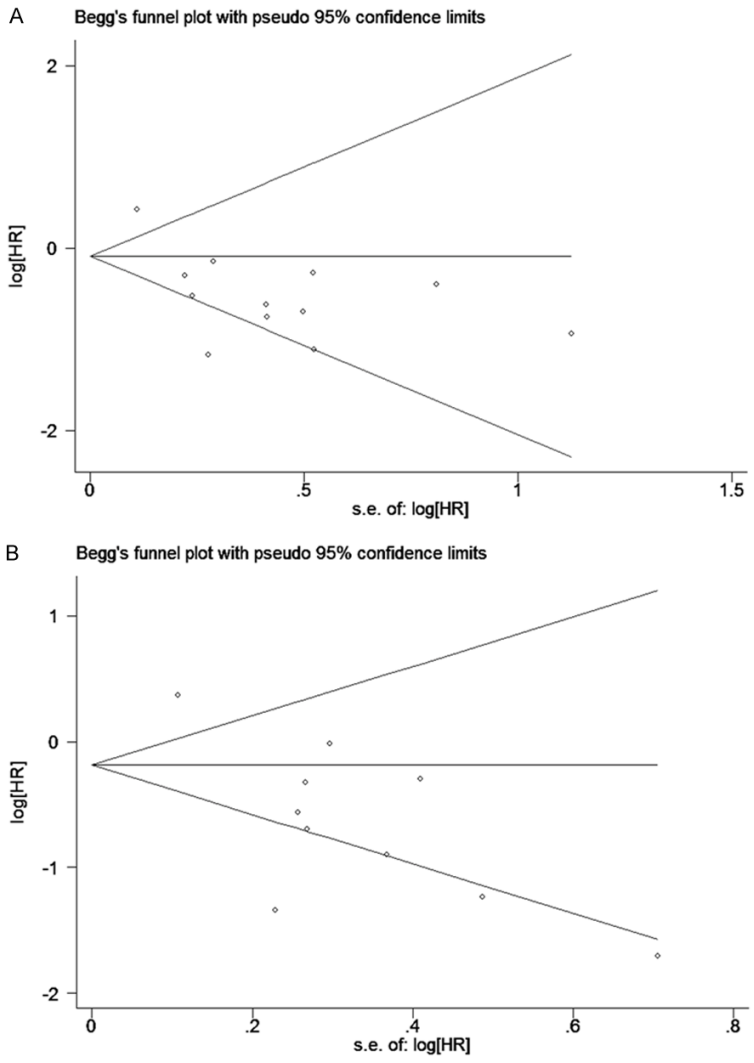

Figure 6. Begg's funnel plot for publication bias test on studies assessing miR-34a overexpression and RFS/PFS/DFS/EFS of solid tumors by univariate analysis (A) and multivariate analysis (B).

Twenty-three eligible studies were included in the present study for qualitative analysis. Our results showed evidence of correlation between miR-34a overexpression and prognosis of cancer patients. Previously, a systemic review published by Wang et al. [46] evaluated the expression level of miR-34 family members in a variety of tumors, and in this study, the levels of miR-34a in various cancers were in controversial. In addition, the article reported by Wang et al. [46] did not explore the prognostic value of miR-34a expression on solid cancer patients. To our knowledge, this is the first meta-analysis to evaluate the association between high level of miR-34a expression and the prognosis of cancers.

In the current study, the results suggested that increased expression of miR-34a could only predict better OS for cancers in univariate analysis (HR: 0.73, 95% CI: 0.54-1.00, P = 0.050.), but not in multivariate analysis due to the existence of obvious heterogeneity (HR: 0.73, 95% CI: 0.47-1.12, P = 0.149, I^2 = 78.8%, $P_{heterogeneity}$ < 0.001). Although there existed heterogeneity in the univariate analysis, the pooled HR remained similar with the one in univariate analysis in the sensitivity analysis. The result concluded by the univariate analysis was in consistent with the previous studies related to prognosis of miR-34a expression in glioma, NSCLC, colorectal cancer, gastric cancer, esophageal cancer and HCC [16, 17, 23, 31, 37, 38].

The quantitative results based on the univariate and multivariate models demonstrated that miR-34a expression was strongly inverse to DFS/RFS/PFS/EFS in tumors (Univariate: HR = 0.62, P = 0.019; Multivariate: HR = 0.55, P = 0.013). After the source of the heterogeneity was removed, the pooled HRs still arrived at the similar ones in favor of miR-34a overexpression related to favorable DFS/RFS/PFS/EFS both in univariate and multivariate analysis. The conclusions verified the results of the previous studies that increased expression of miR-34a contributed to better DFS/RFS/PFS/EFS [14, 16, 17, 34, 38].

The results concluded by univariate analysis identified that decreased expression of miR-34a have unfavorable effect on RFS for cancers (HR: 0.49, 95% CI: 0.28-0.88, P = 0.018) when taking the heterogeneity into consideration. In addition, it was also verified that high miR-34a expression was positive with better PFS and EFS in both univariate and multivariate

analysis, which was in agreement with the previous studies [17, 29, 34, 36, 42]. Nevertheless, the results was opposite in the analysis of miR-34a expression and DFS in cancers, which is in line with the previous study published by Svoboda et al. [45]. Considering the studies involved in the subgroup analyses of DFS/EFS/PFS were less than 3 studies, more related studies are needed in confirming the association between miR-34a and DFS/EFS/PFS in cancer patients.

Except for the results shown by the meta-analysis, some other articles also reported the correlation between miR-34a expression and prognosis of cancer patients. One study demonstrated that inactivation of miR-34a could inhibit the aggression of GBM and contribute to better OS in proneural subtype GBM [25]. Lee et al. [24] also found that high level of miR-34a was associated with longer PFS in SCLC patients, while another report indicated that NSCLC patients with increased expression of miR-34a were more likely to have a longer survival by regulating Axl receptor expression [44]. Meanwhile, two studies identified that miR-34a could serve as a favorable factor for better OS and DFS in gastric cancer through inhibited by JMJD2A (JmjC domain-containing 2A)/PCBP2 (Poly(C)-binding protein-2) [21, 22], which was in contrast to the conclusion calculated by Huang et al. [43]. The study reported by Ogawa et al. [39] suggested that miR-34a down-regulation was closely related to poor DFS and DSS in sinonasal squamous cell carcinomas patients with therapy of cis-diamminedichloroplatinum. However, Svoboda et al. [45] demonstrated that miR-34a expression was not associated with DFS and OS in triple-negative breast cancer patients.

Several limitations existed in our meta-analysis, which must be taken into consideration. Firstly, given other languages were not available to us, thus this meta-analysis was restricted to studies in Chinese and English, which may contribute to a potential publication bias. Secondly, it was not uniform for the sources of tissues samples, which were collected form FFPE, snap frozen in liquid nitrogen or GEO datasets. And the methods of extracting miR-34a varied in different studies, including qRT-PCR and in situ hybridization. Thirdly, although we endeavored to obtain more information on HRs and 95% CIs, it was not possible to calculate the HR and 95% CI in a few studies. Meanwhile, the method to calculated HR and 95% CI by survival curve could affect the results. Fourthly and most importantly, because of limited number of studies included in the present study, it was unable to conduct analyses for all types of tumors. Besides, we were unable to carry out subgroup analysis for certain sort of cancer when considering lack of sufficient studies associated with the same kind of cancer. Fifthly, given the samples size included in the meta-analysis was relatively small; it could not provide strong evidence for the relation between miR-34a and prognosis of tumors (especially for DFS/PFS/EFS). Finally, although the impact of miR-34a on the prognosis of patients with solid tumors was statistically significant, the heterogeneity existed broadly in the meta-analysis, which may confound the results.

In conclusion, our current study indicates that increased expression of miR-34a is correlated with poor OS and RFS in cancer patients, but the indicative value of miR-34a for DFS/RFS/EFS in these patients might be limited. Meanwhile, the results do not identify whether miR-34a expression is specific to certain types of cancer. Therefore, further studies of miR-34a expression and caner prognosis are warranted to confirm these findings, as they could provide novel insights into the clinical implication of miR-34a expression in cancers.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Gang Chen, Department of Pathology, First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road,

Nanning 530021, Guangxi Zhuang Autonomous Region, China. E-mail: chen_gang_triones@163.com

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