

The predictive effect of overexpressed miR-34a on good survival of cancer patients: a systematic review and meta-analysis

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Background: MicroRNA-34a (miR-34a) is a potential prognostic factor for survival in patients with several types of cancer according to previous clinical researches. We conducted a systematic review and meta-analysis to summarize the significance of increased miR-34a expression in the prognosis of patients' overall survival.

Materials and methods: The present systematic review and meta-analysis of 15 researches included 2,597 patients. Overexpression of miR-34a may predict good overall survival ([OS], HR =0.76, 95% confidence interval: 0.55–1.06, $P=0.105$), but the effect was not significant enough. Subgroup analysis results showed miR-34a was an ideal predictor for digestive system cancer (OS, HR =0.50, 95% confidence interval: 0.25–0.99, $P=0.048$). The predictive effects of elevated expression of miR-34a on the OS of untreated and treated patients were not of obvious differences.

Conclusion: This systematic review and meta-analysis showed that miR-34a has a predictive effect on overall survival of patients with digestive system cancer.

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

Introduction

MicroRNAs (miRNAs) are small single-stranded noncoding RNAs of ~19–25 nt in length. They regulate gene expression usually by targeting the 3'-UTR of their target mRNAs for translational repression, degradation, or both.¹ According to the current researches, miRNAs involved in most of the biological processes in mammal cells, including proliferation, differentiation, migration, apoptosis, malignant transformation, metabolism, and so on. It is estimated that approximately 60% genes are regulated by miRNAs.²

miRNAs were first associated with cancer in 2002,³ and the expressions of miRNA-15a as well as miRNA-16-1 were found to be decreased in B-cell chronic lymphocytic leukemia, which implied their potential role in cancer diagnosis and treatment. During the following decade, the abnormal expression pattern of many miRNAs was proved,^{2,4} and gradually, miRNAs were accepted as cancer suppressors or promoters.

MicroRNA-34a (miR-34a) expression was first evaluated in malignant cholangiocytocytes cells in 2006.⁵ In the next year, miR-34a was found to be directly transactivated by p53, and its responsive genes were highly enriched for those that regulate cell cycle progression, apoptosis, DNA repair, and angiogenesis.^{6–9} In colon cancer cells, miR-34a suppressed cell proliferation and induced senescence-like phenotypes by downregulating the E2F pathway in vitro, and its decreased expression was also detected in cancer patients.¹⁰ Some other researches showed the similar results,^{11–15} so miR-34a was considered a tumor-suppressive miRNA.

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In 2009, a clinical research found that miR-34a was downregulated in non-small cell lung cancer (NSCLC) tissue, and its decreased expression was correlated with a high probability of relapse ($P=0.04$), which was the first time that miR-34a was identified as a prognostic marker for cancer patients.¹⁶ Up to now, overexpressed miR-34a has been reported to be related to good survival in NSCLC,¹⁷ pancreatic ductal adenocarcinoma,¹⁸ sinonasal squamous cell carcinoma,¹⁹ Ewing's sarcoma,²⁰ mantle cell lymphoma,²¹ mucosa-associated lymphoid tissue lymphoma and diffuse large B-cell lymphoma,²² as well as glioma.²³ However, there were still insignificant or even opposite results.^{24–30} Therefore, it is necessary to conduct a systematic review and meta-analysis to get a better understanding of the prognostic effect of miR-34a on cancer patients.

In this research, we collected global literatures on the prognostic effect of miR-34a on cancer patients and assessed the value of miR-34a as a biomarker for good survival.

Materials and methods

The current analysis was conducted following the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 Checklist (<http://www.prisma-statement.org/statement.htm>) and Meta-analysis of Observational Studies in Epidemiology group (MOOSE; Table S1).³¹

Identification of eligible studies

We carefully searched the online PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) and Embase (<http://www.embase.com/home>) from January 1st 2002 to April 19th 2014 to collect related literatures. The searching details were “miR-34a (all fields) and (‘neoplasms’ [MeSH terms] or ‘neoplasms’ [all fields] or ‘cancer’ [all fields])”, “miR-34a (all fields) and (‘carcinoma’ [MeSH terms] or ‘carcinoma’ [all fields])”, “miR-34a (all fields) and (‘tumor’ [all fields] or ‘neoplasms’ [MeSH terms] or ‘neoplasms’ [all fields] or ‘tumor’ [all fields])”, “miR-34a (all fields) and (‘neoplasms’ [MeSH terms] or ‘neoplasms’ [all fields] or ‘neoplasm’ [all fields])”, in PubMed and ““mir 34a/exp or ‘mir 34a’ and (‘cancer’/exp or cancer)”, ““mir 34a/exp or ‘mir 34a’ and (‘carcinoma’/exp or carcinoma)”, ““mir 34a/exp or ‘mir 34a’ and (‘tumor’/exp or tumor)”, ““mir 34a/exp or ‘mir 34a’ and (‘neoplasm’/exp or neoplasm)” in Embase. We set no advanced limitations when searching both the databases. All the searching results were checked by going through the titles and abstracts, and the duplications were removed directly.

The eligible studies were collected using our previous methods.³² Information of the eligible reports, such as titles,

abstracts, and full texts, was independently and carefully identified by three reviewers (Jian Wang, Guorong Dan, and Feng Ye), and these extracted articles were checked for a second time by two reviewers (Yu Ding and Jin Cheng). All disagreements were discussed by the aforementioned reviewers or in consultation with two senior reviewers (Fahuan Yuan and Zhongmin Zou).

Quality assessment

All the included studies for survival analysis were evaluated according to the critical review checklist of the Dutch Cochrane Centre proposed by MOOSE.³¹ As described in detail previously,³² studies were excluded if they did not mention all the seven points.

Data extraction and conversion

Data were extracted in standardized data collection form. With regard to the researches on the prognostic effect of miR-34a on cancer survival, the extracted data included the following details: 1) publication information as mentioned earlier; 2) patient characteristics, including sample size, type of disease, stage of disease, histological type, and follow-up time; 3) miR-34a measurement and cutoff value; and 4) hazard ratios (HRs) of elevated miR-34a for overall survival (OS), disease-free survival, disease-specific survival (DSS), event-free survival (EFS), progress-free survival, recurrence-free survival, as well as their 95% confidence intervals (CIs) and P -values. If available, the HRs with their 95% CIs and P -values were directly collected from the original articles or Email correspondence with the corresponding authors. If not, the HRs and their 95% CIs were calculated using the data of observed deaths/cancer recurrences, the data of samples in each group, or the data provided by the authors. If only Kaplan–Meier curves were available, essential data were extracted from the graphical survival plots and the HRs were estimated. All the aforementioned calculations were based on the methods provided by Parmar et al³³ and Tierney et al³⁴ which we used in our previous meta-analysis.³²

Statistical analysis

The test of heterogeneity of combined HRs was carried out using Cochran's Q test and Higgins I -squared statistic. The factors contributing to the heterogeneities were analyzed by subgroup analysis or sensitive analysis according to our previous methods.³² Publication bias was evaluated using the funnel plot with the Egger's bias indicator test.³⁵ The other details were described in our previous analysis.³² All analyses were performed using “STATA: Data Analysis and Statistical Software” V11.

Results

Study characteristics and systematic review

We collected 1,005 records from PubMed and 2,986 from Embase in the primary research with different keywords and excluded 3,015 duplicates from the initial records. After screening the titles, abstracts, publication types, and full texts of the remaining 1,005 records, 990 records were excluded according to the listed criteria. Then, the references of the remaining 15 qualified records were manually checked, and there was no additional record found in the cross-references. So, we got the 15 records included in our analysis.^{16–30} Figure 1 showed the flow diagram of candidate selection records in our study.

All the 15 studies were retrospective cohort researches in design. The collected 2,597 patients were from Austria, Belgium, Brazil, People's Republic of China, Finland, France, Germany, Greece, Italy, Japan, Lithuania, Poland, Romania, Slovakia, Spain, Sweden, UK, US, and Yugoslavia. The sample sizes ranged from 24 to 884 with an average sample size of 173 per study. The patients were diagnosed with a variety of cancers, including acute myeloid leukemia with complex karyotype, glioma, leukemic mantle cell lymphoma, resectable pancreatic ductal adenocarcinoma, glioblastoma, NSCLC, breast cancer, sinonasal squamous cell carcinoma, Ewing's sarcoma of bone, gastric cancer, esophageal adenocarcinoma, ovarian cancer, gastric mucosa-associated lymphoid tissue lymphoma, and diffuse large

B-cell lymphoma. Of all the 15 studies, four studies focused on digestive system tumor (n=351),^{18,22,28,29} three studies on NSCLC (n=742),^{16,17,24} two studies on glioma (n=302),^{23,26} and two studies on lymphoma (n=152).^{21,22} Ten of the studies provided the stage of cancer patients, while the remaining five records did not. Thirteen of the 15 studies detected the expression of miR-34a in cancer tissue (six frozen and seven formalin fixed and paraffin embedded), one study detected in blood sample,²⁹ and one study mentioned nothing about the concerning information.²⁶ Six of the included studies used median value as cutoff value,^{18,20,24,25,27,29} three studies used exact value of miRNA expression,^{17,19,23} two studies used values selected by Maxstat software,^{16,21} one study used the first quartile of the Automated Quantitative Analysis score distribution,²⁶ and three did not mention the concerning information. For criteria of survival assessment, ten of the included studies used OS, two used recurrence-free survival, two used progress-free survival, two used disease-free survival, one used DSS, one used cancer-specific survival, and one used EFS. The HRs were acquired by direct collection of reported data in six records, calculation based on the shown data in three records, evaluation from the survival curve in five researches, and author's Email for one research. The follow-up time ranged from 23 to 150 months and reached 5 years in ten studies. The main information of the 15 studies was summarized in Table 1.

To measure the expression of miR-34a, 12 of the 15 studies used qRT-PCR, two used in situ hybridization,^{22,28} and

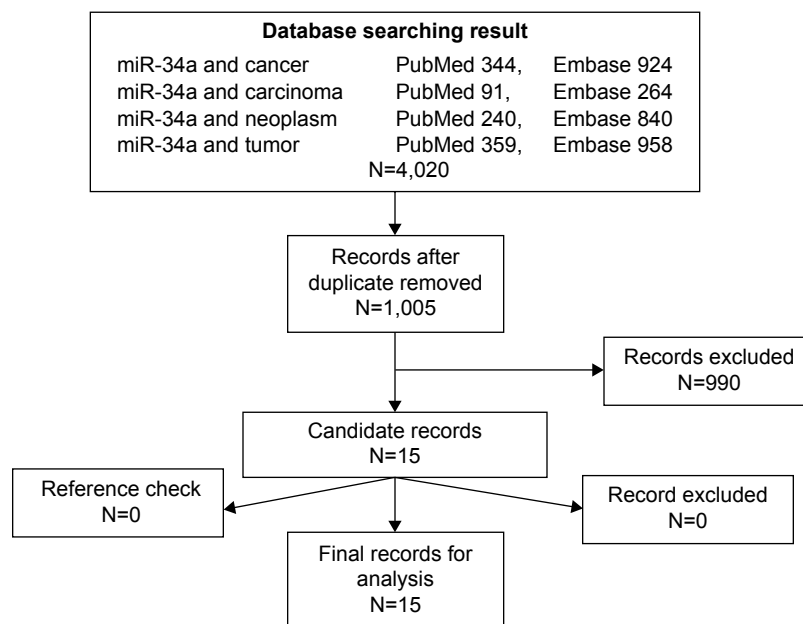


Figure 1 The flowchart showed the selection of studies for meta-analysis.

Abbreviation: miR-34a, microRNA-34a.

Table 1 Summary table of meta-analysis

Study	Year	Origin of population	Study design	Diseases	N	Stage	Sample	Cutoff	Survival analysis	Hazard ratios	Follow-up time (months)
Gallardo et al ¹⁶	2009	Spain	R	NSCLC	70	I–III	FFPE tissue	Selected by the maxstat package of R	SC	RFS	38 (1–127)
Voortman et al ²⁴	2010	14 countries	R	NSCLC	636	I–III	FFPE tissue	Median	RE	OS	96
Jamieson et al ¹⁸	2012	UK	R	Resectable pancreatic ductal adenocarcinoma	48	II–III	Frozen tissue	Median	RE	OS	Median 23.9
Peurala et al ³⁰	2011	Finland	R	Breast cancer	884	I–IV	Tissue	–	RE/CA	CSS	Up to 120
Hu et al ²⁸	2011	US	R	Esophageal adenocarcinoma	99	–	FFPE tissue	–	RE	OS, DFS	16.25 (0.37–256.43)
Reimer et al ²⁷	2011	Austria	R	Ovarian cancer	130	I–IV	FFPE tissue	Median	RE	OS, PFS	23.5 (10.0–91.0)
Mudduluru et al ¹⁷	2011	Italy	R	NSCLC	36	I–III	Frozen tissue	0.75	SC	OS	45.0 (20.8–108.3)
Genovese et al ²⁶	2012	US	R	Glioblastoma	220	–	–	First quartile of the AQUA score distribution	SC	OS	Up to 112
Ogawa et al ¹⁹	2012	Japan	R	Sinonasal SCC	24	II–IVA	Tissue	0.43	RE	DFS, DSS	53 (15–97)
Nakatani et al ²⁰	2012	Italy	R	Ewing's sarcoma of bone	49	–	Frozen tissue	Median	RE/CA	OS, EFS	88 (26–217)
Rücker et al ²⁵	2013	Germany	R	AML with complex karyotype	85	–	Frozen samples	Median	RE	OS, RFS	Up to 52
Navarro et al ²¹	2013	Spain, Germany, US	R	Leukemic MCL	30	–	Tissue	Maxstat software	SC	OS	150
He et al ²²	2013	People's Republic of China	R	Gastric MALT lymphoma and DLBCL	122	II–IV	Frozen tissue	–	RE/CA	OS	63 (3–123)
Gao et al ²³	2013	People's Republic of China	R	Glioma	82	III–IV	Frozen tissue	2 ⁻ DCt = I	SC	OS, PFS	23 (3–72)
Huang et al ²⁹	2014	People's Republic of China	R	Gastric cancer	82	IV	Blood samples	Median	AP	OS	Median 8.2

Notes: The 14 countries are as follows: Austria, Belgium, Brazil, France, Germany, Greece, Italy, Lithuania, Poland, Romania, Slovakia, Spain, Sweden, and Yugoslavia.

Abbreviations: AML, acute myeloid leukemia; AP, author provided; CA, calculate; CSS, cancer-specific survival; DFS, disease-free survival; DLBCL, diffuse large B-cell lymphoma; DSS, disease-specific survival; EFS, event-free survival; FFPE, formalin-fixed and paraffin-embedded; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progress-free survival; RE, reported; RFS, recurrence-free survival; SCC, squamous cell carcinoma; SC, survival curve.

one used microarray.³⁰ In the qRT-PCR studies, three used U6B as their internal reference,^{17,23,25} three used U6,^{18,20,22} and miR-16,²⁹ miR-191,¹⁶ U44,¹⁹ U48,²¹ U66,²⁴ or TBP²⁷ was used in one study separately. With regard to risk evaluation methods, five researches used Kaplan–Meier curves and the rest ten researches used Multiple Cox proportional hazard model. Seven of the 15 researches collected their cancer samples after a period of treatment, seven researches declared that their samples were collected before any clinical treatment, and the rest one research neglected the related information. All the assay details were summarized in Table 2.

Meta-analysis results

HRs could only be retrieved from the unique study, such as DSS (HR =0, 95% CI: 0) in sinonasal squamous cell carcinoma, cancer-specific survival in breast cancer (5-year HR =0.88, 0.65–1.88, $P=0.573$; 10-year HR =0.67, 95% CI: 0.50–0.89, $P=0.073$), and EFS (HR =0.491, 95% CI: 0.40–0.60, $P=0.0004$). If more than two researches fall into one category, the HRs were pooled. For studies evaluating OS of patients, a pooled HR and its 95% CI were calculated with a random model because of the high heterogeneity between studies ($P=0.000$, $I^2=79.4\%$). The result showed that higher expression of miR-34a may predict good OS, and the pooled HR was 0.76 (95% CI: 0.55–1.06); however, the effect did not reach the level of statistical significance ($P=0.105$) (Figure 2).

Furthermore, subgroup analysis was carried out based on the types of cancers, such as digestive system cancer, lymphoma, and sarcoma. First, as an obvious heterogeneity ($P=0.002$ and $I^2=84.0\%$) existed in those researches on OS of digestive system cancer patients, a random model was used to pool the HRs. The combined HR, 0.51 (95% CI: 0.20–1.26, $P=0.145$) (Figure 3A), indicated that overexpressed miR-34a would potentially predict good OS for patients with digestive system cancer. Two researches focused on the prognostic effect of miR-34a on the OS of lymphoma patients and sarcoma patients separately, and meta-analysis was not conducted.

Seeing that the expression of miR-34a in some researches was detected in cancer samples collected before treatment, and some was after treatment, HRs was also pooled according to these two conditions. A random model was used to pool the HRs of researches with untreated patients (low heterogeneity, $P=0.060$ and $I^2=59.5\%$), and the combined HR was 0.63 (95% CI: 0.38–1.06, $P=0.084$) (Figure 4A). Because an obvious heterogeneity ($P=0$ and $I^2=84.4\%$)

existed among six researches with treated patients, a random model was used to pool the HRs. The combined HR, 0.79 (95% CI: 0.51–1.21, $P=0.271$) (Figure 4B), was comparable with the pooled HR of untreated patients, suggesting that the treatment did not significantly influence the predictive effect of miR-34a.

Possible sources of the heterogeneity

Obvious heterogeneity of subjects was found in all the four analysis groups (OS for all, $P=0$ and $I^2=74.9\%$; OS for digestive system cancer, $P=0.004$ and $I^2=77.7\%$; OS for treated patients, $P=0$ and $I^2=84.4\%$; and OS for untreated patients, $P=0.060$ and $I^2=59.5\%$). The most possible sources of the heterogeneity were also analyzed by different methods.

On the one hand, since the heterogeneity of OS analysis group was obvious, we divided the 11 studies into three cancer type-specific analysis groups (four studies on digestive system cancer, two studies for lymphoma, and two studies on sarcoma). The heterogeneity was still obvious in digestive system cancer and sarcoma groups, so the cancer type could not solely explain the heterogeneity in OS analysis group. On the other hand, a meta-regression analysis was conducted to evaluate the potential factors responsible for the obvious heterogeneity. As a result, the publication year ($P=0.617$), cutoff values ($P=0.651$), patient's origin ($P=0.914$), risk evaluation method ($P=0.957$), follow-up time ($P=0.751$), and cancer type ($P=0.112$) contributed little to the heterogeneity, but the sample size ($P=0.023$) was the main source of heterogeneity.

Because meta-regression analysis is not proper to seek the sources of heterogeneity for analysis group with less than ten studies, sensitivity analysis was performed instead. In the OS analysis group for untreated patients, heterogeneity was significant ($P=0.060$ and $I^2=59.5\%$). When Nakatani's study was removed from analysis, the heterogeneity became insignificant ($P=0.704$ and $I^2=0\%$). Using the same method, we found that Jamieson's studies were responsible for the heterogeneity in the digestive system cancer analysis group, and Jamieson's as well as He's studies contributed most to the heterogeneity in the treated patients group.

Publication bias

The publication bias of included studies was evaluated by funnel plots and Egger's tests. As shown in Figure 3B, the funnel plots were almost symmetric. In OS meta-analysis, the P -values of Egger's regression intercepts were 0.175. Hence, there was no evidence for significant publication bias in our meta-analysis.

Table 2 Summary table of miRNA detection, the HRs and their 95% CIs

Author	Year	Disease	HRs	95% CI and P-values	Outcome	miR-34a detection	Internal reference	Risk evaluation method	Before or after treatment
Ogawa et al ¹⁹	2012	Sinonasal SCC	0	0	DSS	qRT-PCR	U44	Cox model	Before
Ogawa et al ¹⁹	2012	Sinonasal SCC	0.005	0–0.29, P=0.011	DFS	qRT-PCR	U44	Cox model	Before
Mudduluru et al ¹⁷	2011	NSCLC	0.01	0	OS	qRT-PCR	U6B	Kaplan–Meier	Before
Jamieson et al ¹⁸	2012	Resectable pancreatic ductal adenocarcinoma	0.15	0.06–0.37, P=0.0128	OS	qRT-PCR	U6	Cox model	After
Nakatani et al ²⁰	2012	Ewing's sarcoma	0.385	0.24–0.60, P=0.0000245	OS	qRT-PCR	U6	Cox model	Before
He et al ²²	2013	Gastric MALT lymphoma and DLBCL	0.44	0.21–0.90, P=0.043	OS	qRT-PCR	U6	Cox model	After
Gallardo et al ¹⁶	2009	NSCLC	0.47	0.20–1.21, P=0.043	RFS	qRT-PCR	miR-191	Kaplan–Meier	After
Nakatani et al ²⁰	2012	Ewing's sarcoma	0.491	0.40–0.60, P=0.0004	EFS	qRT-PCR	U6	Cox model	Before
Navarro et al ²¹	2013	Leukemic MCL	0.61	0.01–26.26, P=0.008	OS	qRT-PCR	RU48	Kaplan–Meier	Before
Peurala et al ³⁰	2011	Breast cancer	0.67	0.50–0.89, P=0.073	10-year CSS	Microarray	–	Cox model	Before
Hu et al ²⁸	2011	Esophageal adenocarcinoma	0.71	0.41–1.24, P=0.23	OS	ISH	–	Cox model	Before
Hu et al ²⁸	2011	Esophageal adenocarcinoma	0.72	0.43–1.22, P=0.22	DFS	ISH	–	Cox model	Before
Gao et al ²³	2013	Glioma	0.75	0.46–1.22, P=0.0254	PFS	qRT-PCR	RNU6B	Kaplan–Meier	After
Gao et al ²³	2013	Glioma	0.8	0.47–1.37, P=0.0074	OS	qRT-PCR	RNU6B	Kaplan–Meier	After
Peurala et al ³⁰	2011	Breast cancer	0.88	0.65–1.88, P=0.573	5-year CSS	Microarray	–	Cox model	Before
Voortman et al ²⁴	2010	NSCLC	0.9	0.72–1.14, P=0.38	OS	qRT-PCR	U66	Cox model	After
Huang et al ²⁹	2014	Gastric cancer	0.973	0.586–1.617, P=0.917	OS	qRT-PCR	miR-16	Cox model	Before
Reimer et al ²⁷	2011	Ovarian cancer	1.237	0.505–3.030, P=0.641	OS	qRT-PCR	TBP	Cox model	–
Reimer et al ²⁷	2011	Ovarian cancer	1.334	0.597–2.978, P=0.482	PFS	qRT-PCR	TBP	Cox model	–
Genovese et al ²⁶	2012	Glioblastoma	1.37	0.99–1.89, P=0.0154	OS	qRT-PCR	–	Kaplan–Meier	After
Rücker et al ²⁵	2013	AML with complex karyotype	1.47	1.06–2.03, P=0.02	OS	qRT-PCR	RNU6B	Cox model	After
Rücker et al ²⁵	2013	AML with complex karyotype	1.9	1.14–3.18, P=0.01	RFS	qRT-PCR	RNU6B	Cox model	After

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; Cox model, Mult Cox proportional hazard model; CSS, cancer-specific survival; DFS, disease-free survival; DLBCL, diffuse large B-cell lymphoma; DSS, disease-specific survival; EFS, event-free survival; HR, hazard ratio; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; miR-34a, microRNA-34a; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progress-free survival; RFS, recurrence-free survival; SCC, squamous cell carcinoma; –, unclear; qRT-PCR, qualitative real-time polymerase chain reaction.

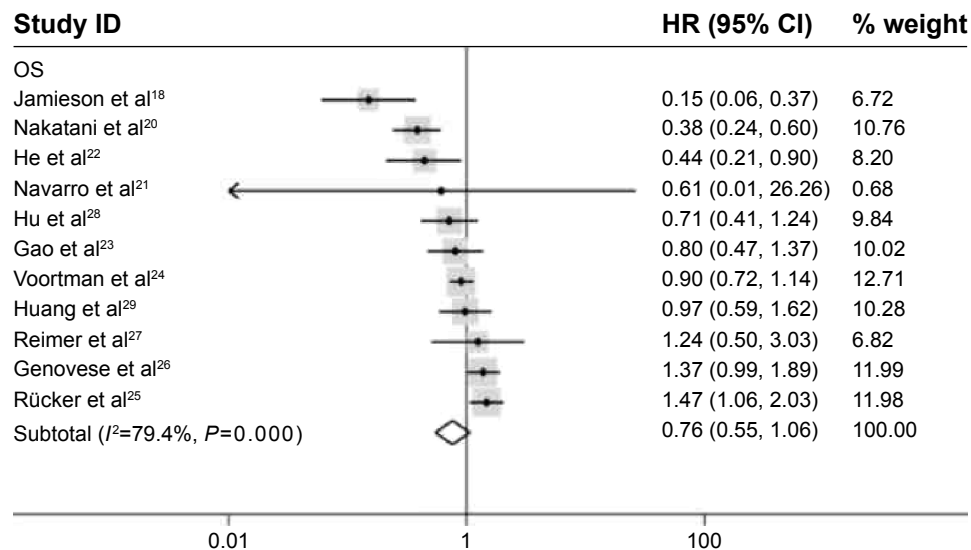
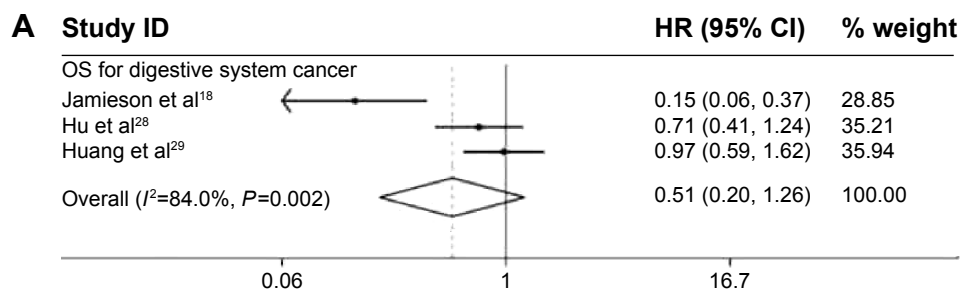


Figure 2 Forrest plots of studies evaluating hazard ratios of high miR-34a expression. The random effects analysis model showed the pooled HR for overall survival is 0.76 with 95% CI: 0.55–1.06, and P -value is 0.105.

Note: Weights are from random effects analysis.

Abbreviations: CI, confidence interval; miR-34a, microRNA-34a; OS, overall survival; HR, hazard ratio; ID, identification.



B Begg's funnel plot with pseudo 95% confidence limits

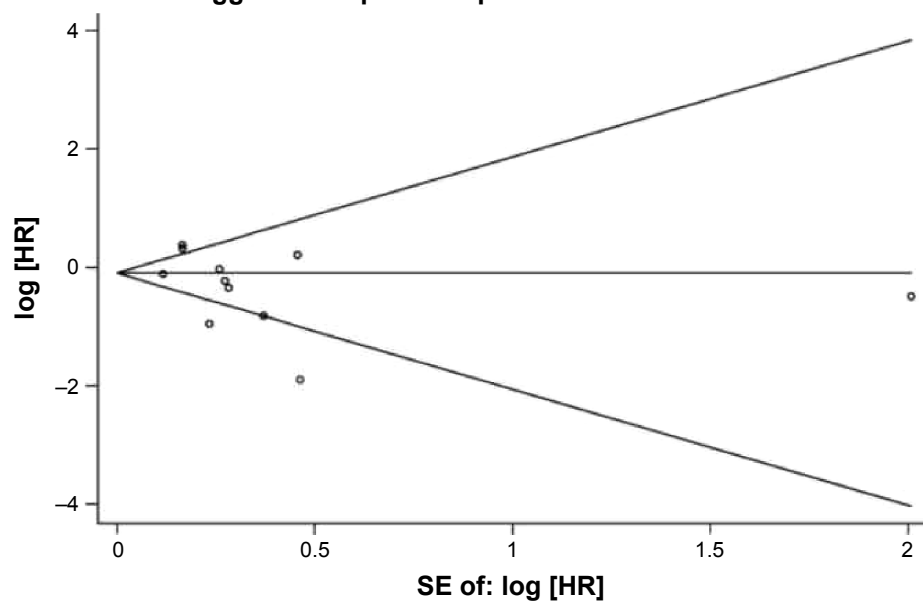


Figure 3 Forrest plots of studies evaluating hazard ratios of high miR-34a expression and Funnel plots of studies included in meta-analysis.

Notes: (A) The random effects analysis model showed the pooled HR for digestive system cancer patients overall survival is 0.51 with 95% CI: 0.20–1.26, and P -value is 0.146. (B) Funnel plots of studies assessed the prognostic effect of miR-34a on overall survival. Weights are from random effects analysis.

Abbreviations: CI, confidence interval; miR-34a, microRNA-34a; OS, overall survival; HR, hazard ratio; SE, standard error; ID, identification.

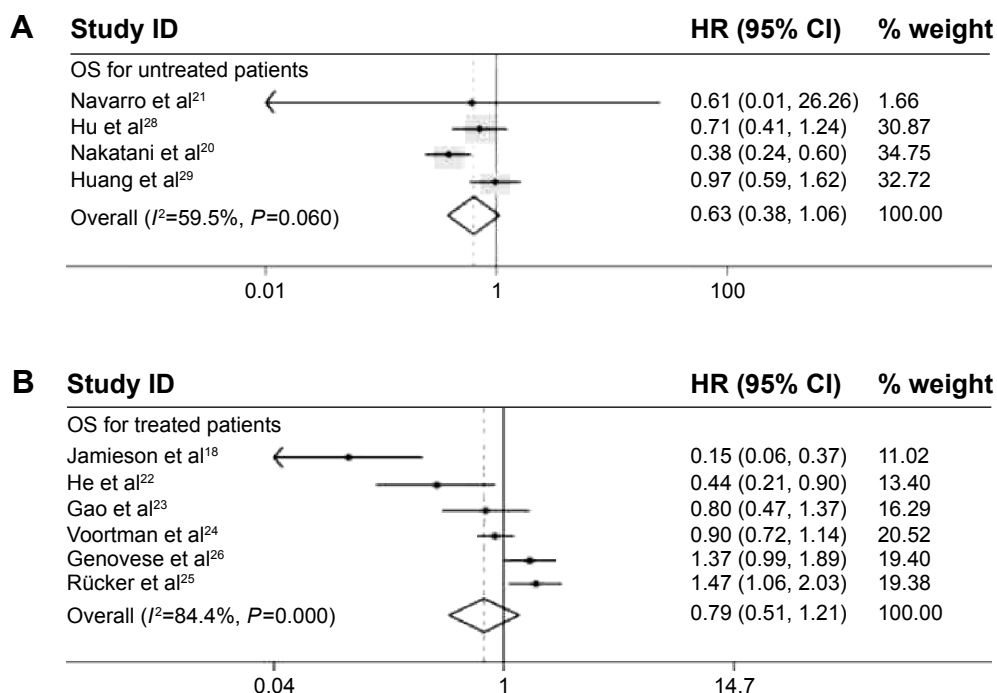


Figure 4 Subgroup analysis results.

Notes: (A) The random effects analysis model showed the pooled HR for overall survival of untreated patients is 0.63 with 95% CI: 0.38–1.06, and P -value is 0.084. (B) The random effects analysis model showed the pooled HR for overall survival of treated patients is 0.79 with 95% CI: 0.51–1.21, and P -value is 0.271. Weights are from random effects analysis.

Abbreviations: CI, confidence interval; OS, overall survival; HR, hazard ratio; ID, identification.

Discussion

miR-34 family includes three members—miR-34a, miR-34b, and miR-34c. In human, miR-34a is located at 1p36 locus of chromosome 1, and miR-34b and miR-34c are located at 11q23 locus of chromosome 11. All the three members are p53-dependent and share the same seed sequence as well as similar functions, but their target genes are different.³⁶ As a downstream element of p53 pathway, miR-34a is always known as a tumor-suppressive miRNA for its repression effect on cell cycle, cell invasion, cell migration, cancer stem cell, and so on.³⁷ miR-34a plays an important role in DNA damage response. Ectopic expression of miR-34a induces cell cycle arrest by downregulating target genes, and as a result, inhibits damaged DNA replication.^{6,10} The expression of miR-34a decreases frequently in some p53 mutant cancer cells, such as U251 and chronic lymphocytic leukemia cells. In these cells, some miR-34a target genes, which are related to cell cycle, tumor invasion, and migration, are upregulated,^{39,40} and restoration of functional miR-34a enhances the chemotherapy susceptibility and inhibits tumor cell growth. Some of the direct downstream targets of miR-34a, such as Bcl-2, Notch, and HMGA2 (high-mobility group AT-hook 2), are all responsible for self-renewal of the cell, so miR-34a may suppress the self-renewal of cancer stem cells.^{36,41}

In animal tumor models, the therapeutic activity of miR-34a was also evaluated in NSCLC, prostate cancer, melanoma, pancreatic cancer, and lymphoma, and the miR-34a treated animal showed a significant tumor growth inhibition.³⁷ These experiment results were consistent to our research.

In the current analysis, we got four pooled HRs from 15 researches on 2,597 patients with 13 different types of cancer from 19 countries. The main conclusions can be summarized as follows: 1) miR-34a did predict good overall survival for cancer patients with a pooled HR = 0.76 (95% CI: 0.55–1.06), though the P -value was not satisfying enough ($P=0.105$); 2) the result seemingly suggested that miR-34a was an ideal biomarker for good outcome in digestive system cancer patients (HR = 0.50, 95% CI: 0.25–0.99, $P=0.048$); 3) the predictive role of miR-34a in cancer tissue collected from patients before (HR = 0.63, 95% CI: 0.38–1.06, $P=0.084$) and after (HR = 0.79, 95% CI: 0.51–1.21, $P=0.271$) treatment, and they were not obviously different. To our knowledge, this was the first meta-analysis on the prognostic effect of miR-34a on cancer patients.

Though the prognostic effect role of miR-34a on cancer patients was proved by experimental results and statistically identified by clinical researches and this meta-analysis,

current conclusions should be cautiously appreciated. First, all the four pooled HRs were of statistical insignificance, suggesting the predictive effect of miR-34a was limited. Second, several HRs were calculated based on the data collected from the survival curves, which would bring errors, although small. Third, HRs were pooled from different articles with different cutoff values due to methods limitations while a general baseline of miR-34a expression level could not be set up. In addition, the HR for OS in Mudduluru's study was 0.01 with 95% CI of 0, which made the direct pooling of this HR with the others using STATA impossible, so the HRs were pooled without Mudduluru's study. Fourth, only researches published in English were included in this analysis, which could lead to the miss of applicable studies in non-English publication.

Because six of the eight analysis groups were with heterogeneity, the HRs were pooled with a random effect model, and the possible resource of heterogeneity was also explored by subgroup analysis, meta-regression, and sensitive analysis. Subgroup analysis based on cancer types as well as the meta-regression result suggested that the cancer type might not be responsible for the heterogeneity. The meta-regression results showed that the sample size of each study was responsible for the obvious heterogeneity in OS analysis group, and the sensitive analysis results figured out responsible studies of heterogeneity in the analysis groups of treated patients, untreated patients, and digestive system cancer patients. Publication bias was not found in the OS analysis group; however, the amount of researches was not large enough to ensure the current conclusion.

Nowadays, miRNAs have been widely considered as oncogene or cancer suppressor, but several concerns should be stressed. First, a set of miRNAs would be more proper to predict the outcome of one type of cancer. There are already studies focused on this issue,^{42,43} and present analysis proposes miR-34a as a candidate miRNA for future study. Second, most of the studies detected the expression of miR-34a in cancer tissue, and this brings many problems. Due to the heterogeneity of cancer, the collected tissue might not precisely reflect the status of cancer, and lack of standard methods of collecting the tissue, isolating the RNA, detecting the expression of miRNA, and even the internal references would obstruct the clinical research of miRNA in cancer. Third, circulating miRNAs would be an ideal choice for their convenient sampling in future clinical cancer research. Circulating miRNA may not only play diagnostic and prognostic role in cancer patients but also can be applied in cancer screening by regularly monitoring miRNA profiles.

Conclusion

Our meta-analysis collected studies on the relationship of miR-34a expression and cancer patient survival found that the overexpression of miR-34a potentially predict good survival of patients with digestive system cancer. Because of the limitation of meta-analysis results when applied to observational or retrospective studies that could hardly be compared, one should be cautious in interpreting the current conclusion. Further clinical researches are needed to testify the association between miR-34a and cancer prognosis as well as the efficiency of therapies.

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Disclosure

The authors declare no conflict of interests in this work.

References

1. Ambros V. The functions of animal microRNAs. *Nature*. 2004; 431(7006):350–355.
2. Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011;12(12):861–874.
3. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature*. 2010;466(7308):835–840.
4. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2006;6(11):857–866.
5. Meng F, Henson R, Lang M, et al. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology*. 2006;130(7):2113–2129.
6. Chang TC, Wentzel EA, Kent OA, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol Cell*. 2007;26(5):745–752.
7. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. *Nature*. 2007;447(7148):1130–1134.
8. He X, He L, Hannon GJ. The guardian's little helper: microRNAs in the p53 tumor suppressor network. *Cancer Res*. 2007;67(23):11099–11101.
9. Raver-Shapira N, Marciano E, Meiri E, et al. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell*. 2007;26(5):731–743.
10. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. *Proc Natl Acad Sci U S A*. 2007;104(39):15472–15477.
11. Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene*. 2007;26(34):5017–5022.
12. Zanette DL, Rivadavia F, Molfetta GA, et al. miRNA expression profiles in chronic lymphocytic and acute lymphocytic leukemia. *Braz J Med Biol Res*. 2007;40(11):1435–1440.
13. Cole KA, Attiyeh EF, Mosse YP, et al. A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. *Mol Cancer Res*. 2008;6(5):735–742.

14. Kumamoto K, Spillare EA, Fujita K, et al. Nutlin-3a activates p53 to both down-regulate inhibitor of growth 2 and up-regulate miR-34a, miR-34b, and miR-34c expression, and induce senescence. *Cancer Res.* 2008;68(9):3193–3203.
15. Rokhlin OW, Scheinker VS, Taghiyev AF, Bumcrot D, Glover RA, Cohen MB. MicroRNA-34 mediates AR-dependent p53-induced apoptosis in prostate cancer. *Cancer Biol Ther.* 2008;7(8):1288–1296.
16. Gallardo E, Navarro A, Viñolas N, et al. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis.* 2009;30(11):1903–1909.
17. Mudduluru G, Ceppi P, Kumarswamy R, Scagliotti GV, Papotti M, Allgayer H. Regulation of Axl receptor tyrosine kinase expression by miR-34a and miR-199a/b in solid cancer. *Oncogene.* 2011;30(25):2888–2899.
18. Jamieson NB, Morran DC, Morton JP, et al. MicroRNA molecular profiles associated with diagnosis, clinicopathologic criteria, and overall survival in patients with resectable pancreatic ductal adenocarcinoma. *Clin Cancer Res.* 2012;18(2):534–545.
19. Ogawa T, Saiki Y, Shiga K, et al. miR-34a is downregulated in cis-diamminedichloroplatinum treated sinonasal squamous cell carcinoma patients with poor prognosis. *Cancer Sci.* 2012;103(9):1737–1743.
20. Nakatani F, Ferracin M, Manara MC, et al. miR-34a predicts survival of Ewing's sarcoma patients and directly influences cell chemo-sensitivity and malignancy. *J Pathol.* 2012;226(5):796–805.
21. Navarro A, Clot G, Prieto M, et al. microRNA expression profiles identify subtypes of mantle cell lymphoma with different clinicobiological characteristics. *Clin Cancer Res.* 2013;19(12):3121–3129.
22. He M, Gao L, Zhang S, et al. Prognostic significance of miR-34a and its target proteins of FOXP1, p53, and BCL2 in gastric MALT lymphoma and DLBCL. *Gastric Cancer.* 2014;17(3):431–441.
23. Gao H, Zhao H, Xiang W. Expression level of human miR-34a correlates with glioma grade and prognosis. *J Neurooncol.* 2013;113(2):221–228.
24. Voortman J, Goto A, Mendiboure J, et al. MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma. *Cancer Res.* 2010;70(21):8288–8298.
25. Rücker FG, Russ AC, Cocciardi S, et al. Altered miRNA and gene expression in acute myeloid leukemia with complex karyotype identify networks of prognostic relevance. *Leukemia.* 2013;27(2):353–361.
26. Genovese G, Ergun A, Shukla SA, et al. microRNA regulatory network inference identifies miR-34a as a novel regulator of TGF-beta signaling in glioblastoma. *Cancer Discov.* 2012;2(8):736–749.
27. Reimer D, Hubalek M, Kiefel H, et al. Regulation of transcription factor E2F3a and its clinical relevance in ovarian cancer. *Oncogene.* 2011;30(38):4038–4049.
28. Hu Y, Correa AM, Hoque A, et al. Prognostic significance of differentially expressed miRNAs in esophageal cancer. *Int J Cancer.* 2011;128(1):132–143.
29. Huang D, Wang H, Liu R, et al. miRNA27a is a biomarker for predicting chemosensitivity and prognosis in metastatic or recurrent gastric cancer. *J Cell Biochem.* 2014;115(3):549–556.
30. Peurala H, Greco D, Heikkinen T, et al. miR-34a expression has an effect for lower risk of metastasis and associates with expression patterns predicting clinical outcome in breast cancer. *PLoS One.* 2011;6(11):e26122.
31. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) Group. *JAMA.* 2000;283(15):2008–2012.
32. Wang J, Zhao J, Shi M, et al. Elevated expression of miR-210 predicts poor survival of cancer patients: a systematic review and meta-analysis. *PLoS One.* 2014;9(2):e89223.
33. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. *Stat Med.* 1998;17(24):2815–2834.
34. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials.* 2007;8:16.
35. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629–634.
36. Hermeking H. The miR-34 family in cancer and apoptosis. *Cell Death Differ.* 2010;17(2):193–199.
37. Bader AG. miR-34 – a microRNA replacement therapy is headed to the clinic. *Front Genet.* 2012;3:120.
38. Ichimura A, Ruike Y, Terasawa K, Shimizu K, Tsujimoto G. MicroRNA-34a inhibits cell proliferation by repressing mitogen-activated protein kinase kinase 1 during megakaryocytic differentiation of K562 cells. *Mol Pharmacol.* 2010;77(6):1016–1024.
39. Zenz T, Mohr J, Eldering E, et al. miR-34a as part of the resistance network in chronic lymphocytic leukemia. *Blood.* 2009;113(16):3801–3808.
40. Luan S, Sun L, Huang F. MicroRNA-34a: a novel tumor suppressor in p53-mutant glioma cell line U251. *Arch Med Res.* 2010;41(2):67–74.
41. Ji Q, Hao X, Meng Y, et al. Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumor spheres. *BMC Cancer.* 2008;8:266.
42. Vosa U, Voorder T, Kolde R, Vilo J, Metspalu A, Annilo T. Meta-analysis of microRNA expression in lung cancer. *Int J Cancer.* 2013;132(12):2884–2893.
43. Cheng C, Wang Q, You W, Chen M, Xia J. MiRNAs as biomarkers of myocardial infarction: a meta-analysis. *PLoS One.* 2014;9(2):e88566.

Supplementary materials

Table S1 MOOSE checklist

Checklist items	Related sections and indications
Reporting of background should include	
Problem definition	Background
Hypothesis statement	Background
Description of study outcome(s)	OS, DFS, PFS, RFS, DSS, EFS, CSS
Type of exposure or intervention used	Cancer
Type of study designs used	Systematic reviews and meta-analysis
Study population	Global
Reporting of search strategy should include	
Qualifications of searchers (eg, librarians and investigators)	Stated in methods
Search strategy, including time period included in the synthesis and keywords	Methods
Effort to include all available studies, including contact with authors	We contact authors and searched reference lists and citations
Databases and registries searched	Methods
Search software used, name and version, including special features used (eg, explosion)	360 secured browser 6.2
Use of hand searching (eg, reference lists of obtained articles)	Methods
List of citations located and those excluded, including justification	Flow diagram in Figure 1
Method of addressing articles published in languages other than English	Method
Method of handling abstracts and unpublished studies	Method
Description of any contact with authors	Method
Reporting of methods should include	
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Method
Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	Methods
Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	Methods
Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	Methods
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	Methods
Assessment of heterogeneity	Methods
Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	Methods
Provision of appropriate tables and graphics	Methods
Reporting of results should include	
Graphic summarizing individual study estimates and overall estimate	Figures 2, 3
Table giving descriptive information for each study included	Tables 1, 2
Results of sensitivity testing (eg, subgroup analysis)	Figures 4
Indication of statistical uncertainty of findings	Discussion
Reporting of discussion should include	
Quantitative assessment of bias (eg, publication bias)	Result
Justification for exclusion (eg, exclusion of non-English language citations)	Discussion
Assessment of quality of included studies	Results and discussion
Reporting of conclusions should include	
Consideration of alternative explanations for observed results	Discussion
Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	Discussion
Guidelines for future research	Discussion
Disclosure of funding source	Acknowledge

Abbreviations: OS, overall survival; DFS, disease-free survival; PFS, progress-free survival; RFS, recurrence-free survival; DSS, disease-specific survival; CSS, cancer-specific survival; MOOSE, Meta-analysis of Observational Studies in Epidemiology group.

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