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The expression of miR-21 and miR-375 predict prognosis of esophageal cancer

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ABSTRACT

Background: MicroRNA is a class of small, well-conserved, non-coding RNAs, and could play a potential role as diagnostic and prognostic biomarkers of esophageal cancers. We aimed to review comprehensively the evidence of microRNA as prognostic biomarkers in esophageal cancers.
Methods: Studies were identified by searching PubMed, Embase and Web of Science until November 2013. Descriptive characteristics of studies were described and an additional meta-analysis for specific microRNAs which were studied most frequently was performed. Pooled hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) were calculated. Fixed model or random model method was chosen depending on the heterogeneity among the studies.
Results: Twenty-two studies including a total of 1946 participants were enrolled after a strict filtering and qualifying process. Among 33 prognostic microRNAs identified for esophageal cancer, miR-21 and miR-375 appeared more frequently. The median study size was 70.5 patients (29–249 patients) and the median HR was 3.305 (IQR = 1.615–7.31). For the studies evaluating miR-21's association with overall survival (OS), the pooled HR suggested that high level of miR-21 has a negative impact on OS (HR = 1.52[1.17–1.98], $P = 0.001$). As for miR-375, the pooled HR for OS (high/low) was 0.53 (95% CI: 0.39–0.73, $P < 0.001$), indicated that low level of miR-375 has a negative impact on OS. These results indicated that microRNAs show promising associations with prognosis in esophageal cancer. Up-regulation of miR-21 and down-regulation of miR-375 can predict unfavourable prognosis in esophageal cancer.

1. Introduction

Esophageal cancer (EC) is the eighth most common cancer in the world and the sixth leading cause of cancer mortality [1]. Despite treatments have been improved recently the prognoses of part EC patients are still poor. How to determine the prognosis of EC patients is key role need to be solved.

Currently, investigators have focused on the potential of microRNAs (miRNAs) to serve as biomarkers for cancer. MiRNAs are small, endogenous, non-coding RNAs with 19–24 nucleotides in length. Aberrant expression of miRNAs in cancer tissue has been reported in various types of cancers, and increasing evidence suggested the potential of miRNA as prognostic markers in cancer.

Many articles have reported promising results for miRNA classifiers in EC prognosis. However, as a kind of biomarker, miRNA in EC patients remains largely unexplored. To clarify the effect of miRNAs as prognostic biomarkers, the data from the studies providing independent assessments of miRNAs in EC were systematically evaluated and synthesized. Furthermore, we performed the meta-analysis of two specific miRNAs (miR-21 and miR-375) as important prognostic classifiers for EC.

2. Materials and methods

2.1. Search strategy, eligibility criteria and data extraction

A comprehensive literature search was done for original articles analyzing the prognostic value of miRNAs in EC with PubMed, Embase and Web of Science. Studies were selected by using the following keywords: esophagus, miRNA, cancer, prognosis. Original and review articles published until November 2013 were sought, considering the latter as an additional source of original works

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otherwise overlooked [2]. Additionally, the bibliographies reported in all the selected studies were reviewed to complete this search. When the reported results were obviously obtained from the same patient population in multiple publications, only the most recent or the most informative studies were included.

Full text of each study was carefully evaluated. Original experimental articles published in English that deal with EC only (any stage or histology) and analyses prognosis in patients stratified by dichotomous miRNA expression levels in tissues or blood were enrolled. Studies investigated on the associations of miRNA expression and malignant potential without survival analysis, as well as absence of key information such as hazard ratio (HR), 95% confidence interval (CI) or survival curve were excluded.

To ensure the quality of meta-analysis, we followed the guidelines of a critical checklist of Dutch Cochrane Centre proposed by Meta-analysis of Observational Studies in Epidemiology (MOOSE) [3]. The extracted data elements included the following: (i) the first author's name and publication year; (ii) characteristics of the studied population including sample size, source, stage and histological type; (iii) miRNA assessments and cut-off values; (iv) follow-up time; (v) HRs of miRNAs for overall survival (OS), recurrence-free survival (RFS), disease-free survival (DFS) or progression-free survival (PFS), along with the 95% CIs and *P* values. If the HRs and their 95% CIs were not directly provided, the numbers of deaths or recurrences and total samples in each study were extracted to calculate [4]. If only Kaplan–Meier curves were available, data were extracted from the survival plots and the HRs were then estimated using the described methods [4–6].

The information such as titles, abstracts, full texts and reference lists of all of the identified reports were carefully identified from all of the publications in duplicate by two reviewers (Fu and Dong). These extracted articles were double checked by Li. Disagreements were resolved by discussion between the three readers (Fu, Dong and Li). We e-mailed to the authors of studies for additional information and data needed for the meta-analytic calculations. A comprehensive data-base was designed to ensure that all data needed for analysis were publicly available (Table 1).

2.2. Statistical analysis

Descriptive statistics were used to describe the characteristics of the eligible studies. Data were presented as the median value with interquartile range (IQR) for continuous variables and as frequencies for categorical variables [7]. To identify the prognostic microRNAs which were defined most frequently, we evaluated the opportunities of each microRNA that had been selected for survival analysis.

The meta-analysis of specific microRNAs was performed finally. A test of heterogeneity of combined HRs was carried out using Cochran's *Q* test and Higgins I-squared statistic. A *P* value of <0.05 or $I^2 > 50\%$ was considered statistically significant. A random effect model (Der Simonian and Laird method) was applied if heterogeneity was observed ($P < 0.05$ or $I^2 > 50\%$), while the fixed effect model was used in the absence of between-study heterogeneity ($P \geqslant 0.05$ or $I^2 \leqslant 50\%$). Publication bias was evaluated using the funnel plot with the Egger's bias indicator test [8]. Finally, sensitivity analysis was carried out by investigating the influence of a single study on the overall HR. All analyses were performed using 'STATA: Data Analysis and Statistical Software' V12.0 (http://www.stata.com/).

3. Results

3.1. Eligible studies

One hundred and sixty-one records for miRNA and were identified from a primary literature search in PubMed, EMBASE and web of science. After manually screening the titles, abstracts and keywords, 131 studies were excluded because they were review articles, letters, non-English articles, laboratory studies or studies irrelevant to the current analysis. And the remaining 30 studies were evaluated in full text. Of the 30 candidate studies, 6 studies lacked the key survival data, one study's sample was the same as others, and one study has two samples only. Therefore, ultimately 22 articles [9–30] identified unique miRNA classifiers associated with survival were considered eligible for inclusion in this review (Fig. 1).

3.2. Characteristics of included studies

We collected data from 22 studies including a total of 1946 participants from the United States, Canada, Japan, Chinese, and German. Most of them were retrospective in design; three studies [11,15,17] were prospective. The median study size was 70.5 patients (29–249 patients). Frozen or formalin-fixed paraffin-embedded (FFPE) tumor tissues were used in 18 studies, serum or plasm was as samples in 4 studies, and cancer tissues and noncancerous tissues were used at the same time in 3 studies. The quantitative real-time polymerase chain reaction (qRT-PCR) assay was used in 20 studies, whereas in situ hybridisation (ISH) assay was used in 2 studies only. The cut-off values of miRNAs were different in each study, with median and mean respectively applied in 6 studies, 75-th, 5-fold, 2-fold, normal tissue's or plasma's miRNA expression value used in other studies. There are 3 studies that did not state the cut-off clearly. Normalization was performed with endogenous controls in almost all studies with U6 and U6B being the most commonly used endogenous controls.

3.3. Outcomes from eligible studies

The outcomes were OS (18 studies), PFS (2 studies), RFS (1 study), DFS (1 study) and TSS (1 study), five studies examined two indicators of them. The number of miRNA analyzed in each study ranged from 1 to 199, and the number of miRNA statistically significantly associated with survival or recurrence ranged from 1 to 6. Eventually, a total of 33 prognostic miRNAs were identified.

The HRs and their 95% CIs of 77 classifiers in the 22 studies were assessed (Fig. 2). The pooled HR was 1.32 (95% CI: 1.13–1.53). Examining adjusted HRs (unless only an unadjusted value was available) revealed that 48 miRNA classifiers in 12 studies [10,12–14,16,21,22,25–27,29,30] were not statistically significantly associated with survival or recurrence. The median HR in the studies that reported statistically significant results at *P*-value level of 0.05 was 3.0412 (IQR = 1.6–3.6545). For all of the reported HRs, regardless of statistical significance, the median HR was 3.305 (IQR = 1.615–7.31). Twelve studies [9,11,14,15,17,20–22,24,25,27,28] were adjusted for potentially prognostic clinical and pathological variables during multivariable regression in the cohorts. Lymph stage ($n = 9$ studies), primary tumor stage ($n = 8$ studies), age ($n = 6$ studies) and sex ($n = 5$ studies) were the most commonly used adjustment factors. Of the 12 studies, 10 of which presented HRs and 95% CIs for both unadjusted and adjusted classifiers, and there was no marked change in the HR estimate with adjustment. Three studies [14,21,25] presented unadjusted HRs and adjusted HRs for partly miRNAs.

3.4. Specific microRNA associated with prognosis

From the 22 studies included in this review, we counted the frequency of each miRNA been selected for survival analysis. A total of 33 prognostic miRNAs were identified in these classifiers, and 2 miRNAs (miR-21, miR-375) appeared most frequently and accounted for 31.82% and 18.18%, respectively. When these two miRNAs were assessed in all miRNA profiles, the probability was

Table 1
Main characteristics and results of the eligible studies.

No.	Study	Year	Origin of population	Source of samples	Histology	Stage	*N*	Gender (male/female)	Age (years)	MiRNAs assay	Endogenous control	Cut-off	Analysis miRNA	Prognostic miRNA(s)	Hazard ratio	Multivariate analysis	Results
1	Chen	2011	China	Frozen tissues	ESCC	I–III	65	57/8	43–75, median 60	qRT-PCR	U6B	75th percentiles	92a	92a	R	YES	OS
2	Feber	2011	USA	Frozen tissues	EAC	I–IV	45	38/7	NR	qRT-PCR	U6	Median	199 miRNAs	143, 145, 199a–3p, 199a–5p,100	R	NR	OS
3	Guo	2008	China	Frozen tissues	ESCC	I–III	31	23/8	NR	qRT-PCR	U6	Median	103/107	103/107	R	YES	OS
4	Hamano	2011	Japan	FFPE	EC	I–IV	98	49/49	High: 63.2 ± 8.5 ; Low: 60.0 ± 8.6	qRT-PCR	U48	Median	Let-7a, let-7g, 21, 134, 145, 155, 200c, 203, 296	200C, 21, 145	SC	NR	OS
5	Harazono	2013	Japan	Frozen tissues	ESCC	NR	29	NR	NR	qRT-PCR	NR	Mean	655	655	SC	NR	OS, RFS
6	Hu	2011	USA	FFPE	EAC/ESCC	0–IV	158	127/31	28–82, median 64	ISH	5S	NR	Let-7g, 9, 16–2, 20, 21, 30e, 34a, 126, 195p, 200a	16–2,30e	R	NR	OS, DFS
					ESCC		59							9, 16–2, 20, 200a	–	YES	OS, DFS
					EAC		99							30e, 200a	R	YES	OS, DFS
7	Komatsu	2012	Japan	Plasma	ESCC	0–IV	50	44/6	⩾65, 25; <65, 25	qRT-PCR	U6	Normal plama	21, 375	21, 375	R	YES	OS
8	Kong	2012	China	Frozen tissues	ESCC	I–IV	60	43/17	⩽66, 23; >66, 37	qRT-PCR	SNORD48	Normal tissue	375	375	SC	NR	OS, DFS
9	Kurashige	2012	Japan	FFPE	ESCC	I–IV	109	90/19	High: 66 ± 9.5; Low: 65 ± 8.4	qRT-PCR	U6		223	223	R	YES	OS
10	Li, J.	2013	China	Tissue microarray	ESCC	I–IV	249	136/113	⩽60, 105; >60, 144	ISH	18s	Normal tissue	375	375	SC	NR	OS
11	Li, P.	2013	China	Tissue	ESCC	I–IV	76	61/15	⩾65, 29; <65, 47	qRT-PCR	GAPDH	5-folds	21	21	SC	YES	DFS
12	Lin	2012	China	Frozen tissues	ESCC	I–III	91	68/23	37–80, median 56	qRT-PCR	U6	Mean	31, 142–3p, 338–3p,1261	142–3p	R	YES	OS
13	Mathe	2009	USA, Canada	Frozen tissues	EAC	0–IV	100	89/11	>62, 49; <62, 51	qRT-PCR	U66	Median	21, 233, 192, 194, 203	None	–	–	–
			USA, Canada	Frozen tissues	EAC with Barrett		58	NR	NR					375	R	YES	OS
			USA, Canada, Japan	Frozen tissues	ESCC		70	52/18	⩾62, 42; <62, 28				375, 21, 146b, 181b, 155, 223, 203, 192, 194	223	R	YES	OS
			USA, Canada, Japan	Noncancerous tissues	ESCC		70	52/18	⩾62, 42; <62, 28					21, 155, 146b, 181b	R	YES	OS
14	Ogawa	2009	Japan	Frozen tissue	ESCC	0–IV	30	24/6	43–75, median 64.1	qRT-PCR	Let7-a, U6B	NR	72 miRNAs	23a, 26a, 27b, 96, 128b, 129	R	Partly YES	OS
15	Qi	2013	China	Tissue	ESCC	I–III	46	23/23	45–71, median 59	qRT-PCR	U6	NR	198	198	SC	YES	OS
16	Takeshita	2013	Japan	Serum	ESCC	I–IV	101	NR	NR	qRT-PCR	miR-16	Mean	1246	1246	R	YES	OS
17	Tanaka	2013	Japan	Serum	ESCC	II–IV	64	49/15	⩾65, 42; <65, 22	qRT-PCR	Cel-miR-39	Median	200c, 21, 145, let-7c	200c	SC	YES	PFS
18	Yokobori	2013	Japan	Frozen tissue	ESCC	I–IV	108	97/ 11	42–81, mean 64.9	qRT-PCR	U6B	Mean	150	150	SC	NO	OS
19	Zhang, M.	2012	China	Frozen tissue	ESCC	I–IV	40	21/9	⩾60, 18; <60, 12	qRT-PCR	U6 and GAPDH	Median	126, 518b, 433	518b	R	YES	PFS
20	Zhang, T.	2011	China	Serum	ESCC	I–IV	44			qRT-PCR	miR-16	Median	31	31	R	YES	RFS, TSS
21	Zhao	2013	China	Frozen tissues	ESCC	I–III	178	108/70	34–78, mean 62.2	qRT-PCR.	U66	Median	21, 181b, 146b, 155, 223	21, 181b, 146b	SC	NR	OS
				Noncancerous tissue										21	SC	NR	OS
22	Zhou	2013	China	Frozen tissues	ESCC	NR	15	76/28	36–74	qRT-PCR.	NR	Mean	483, 214	483, 214	SC	NR	OS

Abbreviations: FFPE, formalin-fixed paraffin-embedded; ESCC, esophageal squamous cell cancer; EAC, esophageal adenocarcinoma; EC, esophageal cancer; qRT-PCR, quantitative real-time PCR; R, reported; SC, survival curve; NR, no reported or calculations not possible for deriving metric; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; TSS, tumor-specific survival; RFS, relapse-free survival.

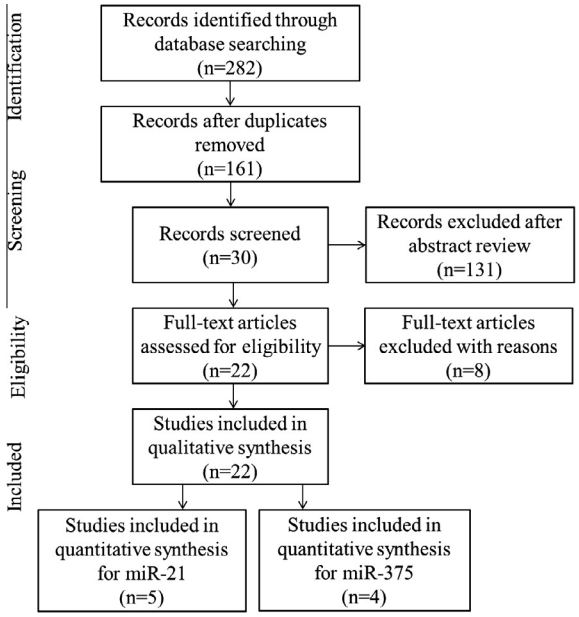

Fig. 1. Methodological flow chart of the review.

12.99% and 7.79%, respectively. Thus, we performed a meta-analysis to identify whether miR-21 and miR-375 could be robust prognostic biomarkers.

For studies evaluating OS for miR-21, no significant heterogeneity have been found in the eight cohorts (I^2 = 36.9%, P = 0.134). Hence, a fixed model was applied to calculate a pooled HR and its 95% CI. We found that higher levels of miR-21 significantly predicted poorer OS, with the pooled HR being 1.52 (95% CI: 1.17–1.98, P = 0.002) (Fig. 3A). The pooled HR was more significantly predictive than a single HR of each study. For each study, the HR varied from 0.79 to 4.71, and 3 studies had a P value bigger than 0.05, which was not statistically significant. Publication bias of the included studies for OS was evaluated by funnel plot (Fig. 3B) and Egger's test. The P value of Egger's regression intercepts were 0.679. Hence, there was no evidence for significant publication bias in the meta-analysis of miR-21. Finally, the sensitive analysis was performed by omitting one study at each time to measure its effects on the pooled HR. As presented in Fig. 3C, no individual study influenced the overall HR dominantly.

For the studies evaluating OS for miR-375 (Fig. 4A), a fixed model was applied since there was no heterogeneity among the seven cohorts (I^2 = 0%, P = 0.547) and the pooled HR was 0.53 (95% CI: 0.39–0.73, P < 0.001). Pooled HR <1, indicated that down-regulated miR-375 may be associated with poor OS in EC. The pooled HR was more reliable because five of seven cohorts had a single P value bigger than 0.05, which was not statistically significant. Publication bias of OS for miR-375 was also evaluated by funnel plot (Fig. 4B) and Egger's test. The funnel plot was almost symmetric, and the P value of Egger's regression intercepts was 0.988. Hence, there was no evidence for significant publication bias in the meta-analysis. The sensitive analysis (Fig. 4C) showed that there is no individual study influenced the overall HR dominantly. No subgroup analysis was performed because of the limitation of the number of eligible literatures.

4. Discussion

MiRNAs were proposed as promising biomarkers for early cancer detection and accurate prognosis as well as targets for more efficient treatment. Emerging studies have demonstrated that aberrant expression of miRNAs was associated with prognosis for EC. The results of their expression feature in cancer tissues or plasma (serum) are inconsistent and controversy still exists in identifying them as new biomarkers of EC prognosis. Therefore, systemically evaluate the most frequently reported miRNAs in EC is important. We gathered complete literatures and pooled the prognostic value. The median HR was 3.305, suggesting a moderately strong discriminatory ability. As little overlap of prognostic miRNAs has been observed, we identified specific miRNAs (miR-21 and miR-375) that appeared repeatedly among the selected classifiers.

By using a meta-analysis approach, we demonstrated that increased expression of miR-21 and decreased expression of miR-375, were significantly associated with poor OS in EC. However, the conclusion is not persuasive enough, and needs to be refined for several reasons. Firstly, the pooled risks of miR-21 and miR-375 for OS, although statistically significant, were not strong, with HRs of 1.52 and 0.53, respectively. Secondly, the number of cohorts included for meta-analysis was insufficient which made the result less convincing. Thirdly, cautions should be taken that the expression levels of miR-21 and miR-375 were measured in both cancerous tissues and noncancerous tissues in Mathe's and Zhao's study [21]. It may introduce overestimation of HR by including these studies in one meta-analysis [31]. Fourthly, the conclusion was somewhat faint because of the difference in cut-off definition. This meta-analysis could not provide a clear clue about how high is high and how low is low. Fifthly, although there was no significant evidence of publication bias in this meta-analysis, cautions should be taken because only studies published in English were selected, which could definitely result in language bias. And the tendency for journals to publish positive results could also make certain bias.

In the past 5 years, miRNAs have been considered as potential biomarkers for cancer prognosis because its unique expression profiles in cancerous tissue compared to normal tissue. In addition, they have more stable expression than mRNA, and they can be easily assessed by qRT-PCR [32]. Our data have shown that miR-21 and miR-375 are very promising for OS prediction. However, several points should be concerned about their clinical application. Firstly, how to define the cut-off value of miRNA expression remains need to decide. To date, most researchers use median or mean value as the cut-off value and the accurate value was different. Lack of abundant miRNA expression data in global population makes it difficult to set a standard cut-off value. Secondly, what kind of sample is better to be used to analysis? In our meta-analysis, samples included cancerous tissue, noncancerous tissue, plasma or serum. Currently, tissues are more widely used for miRNA study. However, circulating markers are more acceptable than tissue markers because they can be acquired before surgery and can be monitored throughout the course of disease. More studies should be conducted in future to evaluate the prognostic value of miRNA level in serum/plasma. Thirdly, which is better for clinical application, a single miRNA or a panel of miRNAs? From our data, miR-21 and miR-375 are both associated with survival. Recently, researchers have considered using a set of miRNAs in place of a single miRNA to increase the prediction power. Komatsu et al. [15] found that plasma miR-21 or miR-375 was not significant associated with survival. When miR-21 and miR-375 were combined, they did find that miR-21/375 was an independent prognostic factor [15]. Their data suggested a set of miRNAs be a stronger predictor for survival than a single miR-21. For routine clinical application in the future, the above-mentioned problems should be solved.

MiRNAs are key players in a wide array of pathological processes, which may partly explain the prognostic associations in EC. The expression of miR-21 and miR-375 association with cancer

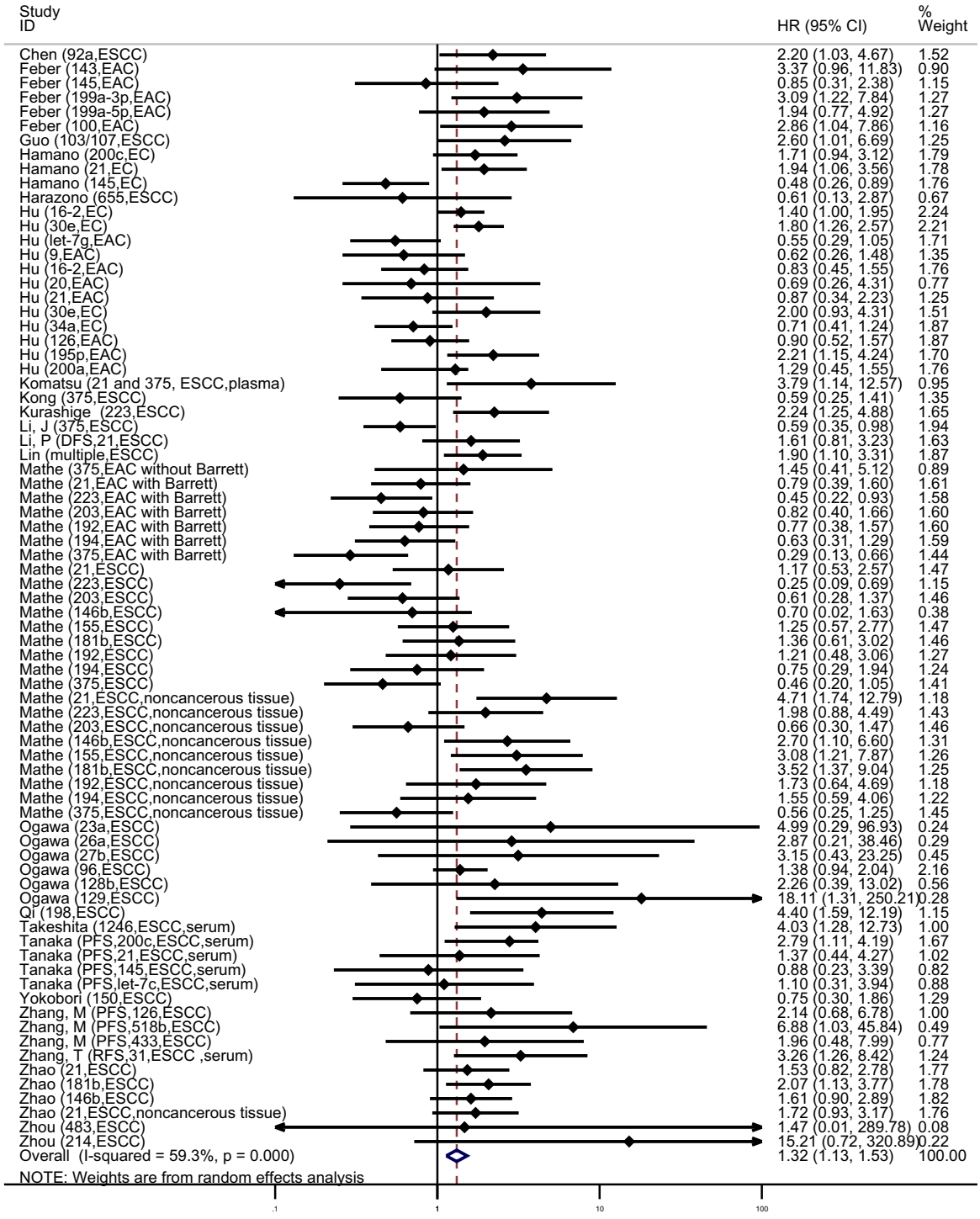

Fig. 2. Forest plots of studies evaluating hazard ratios (HRs) of miRNA in all cohorts.

outcome may be, in part, caused by the biological function of miR-21 and miR-375. MiR-21 is well-known oncogenic miRNA, while miR-375 is anti-oncogenic miRNA. Studies revealed that miR-21 was up-regulated and miR-375 was down-regulated in EC plasma or tissue.

MiR-21 was usually up regulated in EC tissues, plasma or serum. Nouraee et al. [33] found that miR-21 expression is mostly confined to the SCC stroma and its release from fibroblasts influences the migration and invasion capacity of SCC cells. MiR-21 can promote the tumor develop by down-regulating PTEN [19,29], FASL, TIMP3 and RECK [34], activating ERK1/2/MAPK pathway [35]. Unlike miR-21, miR-375 was usually down-expressed in EC. MiR-375 was down-regulated by hypermethylation of the promoter in EC tissues. Anti-oncogenic miR-375 attributed to suppressed tumor proliferation by down-regulating LDHB [36], PDK1 [37], and inhibited tumor growth and metastasis via suppressing IGF1R expression [16]. The function of individual miRNA is different and there are interactions between miRNAs. For instance, Zhang et al. [38] found that miR-203 was significantly down-regulated in EC, and overexpression of miR-203 in EC cells dramatically increased cell apoptosis and inhibited cell proliferation, migration and invasion as well as tumor growth. MiR-203 worked as an EC

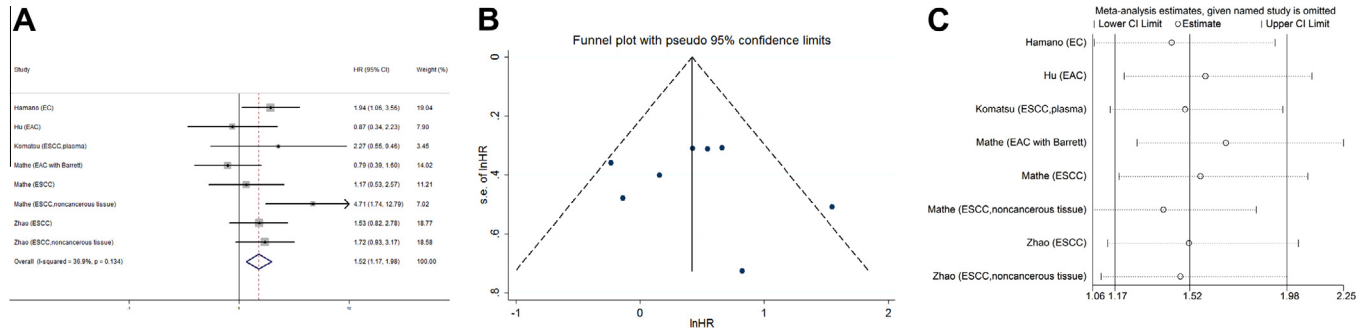

Fig. 3. (A) Forest plots of studies evaluating hazard ratios (HRs) of high miR-21 expression as compared to low expression. (B) Funnel plots of publication bias for meta-analysis of miR-21. (C) Sensitivity analysis for meta-analysis of miR-21.

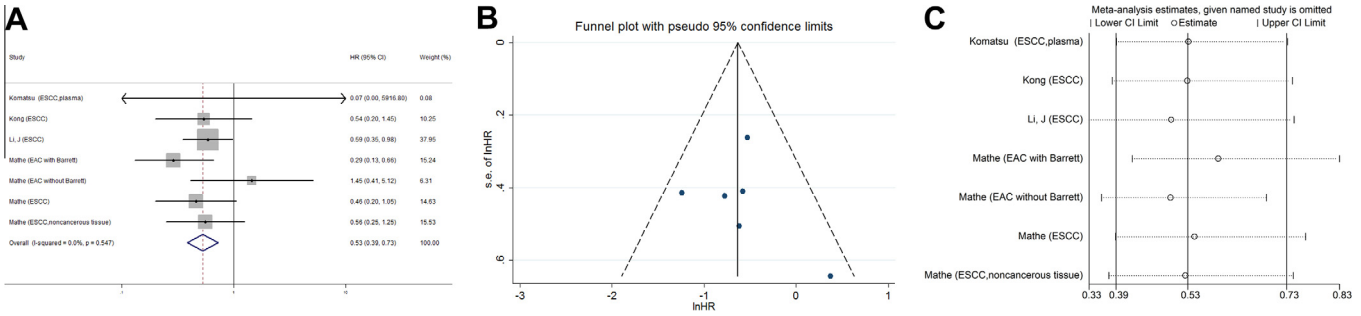

Fig. 4. (A) Forest plots of studies evaluating hazard ratios (HRs) of high miR375 expression as compared to low expression. (B) Funnel plots of publication bias for meta-analysis of miR-375. (C) Sensitivity analysis for meta-analysis of miR-375.

suppressor through down-regulating the expression of Ran and miR-21.

In summary, our meta-analysis, representing a quantified synthesis of all published studies, the result has shown that the up-regulated miR-21 and down-regulated miR-375 expression are significantly associated with poor OS in EC patients. More clinical investigations should be conducted before miR-21 and miR-375 can be implemented in the routine clinical management of cancer.

References

[1] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, 2002, CA Cancer J. Clin. 55 (2005) 74–108.
[2] S. Mocellin, D. Hoon, A. Ambrosi, D. Nitti, C.R. Rossi, The prognostic value of circulating tumor cells in patients with melanoma: a systematic review and meta-analysis, Clin. Cancer Res. 12 (2006) 4605–4613.
[3] D.F. Stroup, J.A. Berlin, S.C. Morton, I. Olkin, G.D. Williamson, D. Rennie, D. Moher, B.J. Becker, T.A. Sipe, S.B. Thacker, Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group, JAMA 283 (2000) 2008–2012.
[4] M.K. Parmar, V. Torri, L. Stewart, Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints, Stat. Med. 17 (1998) 2815–2834.
[5] P.R. Williamson, C.T. Smith, J.L. Hutton, A.G. Marson, Aggregate data meta-analysis with time-to-event outcomes, Stat. Med. 21 (2002) 3337–3351.
[6] J.F. Tierney, L.A. Stewart, D. Ghersi, S. Burdett, M.R. Sydes, Practical methods for incorporating summary time-to-event data into meta-analysis, Trials 8 (2007) 16.
[7] V.S. Nair, L.S. Maeda, J.P. Ioannidis, Clinical outcome prediction by microRNAs in human cancer: a systematic review, J. Natl. Cancer Inst. 104 (2012) 528–540.
[8] M. Egger, G. Davey Smith, M. Schneider, C. Minder, Bias in meta-analysis detected by a simple, graphical test, BMJ 315 (1997) 629–634.
[9] Z.L. Chen, X.H. Zhao, J.W. Wang, B.Z. Li, Z. Wang, J. Sun, F.W. Tan, D.P. Ding, X.H. Xu, F. Zhou, X.G. Tan, J. Hang, S.S. Shi, X.L. Feng, J. He, microRNA-92a promotes lymph node metastasis of human esophageal squamous cell carcinoma via E-cadherin, J. Biol. Chem. 286 (2011) 10725–10734.
[10] A. Feber, L.Q. Xi, A. Pennathur, W.E. Gooding, S. Bandla, M.X. Wu, J.D. Luketich, T.E. Godfrey, V.R. Litle, MicroRNA prognostic signature for nodal metastases and survival in esophageal adenocarcinoma, Ann. Thorac. Surg. 91 (2011) 1523–1530.
[11] Y. Guo, Z. Chen, L. Zhang, F. Zhou, S. Shi, X. Feng, B. Li, X. Meng, X. Ma, M. Luo, K. Shao, N. Li, B. Qiu, K. Mitchelson, J. Cheng, J. He, Distinctive microRNA profiles relating to patient survival in esophageal squamous cell carcinoma, Cancer Res. 68 (2008) 26–33.
[12] R. Hamano, H. Miyata, M. Yamasaki, Y. Kurokawa, J. Hara, J.H. Moon, K. Nakajima, S. Takiguchi, Y. Fujiwara, M. Mori, Y. Doki, Overexpression of miR-200c induces chemoresistance in esophageal cancers mediated through activation of the Akt signaling pathway, Clin. Cancer Res. 17 (2011) 3029–3038.
[13] Y. Harazono, T. Muramatsu, H. Endo, N. Uzawa, T. Kawano, K. Harada, J. Inazawa, K.I. Kozaki, miR-655 Is an EMT-suppressive microRNA targeting ZEB1 and TGFBR2, PLoS ONE 8 (2013) e62757.
[14] Y. Hu, A.M. Correa, A. Hoque, B. Guan, F. Ye, J. Huang, S.G. Swisher, T.T. Wu, J.A. Ajani, X.C. Xu, Prognostic significance of differentially expressed miRNAs in esophageal cancer, Int. J. Cancer 128 (2011) 132–143.
[15] S. Komatsu, D. Ichikawa, H. Takeshita, H. Konishi, H. Nagata, S. Hirajima, T. Kawaguchi, T. Arita, A. Shiozaki, H. Fujiwara, K. Okamoto, E. Otsuji, Prognostic impact of circulating miR-21 and miR-375 in plasma of patients with esophageal squamous cell carcinoma, Expert Opin. Biol. Ther. 12 (2012) S53–S59.
[16] K.L. Kong, D.L. Kwong, T.H. Chan, S.Y. Law, L. Chen, Y. Li, Y.R. Qin, X.Y. Guan, MicroRNA-375 inhibits tumour growth and metastasis in oesophageal squamous cell carcinoma through repressing insulin-like growth factor 1 receptor, Gut 61 (2012) 33–42.
[17] J. Kurashige, M. Watanabe, M. Iwatsuki, K. Kinoshita, S. Saito, Y. Hiyoshi, H. Kamohara, Y. Baba, K. Mimori, H. Baba, Overexpression of microRNA-223 regulates the ubiquitin ligase FBXW7 in oesophageal squamous cell carcinoma, Br. J. Cancer 106 (2012) 182–188.
[18] J. Li, X. Li, Y. Li, H. Yang, L. Wang, Y. Qin, H. Liu, L. Fu, X.Y. Guan, Cell-specific detection of miR-375 downregulation for predicting the prognosis of esophageal squamous cell carcinoma by miRNA in situ hybridization, PLoS ONE 8 (2013) e53582.
[19] P. Li, W.M. Mao, Z.G. Zheng, Z.M. Dong, Z.Q. Ling, Down-regulation of PTEN expression modulated by dysregulated miR-21 contributes to the progression of esophageal cancer, Dig. Dis. Sci. 58 (2013) 3483–3493.
[20] R.J. Lin, D.W. Xiao, L.D. Liao, T. Chen, Z.F. Xie, W.Z. Huang, W.S. Wang, T.F. Jiang, B.L. Wu, E.M. Li, L.Y. Xu, MiR-142-3p as a potential prognostic biomarker for esophageal squamous cell carcinoma, J. Surg. Oncol. 105 (2012) 175–182.
[21] E.A. Mathe, G.H. Nguyen, E.D. Bowman, Y.Q. Zhao, A. Budhu, A.J. Schetter, R. Braun, M. Reimers, K. Kumamoto, D. Hughes, N.K. Altorki, A.G. Casson, C.G. Liu, X.W. Wang, N. Yanaihara, N. Hagiwara, A.J. Dannenberg, M. Miyashita, C.M. Croce, C.C. Harris, MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival, Clin. Cancer Res. 15 (2009) 6192–6200.
[22] R. Ogawa, H. Ishiguro, Y. Kuwabara, M. Kimura, A. Mitsui, T. Katada, K. Harata, T. Tanaka, Y. Fujii, Expression profiling of micro-RNAs in human esophageal squamous cell carcinoma using RT-PCR, Med. Mol. Morphol. 42 (2009) 102–109.
[23] B. Qi, W.J. Yao, B.S. Zhao, X.G. Qin, Y. Wang, W.J. Wang, T.Y. Wang, S.G. Liu, H.C. Li, Involvement of microRNA-198 overexpression in the poor prognosis of esophageal cancer, Asian Pac. J. Cancer Prev. 14 (2013) 5073–5076.

[24] N. Takeshita, I. Hoshino, M. Mori, Y. Akutsu, N. Hanari, Y. Yoneyama, N. Ikeda, Y. Isozaki, T. Maruyama, N. Akanuma, A. Komatsu, M. Jitsukawa, H. Matsubara, Serum microRNA expression profile: miR-1246 as a novel diagnostic and prognostic biomarker for oesophageal squamous cell carcinoma, Br. J. Cancer 108 (2013) 644–652.
[25] K. Tanaka, H. Miyata, M. Yamasaki, K. Sugimura, T. Takahashi, Y. Kurokawa, K. Nakajima, S. Takiguchi, M. Mori, Y. Doki, Circulating miR-200c levels significantly predict response to chemotherapy and prognosis of patients undergoing neoadjuvant chemotherapy for esophageal cancer, Ann. Surg. Oncol. 20 (Suppl. 3) (2013) S607–S615.
[26] T. Yokobori, S. Suzuki, N. Tanaka, T. Inose, M. Sohda, A. Sano, M. Sakai, M. Nakajima, T. Miyazaki, H. Kato, H. Kuwano, MiR-150 is associated with poor prognosis in esophageal squamous cell carcinoma via targeting the EMT inducer ZEB1, Cancer Sci. 104 (2013) 48–54.
[27] M. Zhang, S. Zhou, L. Zhang, J. Zhang, H. Cai, J. Zhu, C. Huang, J. Wang, MiR-518b is down-regulated, and involved in cell proliferation and invasion by targeting Rap1b in esophageal squamous cell carcinoma, FEBS Lett. 586 (2012) 3508–3521.
[28] T. Zhang, Q. Wang, D. Zhao, Y. Cui, B. Cao, L. Guo, S.H. Lu, The oncogenetic role of microRNA-31 as a potential biomarker in oesophageal squamous cell carcinoma, Clin. Sci. (Lond.) 121 (2011) 437–447.
[29] Y. Zhao, A.J. Schetter, G.B. Yang, G. Nguyen, E.A. Mathe, P. Li, H. Cai, L. Yu, F. Liu, D. Hang, H. Yang, X.W. Wang, Y. Ke, C.C. Harris, MicroRNA and inflammatory gene expression as prognostic marker for overall survival in esophageal squamous cell carcinoma, Int. J. Cancer 132 (2013) 2901–2909.
[30] Y. Zhou, L. Hong, Prediction value of miR-483 and miR-214 in prognosis and multidrug resistance of esophageal squamous cell carcinoma, Genet. Test. Mol. Biomarkers 17 (2013) 470–474.
[31] X. Fu, Y. Han, Y. Wu, X. Zhu, X. Lu, F. Mao, X. Wang, X. He, Y. Zhao, Prognostic role of microRNA-21 in various carcinomas: a systematic review and meta-analysis, Eur. J. Clin. Invest. 41 (2011) 1245–1253.
[32] M. Ferracin, A. Veronese, M. Negrini, Micromarkers: miRNAs in cancer diagnosis and prognosis, Expert Rev. Mol. Diagn. 10 (2010) 297–308.
[33] N. Nouraee, K. Van Roosbroeck, M. Vasei, S. Semnani, N.M. Samaei, F. Naghshvar, A.A. Omidi, G.A. Calin, S.J. Mowla, Expression, tissue distribution and function of miR-21 in esophageal squamous cell carcinoma, PLoS ONE 8 (2013) e73009.
[34] N. Wang, C.Q. Zhang, J.H. He, X.F. Duan, Y.Y. Wang, X. Ji, W.Q. Zang, M. Li, Y.Y. Ma, T. Wang, G.Q. Zhao, MiR-21 down-regulation suppresses cell growth, invasion and induces cell apoptosis by targeting FASL, TIMP3, and RECK genes in esophageal carcinoma, Dig. Dis. Sci. 58 (2013) 1863–1870.
[35] F. Liu, S. Zheng, T. Liu, Q. Liu, M. Liang, X. Li, I. Sheyhidin, X. Lu, W. Liu, MicroRNA-21 promotes the proliferation and inhibits apoptosis in Eca109 via activating ERK1/2/MAPK pathway, Mol. Cell. Biochem. 381 (2013) 115–125.
[36] Y. Isozaki, I. Hoshino, N. Nohata, T. Kinoshita, Y. Akutsu, N. Hanari, M. Mori, Y. Yoneyama, N. Akanuma, N. Takeshita, T. Maruyama, N. Seki, N. Nishino, M. Yoshida, H. Matsubara, Identification of novel molecular targets regulated by tumor suppressive miR-375 induced by histone acetylation in esophageal squamous cell carcinoma, Int. J. Oncol. 41 (2012) 985–994.
[37] X. Li, R. Lin, J. Li, Epigenetic silencing of microRNA-375 regulates PDK1 expression in esophageal cancer, Dig. Dis. Sci. 56 (2011) 2849–2856.
[38] F. Zhang, Z. Yang, M. Cao, Y. Xu, J. Li, X. Chen, Z. Gao, J. Xin, S. Zhou, Z. Zhou, Y. Yang, W. Sheng, Y. Zeng, MiR-203 suppresses tumor growth and invasion and down-regulates MiR-21 expression through repressing Ran in esophageal cancer, Cancer Lett. 342 (2014) 121–129.