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Original Article

Prognostic value of circulating microRNA-21 in digestive system cancers: a meta-analysis

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Abstract: Circulating microRNAs show aberrant expression in patients with cancer. The aim of this study was to investigate the prognostic value of circulating microRNA-21 (miR-21) in digestive system cancers. Methods: All the eligible studies were searched by Medline and EMBASE. The hazard ratios (HRs) for overall survival (OS), which compared the expression levels of circulating miR-21 in patients with digestive cancer was extracted and estimated. Pooled HRs and 95% confidence intervals (CI) were calculated. Then a meta-analysis was performed to clarify the prognostic value of the miR-21. Results: A total of seven studies involving 907 subjects were included. The results suggested that higher circulating miR-21 could predict worse OS outcome with the pooled HR of 2.19 (95% CI 1.01-4.75, P = 0.05) in digestive system cancers. Subgroup analysis by ethnicity indicated circulating miR-21 was associated with OS in patients with digestive cancer among Asians with the pooled HR of 2.90 (95% CI 1.30-6.45, P = 0.009). However, subgroup analysis by digestive system site revealed that there is no associated with OS in patients with colorectal cancer with the pooled HR of 1.34 (95% CI 0.45-4.00, P = 0.60). Conclusion: The present findings suggest that circulating miR-21 is associated with poor survival in patients with digestive cancer and could be a prognostic biomarker for those patients.

Keywords: Digestive cancer, circulating miR-21, prognosis, biomarker, meta-analysis

Introduction

MicroRNAs (miRNAs) are endogenous, small noncoding and have a length of 18-25 nucleotides RNAs. These miRNAs could identify post transcriptional gene regulators that paired to complementary sequences in the 3' untranslated region (3' UTR) of target mRNAs, leading to mRNA degradation or translational repression [1]. They can play a significant role in regulation of cell development, metabolism, immunity, proliferation, differentiation, and apoptosis. Since their discovery in 1993, an emerging findings have demonstrated that miRNAs are involved in tumorigenesis and the development of various cancer [2]. miRNAs also present in the cell-free body fluids such as serum, plasma, urine, saliva are termed as circulating miRNAs. They have been exploited as noninvasive biomarkers for cancer in the five past years [3, 4].

microRNA-21 (miR-21) was the representative one since it has been extensively studied in various cancers. Some studies have reported that circulating miR-21 might predict the survival outcome in digestive system cancers [5-8]. However, some other studies showed insignificant or opposite results [9-11]. The relationship between circulating miR-21 and digestive system cancers remains controversial because these studies involved only small study subjects. The aim of this meta-analysis is to investigate the relationship between circulating miR-21 and the survival in patients with digestive cancer.

Materials and methods

Literature search

Two authors independently searched online PubMed and EMBASE from January 1st, 1993 to February 15th, 2014 to identify relevant studies. Two sets of key words were used among that process, namely "circulating/serum/plasma miR-21 and cancer" and "miR-

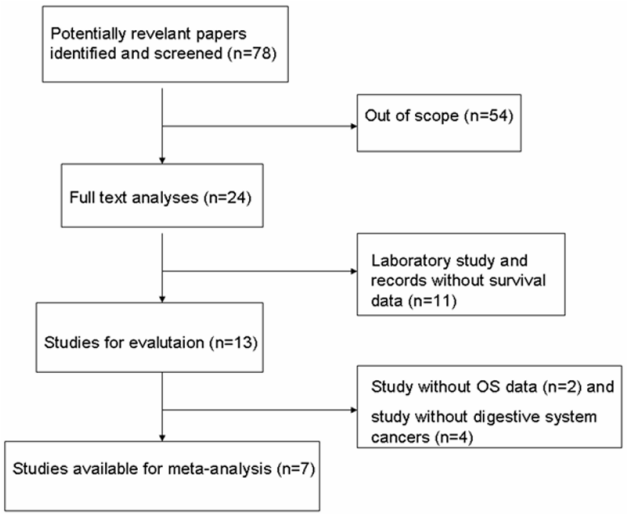

Figure 1. Flow diagram of the studies identification and selection.

21 and cancer prognosis". A manual review of the references of relevant publications was also performed to obtain additional studies. We performed meta-analysis following the guidelines of the Meta-analysis of Observational Studies in Epidemiology group (MOOSE) [12].

Study selection

Eligible studies included in this meta-analysis met the following criteria: (i) they studied the patients with any type of carcinoma; (ii) they measured the expression of circulating miR-21 in plasma or serum; and (iii) they investigated the relationship between circulating miR-21 and cancer survival outcome. Literatures were excluded based on the following criteria: (i) review articles or letters, (ii) non-English articles, (iii) animal or laboratory studies. (iv) studies of non-dichotomous miR-21 expression levels, (v) absence of key information about survival outcome such as hazard ratio (HR), 95% CI and *P* value. When a study reporting the same patient cohort was included in several publications, only the most recent or complete study was selected. If any doubt of suitability remained after the abstract was examined, the full manuscript was obtained.

Quality assessment

Two reviewers critically assessed the quality of all the studies included according to a basic standard as follows: 1) clear report of study population and origin of country, 2) clear definition of study design, 3) clear definition of type of cancer, 4) clear definition of outcome assessment, 5) clear definition of measurement of circulating miR-21, and 6) sufficient follow-up duration. Otherwise, the study was removed in order to enhance the quality of the meta-analysis.

Data extraction

Two reviewers independently extracted the required information from all eligible studies to rule out any discrepancy. The following data were extracted: first author, year of publication, country of origin, tumor type, sample size, method of testing circulating miR-21 and the cut-off, follow-up, HR of circulating miR-21 for overall survival (OS) as well as corresponding 95% confidential interval (CI) and *P* value. Multivariate Cox hazard regression analysis was used in the present analysis. When HR data were not available but appropriate summary statistics or Kaplan-Meier curves were provided, we calculated HR using the described method [13]. Disagreements were resolved by discussion. All the data were subject to consensus.

Statistical analysis

All these HRs and 95% CI were calculated following Tierney's method and the logHR and standard error (logHR) were used for aggregation of the survival results. Generally, an observed HR of > 1 implied worse survival for the group with circulating miR-21 overexpression. This impact of high expression of circulating miR-21 on the survival rate was considered statistically significant if the 95% CI for the overall HR did not overlap in the forest plot. A test of heterogeneity of combined HRs was carried out using Cochran's Q test and Higgins I-squared statistic. Heterogeneity was defined as $p < 0.10$ or $I^2 > 50\%$. Pooled HR was calculated using a fixed effect model or random effect model to evaluate the relationship between circulating miR-21 expression and overall survival. When homogeneity was fine ($p > 0.10$, $I^2 < 50\%$), a fixed effect model was used for secondary analysis. If not, a random effect model was used. Publication bias was evaluated using the funnel plot and Egger's test, $P > 0.05$ was considered indicative of a lack of publication bias. All *P* values were two-sided, and all analyses were performed using the Review

Table 1. Characteristics of the studies included in the meta-analysis

Study	Year	Country	Cancer type	Number	Sample	Cut-off	Method	Survival	HR	Follow-up months
Liu R	2012	China	pancreatic cancer	197	serum	ROC	qRT-PCR	OS	Reported	High (0-8) low (0-24)
Komatsu	2012	Japan	esophageal cancer	50	plasma	ROC	qRT-PCR	OS	DE	0-36
Liu	2013	China	colorectal cancer	200	serum	ROC	qRT-PCR	OS	Reported	36.4 (4-53)
Toiyama	2013	Japan	colorectal cancer	186	serum	ROC	qRT-PCR	OS	Reported	44 (2-84)
Song	2013	China	gastric cancer	103	serum	median	qRT-PCR	OS	Reported	35.9 (24.4-53.1)
Komatsu	2013	Japan	gastric cancer	69	plasma	median	qRT-PCR	OS	Reported	0-40
Menéndez	2013	Spain	colorectal cancer	102	serum	median	qRT-PCR	OS	Reported	23 (0-36)

ROC receiver operating characteristic, qRT-PCR quantitative real-time polymerase chain reaction, OS overall survival, DE data-extrapolated, HR hazard ratio.

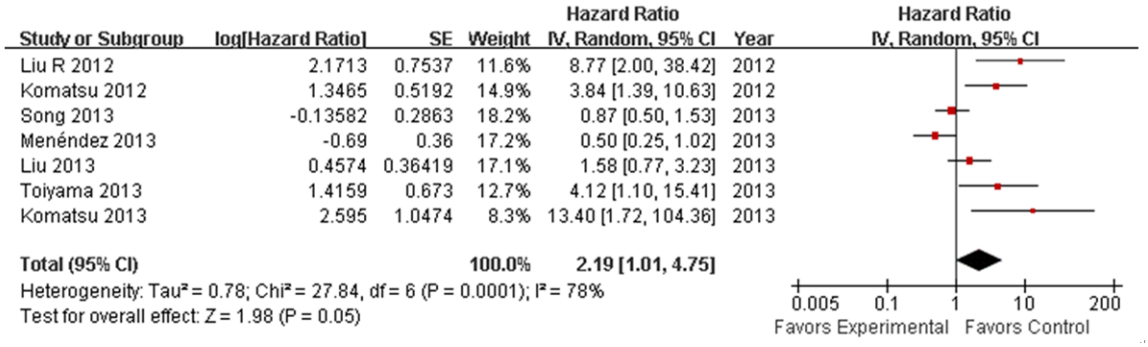

Figure 2. Forest plots of studies evaluating HR of overall survival comparing circulating miR-21 expression.

Manager (v5.0; Oxford, United Kingdom) and Stata soft-ware (StataCorp, College Station, TX, USA).

Results

Studies characteristics

78 publications were identified by initial screening. After manually screening titles, abstracts and key data, 24 records were excluded for not meeting the inclusion criteria. Further review, 11 reports were excluded because they were either laboratory studies or records without survival data. Then, 13 potential candidate studies were evaluated. Two studies only provided disease free survival data without OS were excluded [14, 15]. Four studies were not involved in digestive system cancers. Finally, seven studies were included in this meta-analysis, which were published between 2012 and 2013 [5-11]. Flow diagram of the studies identification and selection was shown in **Figure 1**.

A total of 907 participants were enrolled in the seven studies. There studies were all retrospective in design. Three studies evaluated patients from China, three evaluated patients from Japan, one evaluated patients from Spain. The types of cancers in these studies included pancreatic cancer, esophageal cancer, gastric cancer (n = 2), colorectal cancer (n = 3). The method of circulating miR-21 detection was all quantitative real-time polymerase chain reaction (qRT-PCR). miR-21 expression levels were measured in serum (n = 5) or plasma (n = 2). In each study, the cut-off values of circulating miR-21 appeared to be different. The main characteristics of the included studies were summarized in **Table 1**.

Meta-analysis

For studies evaluating the association between circulating miR-21 expression and OS, there appeared obvious heterogeneity among those studies for circulating miR-21 ($P = 0.0001$, $I^2 = 78\%$). Therefore, the random-effect model was used to calculate the pooled HR with corresponding 95% CI. The result showed that elevated expression level of circulating miR-21 significantly predicted worse OS in digestive system cancers, with the pooled HR of 2.19 (95% CI 1.01-4.75, P = 0.05) (**Figure 2**). In subgroup analysis, when we grouped the meta-analysis by the ethnicity, we found that the pooled HR of Asian studies was 2.90 (95% CI 1.30-6.45, P = 0.009) (**Figure 3**). But subgroup

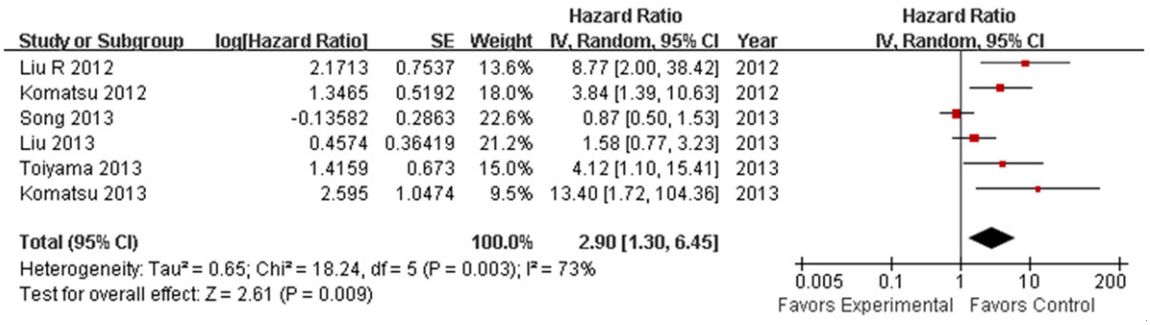

Figure 3. Subgroup analysis evaluating HR of overall survival comparing circulating miR-21 expression in Asian subjects.

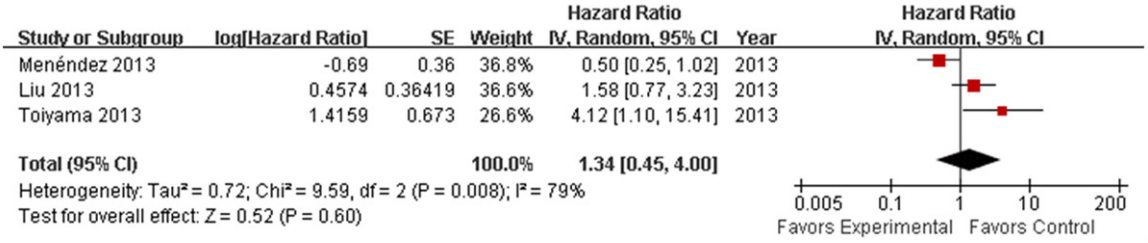

Figure 4. Subgroup analysis evaluating HR of overall survival comparing circulating miR-21 expression in colorectal cancer.

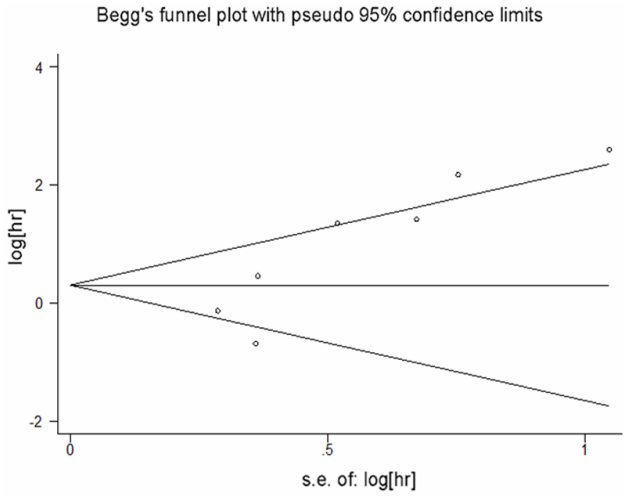

Figure 5. Begg's funnel plot for publication bias analysis.

analysis by digestive system site revealed that there is no significant correlation between circulating miR-21 and OS in patients with colorectal cancer with the pooled HR of 1.34 (95% CI 0.45-4.00, P = 0.60) (**Figure 4**).

Publication bias

Begg's funnel plot and Egger's test were used to assess the publication bias of the included studies. The shape of the funnel plot reveals evidence of asymmetry (**Figure 5**). *P* value of Egger's regression intercept was 0.013, indicating that there was a publication bias in the meta-analysis.

Discussion

During the last decade, miRNAs have been considered as potential biomarkers for cancer prognosis because they have unique expression profiles in cancerous tissue or serum. Compared to normal one, they have more stable expression than mRNA and can be easily assessed by qRT-PCR [16]. Despite early studies on miRNA expression have been performed on tissue specimens, the circulating miRNAs increasingly come to be the focus as biomarkers for diagnosis, prognosis and efficacy of treatment in cancer, because it can be easily achieved without invasive procedure [17-22].

Recent studies have shown that miR-21 acts as an oncogene in cells and the molecular mechanism by which it regulates cellular processes has been studied [23]. Studies have revealed that overexpression of miR-21 could increase cell proliferation, migration, invasion, and sur-

vival in a variety of cancer cell lines [24]. Higher levels of miR-21 expression have been found to be predictive of cancer outcome. Clinical investigation have demonstrated that circulating miR-21 was a potent prognostic biomarker in digestive system cancers, but remained inconsistent. Therefore, we performed this meta-analysis to clarify the prognostic value of circulating miR-21 in digestive system cancers. To our knowledge, it is the most comprehensive meta-analysis concerning the prognostic value of circulating miR-21 in digestive system cancers. The study indicated that elevated circulating miR-21 was significantly associated with poor overall survival in digestive system cancers. Furthermore, the subgroup analysis showed that the association was more prominent in studies of Asian subjects.

However, several limitations of the meta-analysis should be mentioned. First, the samples and studies were limited, with a presence of heterogeneity between the studies. The heterogeneity was probably due to the difference in baseline demographic, characters of population, the tumor types, the disease stages, the cut-off value of circulating miR-21 expression, the duration of follow-up. Second, the result for OS was inconsistent with that for OS in colorectal cancer. This result may be due to the insufficient reports concerning the relationship between circulating miR-21 and prognosis in colorectal cancer. Third, it was observed evidence for possible publication bias. It should be noted that publication bias is not the only explanation for an asymmetrical funnel plot, and the results of formal tests of bias based on the funnel plot should therefore be interpreted with caution. Other possibilities include other selection biases, true heterogeneity, and data irregularities. Finally, in the present meta-analysis, elevated circulating miR-21 was found to have a prognostic role in digestive system cancers, but it was not possible to confirm circulating miR-21 as independent predictive factor. Recently, researchers considered that a panel of miRNAs might have a better predictive role in cancer than a single miRNA.

In conclusion, the study revealed that aberrant overexpression of circulating miR-21 is associated with unfavorable survival in digestive system cancers. The remarkable potential of circulating miR-21 as prognostic biomarkers cannot be underestimated. In the future, well-designed and multi-center prospective studies with larger sample size should be performed before the application of circulating miR-21 in prognosis of digestive system cancers, particularly a single cancer type.

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Disclosure of conflict of interest

None.

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