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Prognostic Role of MicroRNA-21 in Gastric Cancer: A Meta-Analysis

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Background: Mounting evidence shows that microRNAs may be useful as prognostic biomarkers of gastric cancer. The aim of this meta-analysis was to summarize the predictive role of miR-21 for survival in patients with gastric cancer and to verify the association between expression of miR-21 and clinical characteristics.

Material/Methods: All the eligible studies were searched by PubMed and EMBASE and clinical characteristics and survival results were extracted. Then a meta-analysis was carried out to clarify the prognostic role of the miR-21 expression in different subgroups.

Results: We included 8 studies dealing with gastric cancer in this meta-analysis. For overall survival, the pooled hazard ratio of higher miR-21 expression in tumor tissue was 2.00 (95% CI: 1.39–2.88, $P < 0.01$), which could significantly predict poorer survival in gastric cancer patients. Importantly, subgroup analysis suggested that higher expression of miR-21 correlated with tumor differentiation 0.42 (95% CI: 0.25–0.70 $p < 0.01$), lymph node metastasis 6.39 (95% CI: 3.11–13.14, $P < 0.01$), and TNM stage 0.38 (95% CI: 0.21–0.67, $P < 0.01$).

Conclusions: This meta-analysis indicates that miR-21 detection has a prognostic value in patients with gastric cancer. In addition, overexpression of miR-21 is associated with worse tumor differentiation, lymph node metastasis, and TNM stage.

MeSH Keywords: **MicroRNAs • Prognosis • Stomach Neoplasms**

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Background

Gastric cancer is the fourth most commonly diagnosed cancer and the second leading cause of cancer death worldwide. Incidence rates (per 100 000) vary from 3.3 in men and 2.0 in women in Egypt to 65.9 in men and 25.9 in women in Korea [1,2]. In China, there are nearly 460 000 new cases of gastric cancer and 350 000 deaths each year [3]. Despite many advances in GC therapy, the prognosis for patients with gastric cancer remains poor and the 5-year survival rate is 5–20%. Therefore, there is an increasingly urgent need to find prognostic markers that can accurately display biological features of GC and predict the clinical results to heighten the clinical management of GC patients.

MicroRNAs (miRNAs) are a large family of about 22-nucleotide-long RNAs that play important roles by targeting mRNAs as key post-transcriptional regulators of gene expression [4–7]. Mounting evidence suggests that some miRNAs may function as oncogenes or tumor suppressors by regulating cell proliferation and other related biological behaviors [8–10].

MicroRNA-21 (miR-21) is one of the most widely studied oncogenic miRNAs [4,11]. Many studies have investigated the role of miR-21 in the prognosis of GC, with varied results. This study aimed to summarize the evidence for the usefulness of miR-21 in predicting the clinical outcomes of GC patients. We also evaluated the overall risk of raised miR-21 for survival in patients with GC.

Material and Methods

Search strategy

We searched PubMed, Web of Science, and EMBASE from 1966 to 30 February 2014 to identify relevant studies. The search used the following keywords: ‘microRNA- 21’ or ‘miR-21’ and ‘gastric cancer’ or ‘stomach cancer’ and ‘miRNAs and gastric cancer prognosis.’ Studies were considered eligible if they met the following criteria: (I) studied patients with gastric cancer; (II) measured expression of miR-21 in tissue or serum; and (III) investigated the survival outcome or the correlation between miR-21 expression and the clinical variables. Articles were excluded based on the following criteria: (I) review articles or letters, (II) non-English articles, (III) lacked key information for calculation with methods developed by Parmar, Williamson, and Tierney [12–14].

Data extraction

Eligible articles were reviewed independently by 2 investigators (Zhenqiang Wang and Qiang Cai). Any disagreement on a controversial study was resolved by discussion and consensus. Multivariate Cox hazard regression analysis reported in the article

was included in our analysis; if these data were not available, we extracted univariate Cox hazard regression analysis or log-rank p value and Kaplan-Meier survival curves of survival outcomes instead. The above primary information was extracted by 2 investigators working independently. Additional data extracted from the studies included first author, publication year, study size, patient age and sex, TNM stage, lymph node metastasis, histological classification, methods used to detect miR-21, positive miR-21 definition, the conclusion, and other clinical characteristics.

Statistical methods

All these HRs and 95% confidence interval (CI) were calculated following Tierney’s method [13] and the logHR and SE (logHR) (SE) were used for aggregation of the survival results, but these statistical variables were not directly stated in most studies. We calculated the necessary statistics on the basis of available numerical data with methods developed by Parmar, Williamson, and Tierney [13,14]. We also investigate the correlation between miR-21 expression and the clinical variables, including TNM stage, lymph node metastasis, and histological type. According to clinical characteristics, Stage I and Stage II were combined and Stage III and Stage IV were combined. Odds ratio (OR) was used as the measure.

Heterogeneity of combined HRs was conducted using Cochran’s Q test and Higgins I-squared statistic. Heterogeneity was defined as $p < 0.10$ or $I^2 > 50\%$. A random effects model was used if heterogeneity was observed ($P < 0.10$), and the fixed effects model was used in the absence of between-study heterogeneity ($P \geq 0.1$) [15]. Publication bias was evaluated using the funnel plot. Forrest plots were used to estimate the effect of miR-21 expression on survival outcome and the correlation between miR-21 expression and the clinical variables. All of the above calculations were performed using RevMan 5.2 (Cochrane collaboration, Oxford, UK) software.

Results

Characteristics of the eligible studies

We initially searched 180 studies in PubMed and EMBASE. With the review of these abstracts, 13 potentially relevant studies were identified as eligible for full-text review. Five studies were excluded because they lacked the data necessary for calculation. Finally, 8 studies were identified according to the inclusion criteria of the meta-analysis (Figure 1). These eligible studies were published from 2008 to 2013 and included a total of 523 patients. Among all eligible studies, 5 studies [16–20] were used to analyze the correlation between miR-21 and overall survival rate and 5 studies [17,19,21–23] were chosen to demonstrate the connection of miR-21 expression

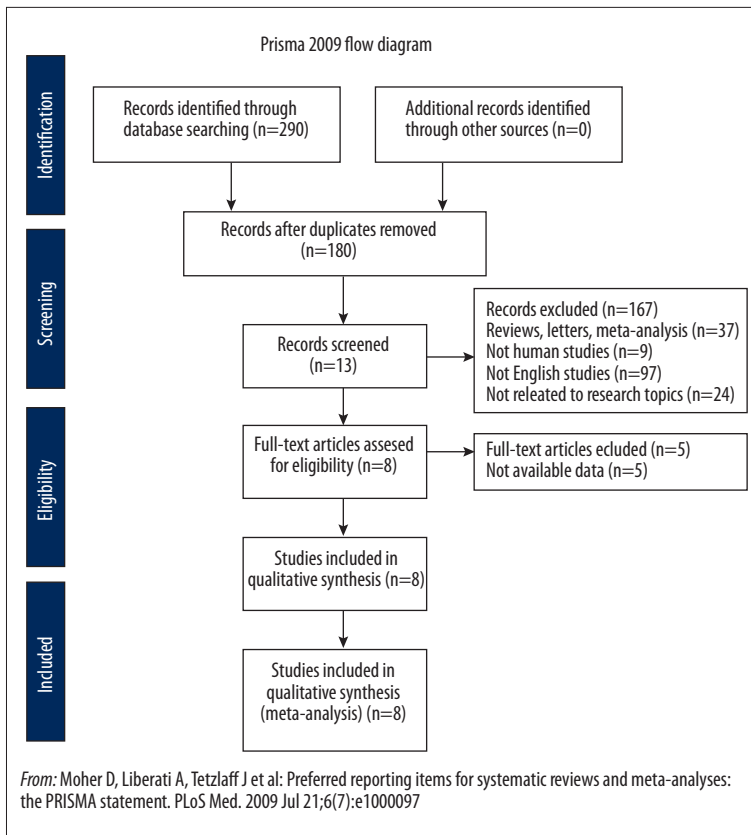


Figure 1. Selection of studies.

Table 1. Clinical characteristics of included studies.

Author	Publication year	Origin of population	Number of patient	Sample	TNM stage	miR-21 assay	Cut-off	Survival analysis	Hazard ratios
Soshi Osawa	2011	Japan	37	Tumor tissue	I-IV	qRT-PCR	Median	OS	Reported
Jianning Song	2013	China	103	Serum	I-IV	qRT-PCR	Median	OS	Reported
Jingting Jiang	2011	China	56	Tumor tissue	I-IV	qRT-PCR	Median	OS	Reported
Yuejuan Xu	2012	China	86	Tumor tissue	I-IV	qRT-PCR	Median	OS	Reported
Shin-hsuan Chan	2008	China	37	Tumor tissue	I-IV	qRT-PCR	2-fold	–	Reported
Kazuo Motoyama	2010	Japan	105	Tumor tissue	I-IV	qRT-PCR	Median	–	–
Baogui Zhang	2011	China	30	Tumor tissue	I-IV	qRT-PCR	Median	–	–
Kamastu.S	2013	Japan	69	Tumor tissue	I-IV	qRT-PCR	Median	OS	Reported

and clinical features. The main features of eligible studies are summarized in Table 1.

High expression of miR-21 predicts poor survival

The meta-analysis of 5 studies for OS showed significant prognostic effect on miR-21 detected in tumor samples and serum. The method for detecting miR-21 expression was quantitative real-time PCR (qRT-PCR). In each study, the cut-off values of

miR-21 appeared to be different. As shown in Figure 2 A, higher expression levels of miR-21 significantly predicted poorer survival, with the pooled HR of 2.00 (95% CI: 1.39–2.88, P<0.01) and heterogeneity of the 5 studies was P=0.17, I²=38%. Observed HR>1 indicated a worse outcome for the positive group relative to the negative group and would be considered statistically significant if the 95% CI did not overlap 1. Based on data displayed in Figure 2A, we conclude that high expression of miR-21 indicates poor survival in gastric cancer patients.

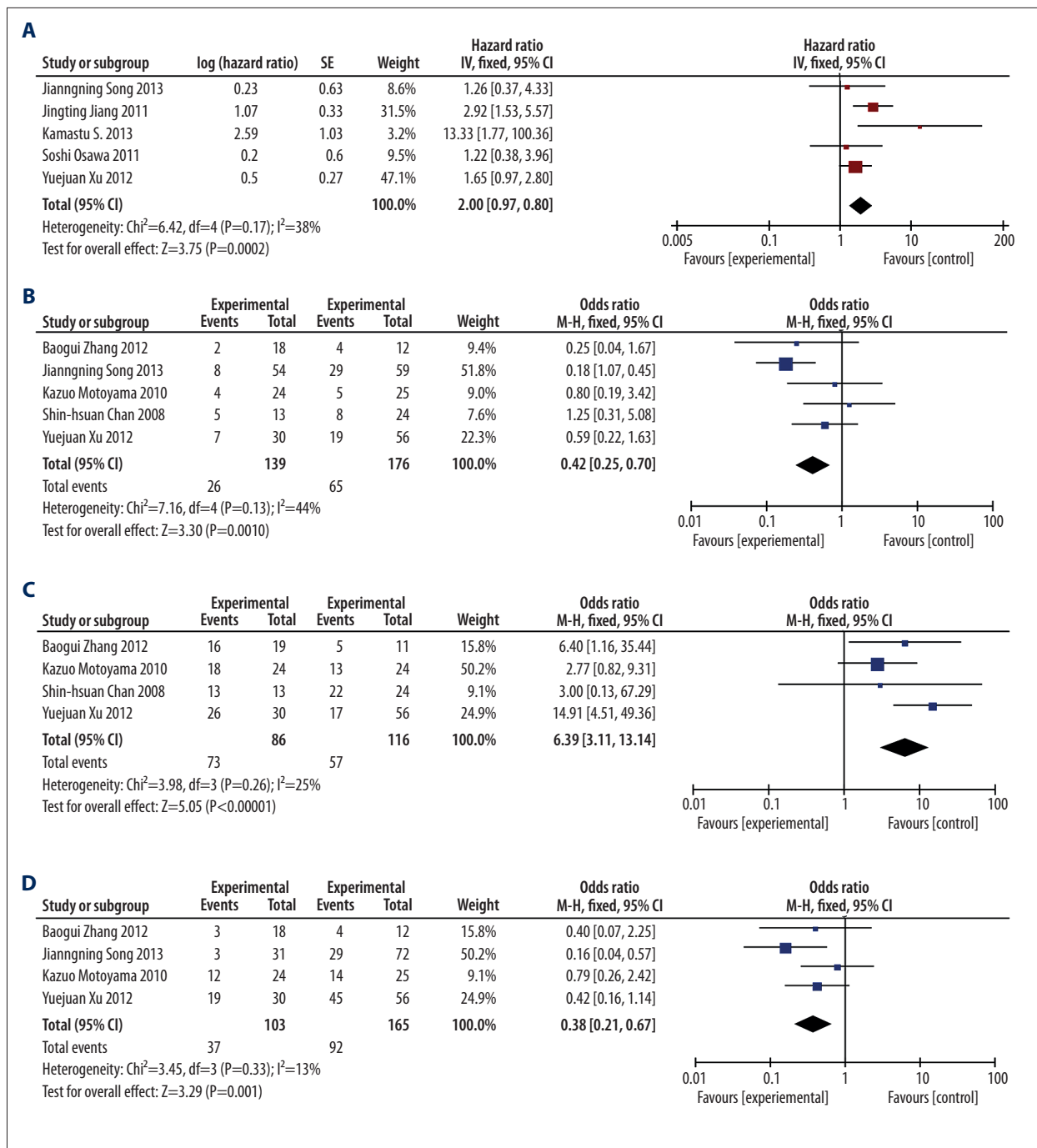


Figure 2. The association between high miR-21 expression and survival of patients with gastric cancer (A). Estimated odds ratios (ORs) Summary for correlation of (B) miR-21 expression and histological differentiation, (C) miR-21 expression and lymph node status, and (D) miR-21 expression and TNM stage.

Correlation between miR-21 expression and clinical characteristics

There were 5 studies that reported correlations between miR-21 expression and some clinical characteristics (TNM stage, lymph node metastasis, and histological type); these

were pooled to calculate the ORs. In our study, we found that miR-21 expression was significantly correlated with tumor differentiation, lymph node metastasis, and TNM stage. The ORs and the corresponding CIs of studies reporting tumor differentiation, lymph node metastasis, and TNM stage were 0.42 (95% CI: 0.25–0.70 $p<0.01$), 6.39 (95% CI:

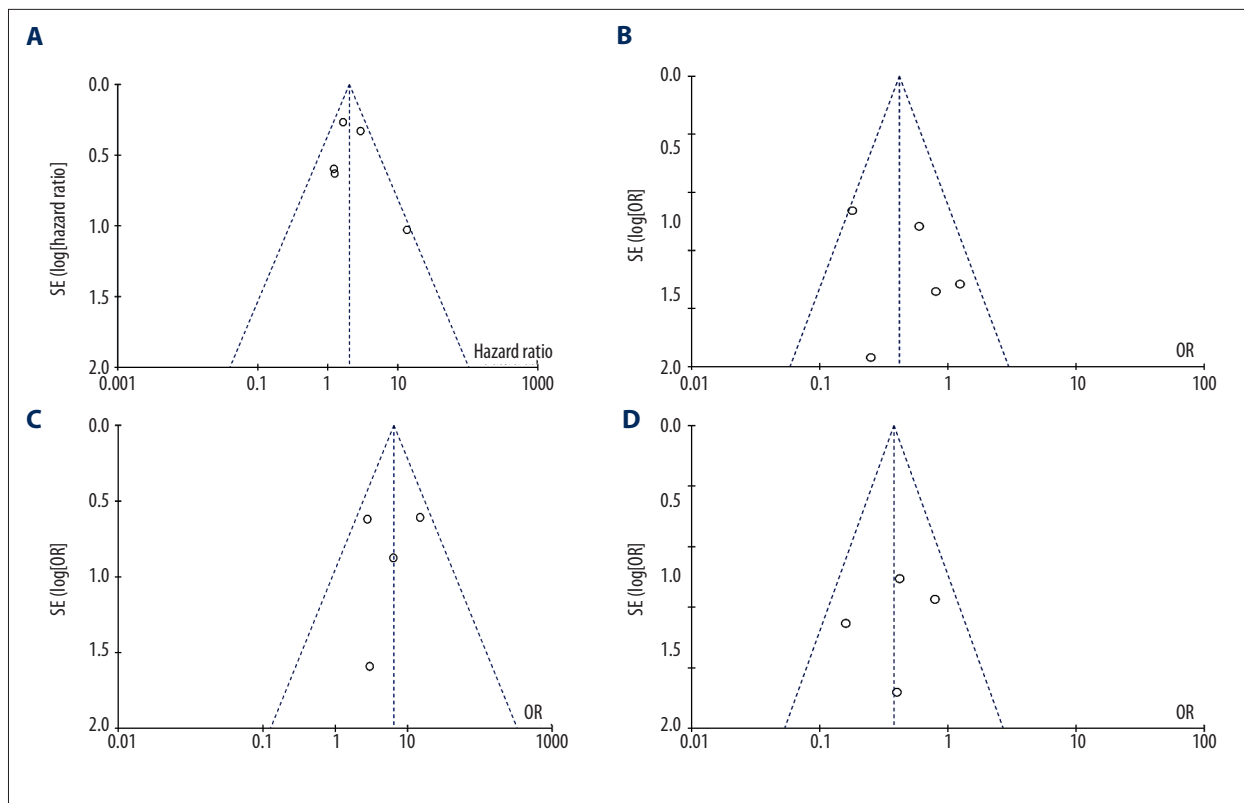


Figure 3. Funnel plots of studies included in the meta-analysis: (A) overall survival, (B) histological differentiation, (C) lymph node status, and (D) TNM stage.

3.11–13.14, $P < 0.01$), 0.38 (95% CI: 0.21–0.67, $P < 0.01$), respectively (Figure 2B–2D).

Assessment of publication bias

The funnel plot test was used to examine publication bias (Figure 3A–3D). No significant publication biases were found in the results of meta-analyses of miR-21 prediction value for OS using tumor and serum samples. There was also no publication bias in the studies for clinical characteristics.

Sensitivity analysis

Sensitivity analyses were performed to assess the contribution of each study to the pooled estimate by excluding individual studies one at a time and recalculating the pooled HR or OR estimates for the remaining studies. Eliminating any one study in each group did not substantially change the pooled point estimate, which indicates the reliability of our results.

Discussion

The increased use of miRNAs in cancer research has substantially changed the understanding of gene regulation. MiRNAs

play a key role in tumor development by regulating the expression of oncogenes and tumor suppressors or by directly exerting their functions as oncogenes or tumor suppressors [24–26]. The prognostic values of miR-21 have been shown in the recent meta-analysis in head and neck squamous cell carcinoma (HNSCC) [27] and non-small cell lung cancer (NSCLC) [28]. Although the potential diagnostic effect of miR-21 in gastric cancer had already been reported [29], the prognostic role of miR-21 in gastric cancer is still unclear. In addition, the relation between gastric cancer patients' clinical characteristics and expression level of miR-21 still needs to be defined. This systematic review and meta-analysis showed that elevated miR-21 did predict poor survival in patients with GC. Although the number of relevant prognostic studies is limited, the available data show that miR-21 is associated with the OS of gastric cancer. As far as we know, this is the first meta-analysis of the prognostic value of miR-21 for GC.

In this meta-analysis, we calculated pooled HRs from 454 patients with gastric cancer from 5 studies. Our result of pooled HR for OS was 2.00 (95% CI: 1.39–2.88, $P < 0.01$). The interval of HR did not overlap 1, which demonstrated that the miR-21 expression did have prognostic significance on GC. In the correlation study of miR-21 expression with patients' clinical characteristics, ORs for TNM stage, lymph node metastasis,

and histological type were significant. The over-expression of miR-21 is positively related to poor histological type, lymph node metastasis, and high-grade of TNM stage, which means gastric cancer patients with the clinical characteristics mentioned above were more likely to have shorter survival time and poorer prognosis after the diagnosis was made.

The association between miR-21 expression and cancer outcome may be partly caused by the biological function of the miR-21. Recent studies have provided supporting evidence for an oncogenic role of miR-21 and shed light on the molecular mechanisms by which it regulates cellular processes. Elevated miR-21 can increase cell proliferation, colony formation, and migration and miR-21 had been reported to regulate the metastatic behavior in both mice and humans by activating various signaling pathways [22,30].

Although we tried to avoid biases in performing this meta-analysis, there were still several limitations to our study. Firstly, appreciation of the oncogenic role of miR-21 in GC has grown in recent years, but only limited research has been done on its relationship to prognosis and clinical characteristics. Results regarding GC outcomes were less powerful because only 5 studies were included for OS meta-analysis, with a relatively small sample size of 351 patients. Secondly, funnel plots showed no evidence of publication bias, but these studies are all from

China and Japan, indicating possible geographic publication bias. Thirdly, this meta-analysis could not provide a clear definition about “how high is high” and a clear definition is needed of the cut-off value of miR-21 level for increased survival risk. To date, most researchers use median or mean value in their laboratory or hospital as the cut-off value but the actual value was different. Lack of abundant miR-21 expression data by qRT-PCR in the global population makes it difficult to set a standard cut-off.

Conclusions

High expression of miR-21 was significantly associated with poor survival in patients with gastric cancer. In addition, over-expression of miR-21 is associated with worse tumor differentiation, lymph node metastasis, and TNM stage. Given its limitations, the results of the present analysis should be interpreted with caution. Further clinical investigations are needed to determine the association between miR-21 and cancer prognosis, as well as the efficiency of therapies. More clinical investigations should be conducted before miR-21 can be implemented in the routine clinical management of cancer.

Conflict of Interest

The authors have declared that no competing interests exist.

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