Original Article
Prognostic role of microRNA-25 in cancers: evidence from a meta-analysis

Jingjing Qu, Min Li, Wen Zhong, Chengping Hu

Department of Respiratory Medicine, Xiangya Hospital, Central South University, Changsha 410008, Hunan, China

Abstract: Background: miRNAs have been participated in human carcinogenesis as tumor oncogenes or suppressors, and have a prognostic significant for patients with cancers. In recent years, miR-25 was found associated with clinical value of cancerous patients. Meanwhile the miR-25 showed a different expression in related studies. Therefore, we summarize the results from a variety of studies using a meta-analysis, in order to explain the prognostic value of miR-25 in many human cancers. Methods: The articles were retrieved from the on-line databases, PubMed, EMBASE and CNKI. We extracted and estimated the hazard ratios (HRs) for overall survival (OS), which compared the high and low expression levels of miR-25 in patients with a different of cancers. Pooled HRs and 95% confidence intervals (CIs) were calculated. Results: Eight studies of 868 patients were selected into the final meta-analysis after a strict filtering and qualifying process. Fixed model or random model method was chosen depending on the heterogeneity between the studies. For OS, higher miR-25 expression could significantly predict worse outcome with the pooled HR of 2.434 (95% CI 1.330-3.539, *P*=0.000). The subgroup analysis showed that high expressed miR-25 could worsen OS in Chinese patients with pooled HR of 1.895 (95% CI 1.096-2.693, *P*=0.007). The sensitive analysis showed that removing the document one by one, there no obvious change of HR of 0.823 (95% CI 0.385-1.260). No bias existed in the group. Conclusion: Our study indicates that high expression level of miR-25 corresponds with poor survival in cancerous patients, and the expression of miR-25 could be a promising prognostic biomarker in the future.

Keywords: miR-25, cancer, biomarker, prognostic factor, meta-analysis

Introduction

microRNAs (miRNAs) are small noncoding endogenous RNA molecules of 19-25 nucleotides. They could bind to complementary sequences in the 3' untranslated region of target mRNAs, leading to mRNA degradation or translational repression. They suppress gene expression by binding to the complementary regions of mRNA, which either blocks translation or degrades mRNA through the RNA-induced silencing complex [1]. Recently, evidence from clinical studies has demonstrated that many miRNAs deregulated in variety of cancers, and the expression levels of some miRNAs correlated with clinicopathological features of diseases [2, 3]. Thus, these miRNAs could be potential biomarkers for cancer.

miR-25 is a member of the miR-106b-25 cluster which consists of miR-106b, miR-25 and miR-93, and is located within intron 13 of the minichromosome maintenance complex component 7 (MCM7) gene on chromosome 7q22.1 [4, 5]. The effects of miR-25 on cancer are complicated because it can be oncogenic or tumor suppressive in different types of cancer. Therefore, the prognostic value of circulating miR-25 in patients with cancer remains controversial. In terms of the limits of the single study, the aim of this meta-analysis was to evaluate the relationship between miR-25 and the survival outcome in patients with cancer.

Materials and methods

Search strategy

We performed a meta-analysis following the guidelines of the Meta-analysis of Observational Studies in Epidemiology group (MOOSE) [14]. We carefully searched online database PubMed,
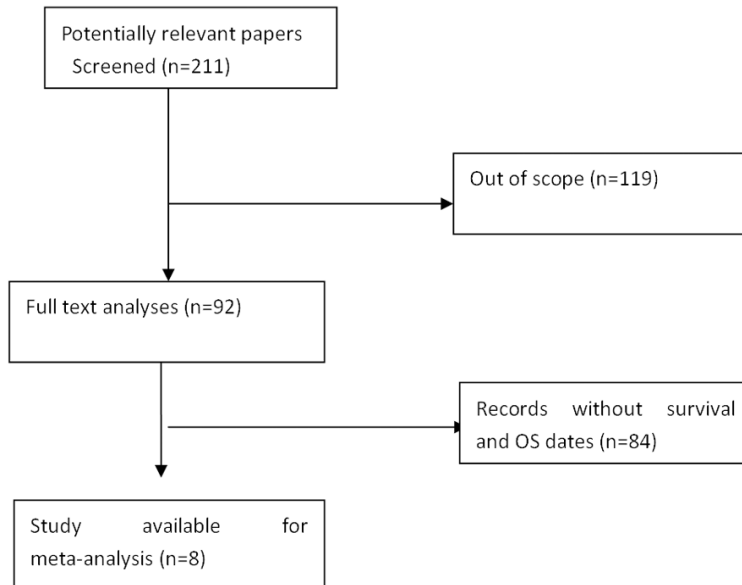
Figure 1. Flow diagram of the identification and selection of studies.

EMBASE and CNKI to identify relevant published studies from Jan 1st, 1993 to Dec 12, 2014. Two sets of key words were used among that process, namely "miR-25 and cancer" and "microRNA-25 and cancer". Annual review of the references of relevant publications was also performed to obtain additional studies.

Inclusion and exclusion criteria

We used references manager software EndNote to check out duplications. And according the follow inclusive criteria to choose studies: (1) it studied miR-25 in a variety of human cancers; (2) it must be human samples; (3) it studied the association between miR-25 and survival outcome. The candidate articles were manually chosen by 2 authors (JJ Qu and W Zhong) independently. The studies were excluded if they were: (1) investigation of a set of miRNAs but not miR-25 alone; (2) review or letters; (3) non-dichotomous miR-25 expression levels; (4) or lack of important information such as hazard ratio (HR), 95% CI and *P* value. If we needed addition information and data which cannot been found in article, we will e-mailed the authors. M Li supervised the entire process.

Quality assessment

All of the quality of studies were assessed by the two authors according to a basic standard as follows: (1) clear report of study origin of country and population, (2) clear method of

study design, (3) clear definition of outcome assessment, (4) clear definition of type of cancer. The study was removed if not including the basic standard 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, sample size, tumor type, method of testing miR-25, and HR of miR-25 for overall survival (OS), as well as the corresponding 95% CI. If the HR and CI were not reported directly, the total observed death events and the numbers of patients in each group were extracted to calculate HR and its variance indirectly [15]. If only Kaplan-Meier curves are available, data was extracted from the graphical survival plots. Disagreements were resolved by discussion. All the data were resolved by consensus.

Statistical analysis

Firstly, HR with 95% CI was used to combine the pooled data. We used the chi-square based Q-test to test the statistical heterogeneity of studies, then identified the absence of heterogeneity across studies, after used fixed-effects model (the Mantel-Haenszel method), otherwise, we will performed the random effect model. Heterogeneity was defined as $P<0.10$ or $I^2>50\%$. Secondly, subgroup and sensitivity analysis was carried out by investigating the influence of a single study on the overall HR. Finally, we used the methods of Begg plots and Egger test to analysis publication bias ($P<0.05$ was considered representative of statistically significant publication bias). All of the analyses were carried out using STATA v11.0 (Stata Corp., College Station, TX).

Results

Study inclusion and characteristics

A total of 211 articles were identified by initial search. After screening titles, abstracts and

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

| Study	Year	Country	Disease	Number	Sample	Assay	Survival	HR Reported
X Wang [11]	2014	China	Ovarian cancer	86	Tissue	qRT-PCR	OS	2.119 (1.568-3.221)
Zhongxue Su [12]	2014	China	Hepatocellular	131	Tissue	qRT-PCR	OS	2.179 (1.876-4.335)
Fangxiu Xu [9]	2014	China	Lung adenocarcinoma	100	Tissue+Plasma	qRT-PCR	OS	2.34 (1.02-5.35)
Xiaojun Li [8]	2014	China	Colorectal	186	Tissue	qRT-PCR	OS	1.90 (1.22-2.97)
Yungui Wang [10]	2011	China	AML	53	Bone marrow samples	qRT-PCR	OS	0.648551 (0.36-1.45)
Caiyun Wu [7]	2014	China	Oesophageal Squamous cell carcinoma	111	Serum	qRT-PCR	OS	3.84 (1.02-14.41)
Baek-Hui Kim [6]	2012	Korea	Gastric carcinoma	91	Tissue	qRT-PCR	OS	4.52 (3.39-5.38)
Xiaohui Xu [13]	2013	China	Esophageal Squamous cell carcinoma	86	Tissue	qRT-PCR	OS	3.87 (0.65-5.83)
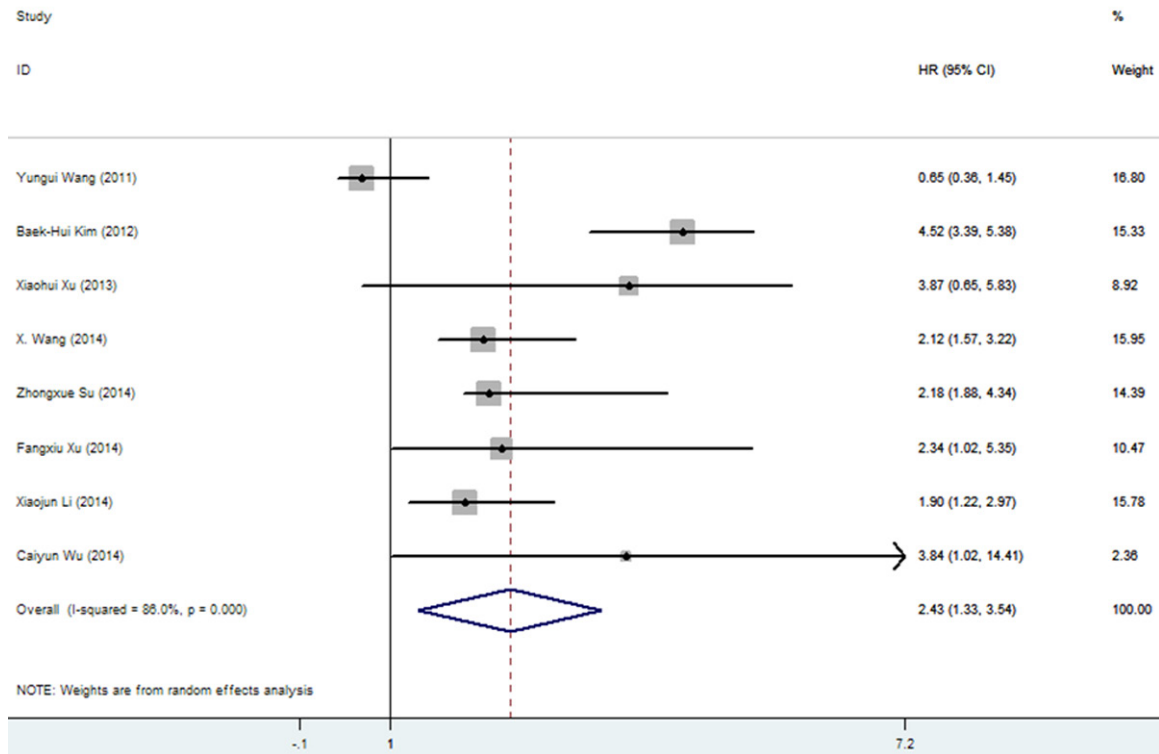
Figure 2. Forest plots of studies evaluating HR of overall survival comparing high- and low miR-25 expression.

key data, 119 articles were excluded for not meeting the inclusion criteria. After reading the full texts of the remaining, 84 reports were excluded because they were not records without survival data. Then, 8 potential candidate studies were evaluated between 2011 and 2014 (**Figure 1**).

Table 1 shows the main results of this meta-analysis. A total of 868 participants were enrolled in the 8 studies. Seven studies evaluated patients from China, 1 evaluated from Korea, The types of cancers in these studies included ovarian cancer, hepatocellular cancer, non-small cell lung cancer, colorectal cancer, acute myelocytic leukemia, esophageal cancer, gastric cancer. The method of miR-25 detection was all quantitative real-time polymerase chain reaction (qRT-PCR). miR-25 expression levels were measured in plasma or tissue.

Meta-analysis

For evaluating the association between miR-25 expression and OS, there appeared obvious heterogeneity among those studies for miR-25 (P=0.000, I^2=86.0%). Therefore, the random-

effect model was used to calculate the pooled HR with the corresponding 95% CI. The result showed that a higher expression level of miR-25 significantly predicted worse OS in various carcinomas, with the pooled HR of 2.434 (95% CI 1.330-3.539, P=0.000) (**Figure 2**). Interestingly, subgroup analysis was performed in the ethnicity, it was found that the I^2=66.1%, pooled HR of OS was 1.895 (95% CI 1.096-2.693, P=0.007) in China population with cancers, indicating racial maybe the source of heterogeneity (**Figure 3**). Meanwhile, the sensitive analysis was performed by omitting one study at each time to measure its effect on the pooled HR. As presented in **Figure 4**, no individual study influenced the overall HR dominantly.

Publication bias

Finally, Begg's funnel plot and Egger's test were used to evaluate the publication bias of the included studies. The shape of the funnel plot did not reveal any evidence of obvious asymmetry. The P value of Egger's regression intercept was 0.242, indicating that there was no significant publication bias in the meta-analysis.
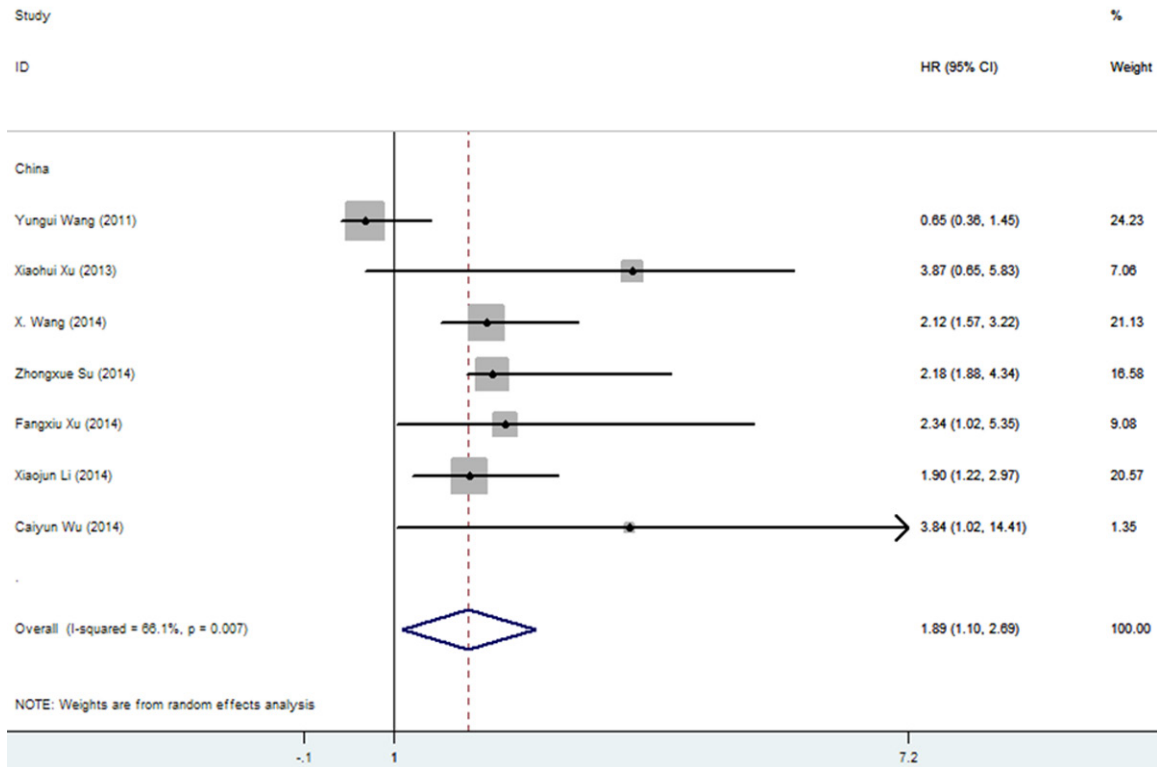
Figure 3. Subgroup analysis evaluating HR of overall survival comparing high- and low-miR-25 expression in Chinese.

Discussion

miRNAs regulate gene expression at post-transcriptional level and play a pivotal role in the regulation of cell development, metabolism, proliferation, differentiation and apoptosis. Thus, they may serve as potential biomarkers to indicate different disease courses and outcomes [16, 17]. Previous studies have shown that the expression of miR-25 was up-regulated significantly in several types of cancers, such as small cell lung cancer, gastric cancer, liver cancer [18-20]. Zhao and his colleagues [18] find that miR-25 was overexpressed in both SCLC cells and human SCLC tumor tissues. Down-regulation of miR-25 significantly reduced cancer cell growth, invasive capability and resistance to cisplatin. Also, it induced G1 cell cycle arrest and downregulated cell cycle related proteins cyclin E2 and CDK2. Li and his colleagues [19] find that miR-25 was overexpressed in plasma and primary tumor tissues of gastric cancer (GC) patients with tumor node metastasis stage (III or IV) or lymph node metastasis. miR-25 inhibition significantly decreased the metastasis, invasion and prolifera-

tion of GC cells in vitro, and reduced their capacity to develop distal pulmonary metastases and peritoneal dissemination in vivo. In the study by Li [20], more than 50% of the hepatocellular carcinoma samples showed a greater than two fold increase in the expression of the miR-106b-25 cluster when compared with the corresponding paired (or counterpart) non-tumor samples. Knock-down studies for the miR-106b-25 cluster, which includes miR-106b, miR-93 and miR-25, showed that the expression of the cluster was necessary for cell proliferation and for inhibited independent growth. However, some investigators found that the expression of miR-25 had a reverse expression in other cancers. For example, Zhou [21] found that high expression of miR-25 resulted in inhibit colony formation, metasomatic in both cell lines. It is reported the overexpression of miR-25 led to a decreased ANGPTL2 mRNA and protein expression, whereas there was an adverse efficiency when down regulated miR-25. Although miR-25 was found to be expressed in most human cancers and widely studied in tissue or plasma, indicat-
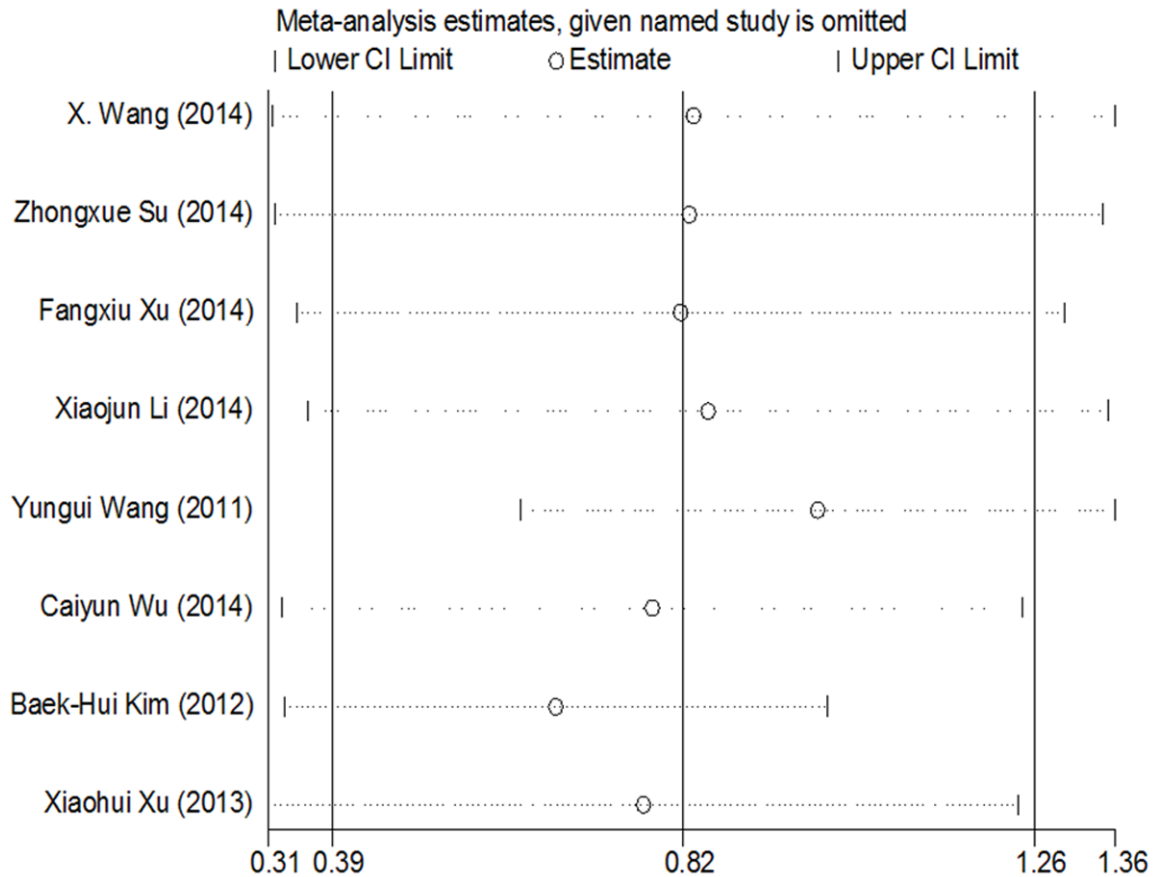
Figure 4. Sensitivity analysis. Removing the document one by one, there no obvious change.

ing it can be used as a potential biomarker for cancer. However the prognostic value of miR-25 in cancers is still a puzzle. Therefore, we conduct this meta-analysis to answer this question, which is the first evaluation we know concerning the relationship between miR-25 expression and the survival in patients with various cancers.

The present meta-analysis showed that miR-25 indeed predict poor survival in carcinomas patients. Furthermore, the subgroup analysis showed that the association was more prominent in Chinese. Because there was some limitations exist in present meta-analysis. Since the number of included studies was relatively small with only about 868 cases and no random clinical trials had been found. Finally, the heterogeneity existed in this meta-analysis and was probably because of the differences in types of cancer, demographic characters of population, disease stages etc. It would be more valuable if data are from different sample

origins. However, it is difficult to perform a meta-analysis in subgroups currently due to the limited sample size.

In summary, the present meta-analysis shows that the highly expressed miR-25 is significantly associated with poor survival in patients with various types of carcinomas. However, the role that miR-25 acts in the process of oncogenes is still unclear to us. Our study could aid in the delineation of this issue by demonstrating miR-25 performance in clinic and provide clues for future investigations.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chengping Hu, Department of Respiratory Medicine, Xiangya Hospital, Central South University, Changsha 410008, Hunan, China. Tel: 086-731-84328029; Fax: 086-731-84328029; E-mail: huchengp28@126. com

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