The prognostic and diagnostic values of MicroRNA-10b in gastric cancer
A comprehensive study based on meta-analysis and TCGA database

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Abstract
We conducted a study to evaluate the prognostic and diagnostic values of microRNA-10b (miR-10b) in gastric cancer (GC) based on meta-analysis and TCGA database. Relevant studies were searched in English and Chinese database and meta-analysis was conducted on Stata 12.0. The expression value of miR-10b and clinical parameters of GC patients were downloaded from TCGA database, and relevant analyses were conducted on SPSS. High expression of miR-10b was linked with unfavorable overall survival (OS) in GC (HR=1.572, 95% CI: 1.240–1.992, P<.001). However, the meta-analysis was significant for patients in early stage, but not for patients in advanced stage. The expression of miR-10b-3p was significantly lower in cancer tissue compared with adjacent tissue (P<.001). Meanwhile, the area under the ROC curve (AUC) value was 0.652 (0.562–0.742, P=.001). Disease-free survival analysis showed increasing miR-10b-5p was correlated with worse survival outcome (HR=2.366, 95% CI: 1.414–3.959, P=.001). In conclusion, miR-10b acts as a tumor suppressor with prognostic and diagnostic values for GC.

Abbreviations: CBM = China Biology Medicine disc, CNKI = China National Knowledge Infrastructure, DFS = disease free survival, GC = gastric cancer, ISH = in situ hybridization, miRNAs, microRNAs, OS = overall survival, TCGA = The Cancer Genome Atlas.

Keywords: gastric cancer, meta-analysis, MicroRNA-10b, TCGA

1. Introduction
Gastric cancer (GC) is the fifth most common cancer worldwide, and the third most common cause of all cancer deaths.[1] About 1 million people are diagnosed with GC worldwide each year.[2]

which cause a high disease burden worldwide.[3] Both genetic and environmental factors are important to the development of GC. Even if improvements in chemotherapy and radiotherapy have been achieved, the average 5-year survival rate for GC patients is less than 40% because of late diagnosis.[4,5] Identifying a reliable marker is important for GC diagnose and prognosis.

MicroRNAs (miRNAs) are small non-coding RNAs, which play a vital role in the pathogenesis of GC.[6–8] Several miRNAs, including miR-1246,[9] miR-421,[10] and miR-515-3p,[11] have been identified as diagnostic markers for GC. miR-10b is located in the homeobox gene cluster which belongs to the transcriptional regulator family.[12] The impact of miR-10b has been explored in several cancers, including colorectal cancer,[13] hepatitis B-related liver cancer,[14] and breast cancer.[15] Recently, several studies[16,17] have explored the relationship between miR-10b and GC. However, the sample size is not enough.

Meta-analysis is a well methodology for pooling the results of different research.[18] Thus, a meta-analysis on the impact of miR-10b on GC was conducted in this study, and data from The Cancer Genome Atlas (TCGA) was used to verify the results. This study is aimed at clarifying the diagnostic and prognostic values of miR-10b in GC.

2. Methods
This study was conducted following the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines. The ethical approval is not required, because this meta-analysis was conducted through reviewing issued papers.
2.1. Search strategy

Relevant studies were searched in PubMed, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, Wiley Online Library and Chinese Databases, including China National Knowledge Infrastructure (CNKI), China Biology Medicine disc (CBM), Chongqing VIP and Wan Fang Data (updated on November 5, 2019) using the following keywords (miR-10b OR miRNA-10b OR microRNA-10b OR miR10b OR miRNA10b OR microRNA10b OR “miR 10b” OR “miRNA 10b” OR “microRNA 10b”) and (malignant OR cancer OR tumor OR tumor OR carcinoma OR adenocarcinoma) and (digestive OR gastric OR stomach). Similar meta-analyses, reviews and references cited in these studies were also evaluated for eligible studies. The searches were performed by 2 authors independently, and any disagreement was resolved through discussion.

2.2. Inclusion and exclusion criteria

Included studies met the following inclusion criteria:

1. study of GC patients, and the expression value of miR-10b was detected;
2. survival analysis or clinicopathological parameters were assessed based on miR-133a expression, and
3. sufficient data was provided to conduct meta-analysis.

The exclusion criteria:

1. miR-10b was combined with other biomarkers to be investigated, and
2. no sufficient data for meta-analysis.

2.3. Data extraction

All data was acquired independently by 2 authors. The following information was collected from included studies: first author, year of publication, country, sample source, stage, detection method, case, follow-up time, survival index, statistical method, HR as well as 95% CI, and the survival outcome of the high miR-10b expression group. When both univariate and multivariate analyses, the result showed that high expression of miR-10b was significantly associated with unfavorable OS (HR = 1.572, 95% CI: 1.240–1.992, P < .001, I² = 47.1%) (Fig. 2A, Table 2). Besides DFS which had been investigated in 2 studies was also analyzed, but no statistical significance was detected (HR = 1.497, 95% CI: 0.795–2.819, P = .212, I² = 40.7%) (Fig. 2B, Table 2). According to different test methods, we conducted further analysis on qRT-PCR and IHC studies, and HR was 1.272 (95% CI: 0.569–2.841, P = .538, I² = 73.4%) and 1.664 (95% CI: 1.320–2.098, P < .001, I² = 32.0%), respectively (Fig. 2C-D, Table 2). In addition, subgroup meta-analyses were conducted on specific cancer stage I-IV, and pooled HRs in these 3 subgroups were found to be 2.023 (95% CI: 1.493–2.74, P < .001, I² = 0%), 2.632 (95% CI: 1.557–4.446, P = .001, I² = 0%), 1.363 (95% CI: 0.959–1.937, P = .084, I² = 0%) and 1.727 (95% CI: 0.799–1.727, P = .412, I² = 83.9%), respectively (Fig. 3, Table 2). Meanwhile, survival data of GC patients from TCGA was also analyzed based on miR-10b expression, and high expression of miR-10b-3p was significantly linked with worse DFS (HR = 2.366, 95% CI: 1.414–3.959, P = .001) (Fig. 4A, Table 3). However, no statistical significance was observed in other survival outcomes, and the HRs were 1.296 (95% CI: 0.933–1.801, P = .122), 1.254 (95% CI: 0.905–1.736, P = .173) and 0.848 (95% CI: 0.53–1.356, P = .49) for miR-10b-5p in OS, miR-10b-3p in DFS, and miR-10b-3p in DFS, respectively (Fig. 4B-D, Table 3).

4.2. MiR-10b with diagnostic value for GC

Three hundred eighty six GC patients were enrolled from TCGA. As shown in Table 4, the expression of miR-10b-3p was assessed by the ROC curve. Survival analysis was investigated by Cox regression. Patients were divided into high or low expression group according to the mean expression level of miR-10b. All statistical analyses were conducted by SPSS statistical software package, version 21.0 (IBM Corporation, Armonk, NY, USA), and P < .05 indicated statistically significant.
significantly lower in cancer tissue compared with adjacent tissue \((P < .001)\). The ROC curve showed a diagnostic value of miR-10b-3p for GC \((P = .001)\), and the optimum diagnostic point with sensitivity and specificity were also showed in Table 3. Corresponding information of miR-10b-5p for GC was summarized in Table 3, and the relationships between miR-10b and clinicopathological parameters were also showed in Table 4.

5. Discussion

Studies have identified that aberrant expression of miRNAs can be used for diagnosis and prediction of prognosis in many cancers, including GC. miR-10b is imbedded in HOX gene clusters on chromosomes 2q.\(^{[23]}\) Until now, several meta-analyses concluded that expression of miR-10b can predict outcomes in some types of cancer.\(^{[24]-[26]}\) However, these meta-analyses did not include GC study. In 2017, Huang et al.\(^{[27]}\) included 1 GC study\(^{[22]}\) and conducted a meta-analysis to show expression of miR-10b strongly predicts poor prognosis for patients with cancers. To the best of our knowledge, this is the first meta-analysis to explore prognostic value of miR-10b in GC. Moreover, data from TCGA was used for validation. We also analyzed diagnostic value of miR-10b in GC by TCGA data analysis. In this study, our meta-analysis demonstrated that high expression of miR-10b was associated with poor OS, but not with DFS. Further TCGA data analysis showed that high expression of miR-10b-5p was related with poor DFS and miR-10b-3p can be a diagnostic marker for GC.

Overall, we observed an association between expression of miR-10b and OS in GC patients. Further subgroup analyses showed high expression of miR-10b related with poor OS in GC patients with stage I or stage II. These results suggested that expression of miR-10b in GC should be noted in stage I or stage II patients, which can be a prognostic marker for those patients. In addition, we found that there was no difference between the expression of miR-10b and DFS in GC patients. This might be caused by the small sample size. Thus, more studies about the relationship between miR-10b and DFS are needed in the future. Further subgroup analysis was conducted based on test methods.

![Flow chart showing the selection process for the including studies.](Figure 1)
Result showed expression of miR-10b related with OS in ISH group, but not in qRT-PCR group. One possible reason is that ISH group has more patients than qRT-PCR group, which can bring more statistical power.

In fact, there are 2 kinds of miR-10b, one is miR-10b-3p, and the other is miR-10b-5p. The 3p strand exists in the reverse position (3' → 5') and the 5p strand is located in the forward position (5' → 3'). The role of miR-10b-3p has been investigated in

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**Table 1**

| Author       | Year | Country | Sample source | Stage | Test method | Case | Follow-up (month) | Survival | Statistic method | HR     | LL     | UL     | Outcome | NOS |
|--------------|------|---------|---------------|-------|-------------|------|-------------------|----------|------------------|--------|--------|--------|---------|-----|
| Wang Y       | 2013 | China   | Tissue        | I     | ISH         | 393  | 60–132            | OS       | Survival curve   | 1.868  | 1.245  | 2.532  | Worse   | 6   |
|              |      |         |               | II    | ISH         |      |                   |          |                  | 2.178  | 1.231  | 3.267  | Worse   |     |
|              |      |         |               | III   | ISH         |      |                   |          |                  | 1.254  | 1.106  | 3.521  | Worse   |     |
|              |      |         |               | IV    | ISH         |      |                   |          |                  | 1.147  | 0.893  | 2.385  | NS      |     |
| Huang Z      | 2017 | China   | Serum         | I-N   | qRT-PCR     | 188  | 50–65             | OS       | Survival curve   | 0.877  | 0.531  | 1.352  | NS      | 7   |
|              |      |         |               |       |             |      |                   |          |                  | 1.124  | 0.857  | 2.964  | NS      |     |
| Gao Y        | 2018 | China   | Tissue        | I     | ISH         | 120  | 60                | OS       | Survival curve   | 2.512  | 1.352  | 4.364  | Worse   | 6   |
|              |      |         |               | II    | ISH         |      |                   |          |                  | 3.225  | 1.278  | 5.792  | Worse   |     |
|              |      |         |               | III   | ISH         |      |                   |          |                  | 1.431  | 1.025  | 2.461  | Worse   |     |
|              |      |         |               | IV    | ISH         |      |                   |          |                  | 1.221  | 0.705  | 2.437  | NS      |     |
| Obermannova  | 2018 | Czech   | Tissue        | I-N   | qRT-PCR     | 67   | 100               | OS       | Univariate analysis | 2.000  | 1.003  | 3.964  | Worse   | 6   |
|  R           |      |         |               |       |             |      |                   |          |                  | 2.155  | 1.053  | 4.831  | Worse   |     |

ISH = in situ hybridization, OS = overall survival, DFS = disease-free survival, HR, hazard ratio, LL = lower limit, UL = upper limit, ∗ = outcome was for patient with high miR-10b expression, NS = not significant, NOS, the scores of Newcastle-Ottawa quality assessment scale.

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**Figure 2.** (A) Forest plot for the association between miR-10b expression and OS of GC; (B) Forest plot for the association between miR-10b expression and DFS of GC; (C) Forest plot for the association between miR-10b expression and OS of GC in qRT-PCR group; (D) Forest plot for the association between miR-10b expression and OS of GC in ISH group.
various cancers, and 1 study showed that miR-10b-3p expression levels were significantly unregulated in the esophageal squamous cell carcinoma tumor tissues.\(^2\) Moreover, Yoon et al.\(^2\) identified that expression of serum miR-10b-3p may prove valuable in the diagnosis of hepatocellular carcinoma. Our TCGA data analysis found that miR-10b-3p is down-regulated in GC tissues compared with normal tissues. Further survival analysis showed that miR-10b-3p is not associated with OS and DFS in GC patients. These findings suggested that miR-10b-3p may not suitable as GC prognostic marker. Notably, we also analyzed the role of miR-10b-5p in GC. Wang et al indicated that miR-10b-5p is down-regulated in breast cancer.\(^3\) Moreover, some studies suggested that miR-10b-5p is an independent prognostic biomarkers for non-small-cell lung cancer\(^4\) and lower grade glioma.\(^5\) Based on TCGA data, our survival analysis found that high expression of miR-10b-5p is related with poor DFS. Thus, miR-10b-5p can be a prognostic biomarker for GC. However, the mechanism by which miR-10b-5p affects the pathogenesis of GC needs to be further illustrated.

The diagnostic value of miR-10b in cancer has been reported in some studies. Lai et al\(^6\) showed that the expression of plasma miR-10b distinguished normal controls from pancreatic ductal adenocarcinoma patients, with a sensitivity and specificity of 100% and 100%, respectively. miR-10b also showed diagnostic

### Table 2

| Survival (stage and method) | Number of patients | HR (95% CI) | P value | Heterogeneity test |
|----------------------------|--------------------|-------------|---------|--------------------|
| OS (pooled stages and methods) | 768 | 1.572 (1.240-1.992) | <.001 | 47.10% | .049 Random effect model |
| OS (pooled stages and qRT-PCR) | 255 | 1.272 (0.569–2.841) | .5580 | 73.40% | .052 Random effect model |
| OS (pooled stages and ISH) | 513 | 1.664 (1.320–2.098) | <.001 | 32.00% | .172 Random effect model |
| OS (stage I and ISH) | 115 | 2.023 (1.490–2.74) | <.001 | 0.00% | .397 Random effect model |
| OS (stage II and ISH) | 133 | 2.632 (1.557–4.446) | <.001 | 0.00% | .464 Random effect model |
| OS (stage II and ISH) | 221 | 1.363 (0.959–1.937) | .0840 | 40.70% | .194 Random effect model |
| OS (stage IV and ISH) | 44 | 1.175 (0.799–1.727) | .4120 | 0.00% | .877 Random effect model |
| DFS (pooled stages and qRT-PCR) | 255 | 1.497 (0.796–2.819) | .2120 | 40.70% | .194 Random effect model |

OS = overall survival, DFS = disease-free survival, ISH = in situ hybridization, HR = hazard ratio.
accuracy for esophageal squamous cell carcinoma, with a sensitivity and specificity of 76% and 84%, respectively.\textsuperscript{[34]} Regarding GC, the diagnostic accuracy of miR-10b has not been explored. In this study, we analyzed the diagnostic value of miR-10b-3p and miR-10b-5p in GC based on TCGA data. ROC curve analyses revealed that the AUC value for miR-10b-3p and miR-10b-5p were 0.652 (95% CI: 0.562–0.742; $P=0.001$) and 0.565 (95% CI: 0.471–0.660; $P=0.165$), respectively. This suggested

Table 3

| miR-10b | AUC (95% CI) | P  | The optimum diagnostic point | Sensitivity | Specificity | Overall survival | Disease-free survival |
|---------|--------------|----|-----------------------------|-------------|-------------|------------------|----------------------|
| 5p      | 0.565 (0.471–0.660) | .165 | 13.7816                     | 0.524       | 0.663       | 1.296 (0.933–1.801) | .122                 |
| 3p      | 0.652 (0.562–0.742) | .001 | 1.8621                      | 0.849       | 0.429       | 1.254 (0.905–1.736) | .173                 |

AUC = the area under the ROC curve.
that miR-10b-3p has potential to be noninvasive screening tools for GC detection.

There are several limitations in this study. First, only 4 studies were included in this meta-analysis, the number of patients was limited. Second, sensitive analysis and meta-regression were not conducted due to limited studies. Third, we only included studies published in English, the language bias is inevitable. Last but not least, this study lack of experiments to confirm our finding based on our own patient samples. We plan to perform experimental validation in the future study in subsequent years.

In conclusion, this is first meta-analysis indicated that expression of miR-10b is associated with OS in GC patients. Moreover, miR-10b-3p is promising to be a new biomarker for diagnosis of GC and high expression of miR-10b-5p is associated with poor DFS in GC patients. Considering above limitations, more larger sample size studies can help to verify the diagnostic and prognostic value of miR-10b in GC.

**Author contributions**

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Writing – review & editing: Zhangguo Shen, Sumei Xu.

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