Diagnosis value of miR-181, miR-652, and CA72-4 for gastric cancer

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Abstract

**Purpose:** To find a useful disease marker for early diagnosis of gastric cancer, we tried to explore the expression of serum miR-181, miR-652, and carbohydrate antigen 72-4 (CA72-4).

**Patients and Methods:** According to clinical pathologic stages, 112 patients with gastric cancer were divided into early gastric cancer group (n = 60) and advanced gastric cancer group (n = 52), stage I-II (n = 65), and stage III-IV (n = 47). Another 50 cases of gastric benign lesions and 40 healthy controls were also selected. Real-time quantitative PCR together with chemiluminescence were applied to detect expression levels. ROC curve was applied to judge their diagnostic efficiency. Pearson's correlation analysis was put into use to investigate the relevance of three indicators.

**Results:** Compared with benign lesions group and control group, significantly higher expression levels were found in patients of gastric cancer (all \( p < 0.001 \)). Similarly, compared with early gastric cancer group, significantly higher expression levels were found in advanced gastric cancer group (all \( p < 0.001 \)). The same result was also found in stage III-IV (all \( p < 0.001 \)). The best cutoff values were 0.93, 2.38, and 16.94 U/ml, respectively. The area under the curve (0.917, 95%CI: 0.856–0.975) of the three combined diagnosis of early gastric cancer was the largest, and its sensitivity and specificity were 92.5% and 86.8%. And miR-181 and miR-652 were positively correlated with CA72-4 (\( r = 0.772, p < 0.001 \); \( r = 0.853, p < 0.001 \)).

**Conclusion:** Serum miR-181, miR-652, and CA72-4 are closely linked to the occurrence and development of gastric cancer. Combination of three indicators has diagnostic value for early gastric cancer.

**KEYWORDS**
carbohydrate antigen 72-4, diagnostic value, gastric cancer, miR-181, miR-652
1 | INTRODUCTION

Gastric cancer (GC) has the highest incidence rate in digestive tumors globally.1 Due to lack of obvious clinical symptoms of GC in the early stage, too many patients are diagnosed at advanced-stage, resulting in poor prognosis and losing their lives.2-4 Thus, diagnosis and treatment in early stage are crucial to improving life quality and reducing death rate.5

The etiology and pathogenesis of psoriasis vulgaris is complex, involving multiple factors such as heredity, immunity, infection, and environment.6 miRNA is a small non-coding RNA, 18–25 nucleotides in length, it participates in the post-transcriptional regulation of target genes by shearing miRNA and inhibiting protein translation.7 miRNA plays an important regulatory role in various physiologic processes in the organism.8,9 It is becoming important targets in the diagnosis and treatment in cancers.10 Researches have reported that miRNA may participate in the tumorigenesis and occurrence of gastrointestinal cancer.11 For gastrointestinal cancer, recent insights have proved abnormal expression level in miR-181 and miR-652, as well as its role in the tumorigenesis and development of other cancers though adjusting several cellular signaling pathways.12-14 Thus, miR-181 and miR-652 may have implications for gastrointestinal cancer. CA72-4 (carbohydrate antigen 72-4), a common available tumor marker, has been considered as an important and useful marker for diagnosis and prognosis of GC.15,16 In this research, we analyzed the expression level of miR-181, miR-652, and CA72-4 in gastric diseases to evaluate the value for early diagnosis and prognosis of GC.

2 | MATERIAL AND METHODS

2.1 | Clinical specimens

112 patients with gastric cancer admitted to our hospital were selectively enrolled from January 2019 to October 2020, including 70 male and 42 female (median age, 58.6 years; range, 33–79 years). Inclusion criteria were as follows: (1) gastric adenocarcinoma confirmed histopathologically; (2) having not received radiotherapy, chemotherapy, immunotherapy, and surgical resection before test. Exclusion criteria were as follows: a history of other malignant cancers, pathological angiogenesis, immune system disease, serious liver and kidney diseases. In addition, 50 patients with benign gastric diseases were recruited for the controls group, including 32 gastric ulcers and 18 gastric polyposis (33 male and 17 female). 40 healthy individuals were recruited for the control group (healthy controls) (25 male and 15 female). Based on the progress of gastric cancer and the clinical pathological stages, the patients were classified for two groups including early gastric cancer group and advanced gastric cancer group. The clinical tumor stage was determined on the basis of seventh edition of the TNM classification.17

All patients signed written consent form and study protocol was approved by the Ethics Committee of First Affiliated Hospital of Soochow University.

2.2 | Quantitative real-time PCR

Two milliliters of peripheral blood was placed in tubes with no anticoagulant, and the serum was separated by centrifugation. RNA extraction was applied a standard liquid–liquid extraction protocol using TRizol LS (Invitrogen, Life Sciences, Carlsbad, CA). cDNA synthesis was based on the TaqMan miRNA reverse transcription kit (Applied Biosystems, Life Sciences, Foster City, CA). The reaction system was 15 μl, which contained 5 μl of eluted plasma miRNA, 10 μl of master mix (3 μl of reverse transcription-specific primer, 1.5 μl of buffer, 4.16 μl of nuclease-free H2O, 1 μl of 50 U/μl MultiScribe Reverse Transcriptase, 0.15 μl of 100 mmol/L dNTPs, 0.19 μl of 20 U/μl RNase inhibitor). We used real-time PCR to calculate the miR-181 and miR-652 expression on ABI 7500. 20 μl reaction systems with iQ SYBR Premix Ex Taq Perfect Real Time was built. 2^△△Ct formula was applied to calculate relative mRNA expression, and the U6 was applied as an endogenous control. Additionally, chemiluminescence method was used to quantitate CA72-4 level on Roche Electrochemical luminescence Immunoassay Analyzers.

2.3 | Survival analysis

Online tool Kaplan-Meier plotter (KM plotter, www.kmplot.com) can assess the impact of gastric (nasty 1440) cancer on survival rate. OC patients were divided into the expression and low expression group, according to the median expression level of specific genes. Kaplan–Meier was used to analyze the overall survival of patients with OC. Calculate and show the hazard ratio (HR) of the 95% confidence interval (CI).

2.4 | Statistical analysis

The SPSS 12.0 was applied for whole analysis, the measurement data was represented by (X ± S), and the comparison of the means of two independent samples was performed by t test. The counting data were expressed as percentage (%) and was performed by the χ2 test. Pearson's correlation analysis was also applied. p < 0.05 indicated the difference was statistically significant. Bioinformatics analysis was based on common data base and published literatures.18,19

3 | RESULTS

3.1 | miR-181, miR-652, and CA72-4 expression

Compared with benign lesions group and control group, significantly higher expression levels were found in patients of gastric cancer (all p < 0.001) (Figure 1). Similarly, compared with early gastric cancer group, significantly higher expression levels were found in advanced gastric cancer group (all p < 0.001) (Figure 2). The serum miR-181 (r = 0.772, p < 0.001) and miR-652 (r = 0.853, p < 0.001)
was positively associated with CA72-4 in patients with gastric cancer (Figure 3). Compared with stage III–IV, significantly higher expression levels were found in stage III–IV (all \( p < 0.001 \)) (Figure 4).

### 3.2 | The diagnostic value of serum miR-181, miR-652, and CA72-4 levels patients with early gastric cancer

AUC of miR-181, miR-652, and CA72-4 generated by ROC analysis was 0.820, 0.842 and 0.769. The cutoff points of above three indicators is 0.93, 2.38, 16.94 U/ml.

Moreover, AUC of combination of miR-181, miR-652, and CA72-4 is 0.917 (95% CI = 0.856 to 0.975, sensitivity = 92.5%, and specificity = 86.8%) (Figure 5 and Table 1).

### 3.3 | The prognostic value of serum miR-181 and miR-652 levels patients with gastric cancer

Two selected miRNAs, namely, miR-181 and miR-652, were significantly associated with the prognosis of patients (Figure 6). High expression of miR-181 in STAD patients was associated with poor OS (HR: 1.35; 95% CI: 0.99–1.84; \( p = 0.045 \)). High expression of miR-652 in STAD patients was associated with poor OS (HR: 1.45; 95% CI: 1.03–2.04; \( p = 0.034 \)).

### 4 | DISCUSSION

CA72-4, the most common serum tumor biomarker, was widely used in the diagnosis, metastasis monitoring, and prognosis judgment of gastric cancer.\(^{20,21}\) Emoto et al.\(^{22}\) reported that, CA72-4 is associated with advanced stages of gastrointestinal cancer and poor prognosis. Similarly, the serum level of CA72-4 can provide material circumstances about prognosis such as the extent of tumor invasion, metastases. However, CA72-4 has low sensitivity in the early diagnosis of GC, and the value of single index in the diagnosis of early gastric cancer is limited. Therefore, identification of biomarkers with higher sensitivity and combined markers would be of great value.

Numerous miRNAs in GC have been found to be upregulated including miR-15b, miR-16, miR-21, miR-23a, miR-27, miR-43c, miR-106a, miR-107, miR-130b, miR-150, and miR-223.\(^{23–35}\) Meanwhile, various miRNAs in GC have been found to be downregulated including miR-9, miR-34b, miR-124a, miR-126, miR-129-2, miR-143, miR-145, miR-146a, miR-148b, miR-181c, miR-212, miR-218, miR-375, and miR-451.\(^{36–52}\)

Although increasing studies have indicated that miRNAs play key roles in the progression of gastric cancer, the clear mechanism and functions of numerous miRNAs remains unknown. Therefore, the role and pathway of miRNAs in gastric cancer need to be further explored in future study. A majority of miRNAs were found be associated with cell proliferation and a small minority of miRNAs...
were found be associated with cell survival, epigenetic regulation, cell cycle regulation and cell cycle arrest. miR-181 was considered to promote cell proliferation and inhibit apoptosis in gastric cancer and miR-652 was served as a prognostic biomarker in gastric cancer.\textsuperscript{14,53} Furthermore, CA72-4 was considered highly specific to gastric cancer.\textsuperscript{21} As far as we know, this is the first study that investigates the diagnosis of gastric cancer by combining three indicators.

In this research, our data indicated that serum miR-181, miR-652, and CA72-4 are closely linked to the occurrence and development of gastric cancer. Combination of three indicators has diagnostic value for early gastric cancer. Thus, miRNA could be biomarkers for GC to predict its diagnosis and prognosis. The expression level of miR-181 and miR-652 were in positive relation to serum CA72-4 level, suggesting the similar roles in gastric cancer. Moreover, the expression of miR-181, miR-652, and CA72-4 in advanced GC were significantly higher than in gastric benign disease cases and healthy group. Their expression level in III–IV stage GC were significantly higher than in I–II. All the evidence suggested that increased expression level of miR-181 and miR-652 in Patients were correlated to the progression of GC.

Multiple marker combination may contribute to improving the diagnostic accuracy of various diseases, including GC. The present study suggested that three indictors combined improved the diagnostic accuracy of GC. The diagnosis value of early gastric cancer was enhanced when combined with the conversed biomarker CA72-4, providing a new strategy for elucidating the mechanism underlying GC. N4-acetylcytidine (ac4C) has been subject to widespread attention as comprehensive modifications have been detected in mRNAs of human and yeast.\textsuperscript{54} It contributes to
accurately reading codons in the process of translation and improving translational efficiency. Furthermore, there is a direct correlation between ac4C and occurrence, development, progression of number diseases. This may be an interesting and meaningful research topic to investigate the relationship between ac4C and miRNA expression.

5 | CONCLUSIONS

In summary, serum miR-181, miR-652, and CA72-4 were significantly associated with the progression and stage of gastric cancer. Combination of miR-181, miR-652, and CA72-4 had the greater diagnostic value for early diagnosis of GC.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

TABLE 1 Serum miR-181, miR-652, and CA72-4 cutoff points and the corresponding sensitivity and specificity for predicting EGC

| Indicator | Cutoff point | AUC (95%CI)  | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | PLR | -LR |
|-----------|--------------|-------------|----------------|----------------|---------|---------|-----|-----|
| miR-181   | 0.93         | 0.820 (0.761–0.879) | 83.6           | 78.5           | 80.7    | 81.5    | 3.888 | 0.209 |
| miR-652   | 2.38         | 0.842 (0.783–0.902) | 86.2           | 80.4           | 83.5    | 83.6    | 4.398 | 0.172 |
| CA72-4    | 16.94 U/ml   | 0.796 (0.741–0.854) | 80.4           | 73.5           | 76.2    | 77.8    | 3.034 | 0.267 |
| Combinations | /          | 0.917 (0.856–0.975) | 92.5           | 86.8           | 89.3    | 90.4    | 7.008 | 0.086 |

FIGURE 6 Relationship between miR-181, miR-652 expression and prognosis

AUTHOR CONTRIBUTIONS

Fujuan Luan conceived study design; Xueqin Pang conceived the content concept; Wenyang Tian and Xueqin Pang performed the data collection, extraction, and analyzed the data. Wenyang Tian interpreted and reviewed the data and drafts. Fujuan Luan reviewed the final draft. All authors were involved in literature search, writing the paper and had final approval of the submitted and published versions.

DATA AVAILABILITY STATEMENT

All the data generated or analyzed during this study are included in this published article.

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