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ORIGINAL ARTICLE

Prognostic potential of miR-21-3p in gastric cancer

Xiaofen Sun, Kanru Zhang, Duoqiao Li

Department of the Second Clinic of Internal Medicine, 943 Hospital of the Joint Logistics Support Unit of the Chinese People's Liberation Army, Wuwei, China.

Summary

Purpose: *This study aimed to clarify the role of microRNA (miR)-21-3p in regulating progression and prognosis in gastric cancer (GC).*

Methods: *One hundred patients with primary GC were included in this study. Their primary GC tissues and paracancer normal mucosa were collected for detecting miR-21-3p levels. Receiver operating characteristic (ROC) curves were depicted for analyzing the predictive ability of miR-21-3p in GC. Subgroup analyses were conducted based on tumor size, lymph node metastasis status and TNM staging in GC patients. All GC patients were followed up for 5 years, and survival analysis was conducted using Kaplan-Meier method with log-rank test. Univariate and multivariate Cox regression analyses were performed for exploring potential prognostic factors for GC.*

Results: *MiR-21-3p was highly expressed in GC tissues. Subgroup analyses were conducted based on tumor size, lymph node metastasis status and tumor staging. Subgroup analyses showed higher level of miR-21-3p in GC tissues collected from patients with large tumor size, lymph node metastasis or advanced TNM staging. ROC curves confirmed the diagnostic potential of miR-21-3p in GC. In addition, Kaplan-Meier and log-rank test revealed lower progression-free survival (PFS) and overall survival (OS) in GC patients overexpressing miR-21-3p. Tumor size, lymph node metastasis, TNM staging and miR-21-3p level were independent risk factors for the prognosis of GC.*

Conclusions: *MiR-21-3p is upregulated in GC samples, which is closely related to GC progression. MiR-21-3p can be used to predict the prognosis of GC.*

Key words: *gastric cancer, MiR-21-3p, prognosis*

Introduction

Globally, gastric cancer (GC) is the most-common tumor in the digestive system [1]. With the progress made on experimental and clinical explorations, the diagnostic and therapeutic levels have been largely improved. However, the prognosis of GC patients is unsatisfactory [2]. The low detection rate of early stage GC is the major reason for the high mortality [3]. Timely diagnosis of malignant tumors is of significance to improve the clinical outcomes [4]. So far, clinically used serum biomarkers for GC have relatively low sensitivity and specificity [5].

MicroRNAs (miRs) are a type of noncoding RNAs that exert vital functions in epigenetic regulation. A mature miR is 22-25 nucleotides long, which inhibits the translation of target gene or directly induces mRNA degradation by complementary base pairing to mRNA 3'untranslated region (3'UTR) [6]. Accumulating studies have identified abnormally expressed miRs in malignant tumors. Serving as vital regulators on oncogenes or tumor suppressors, miRs are involved in tumor cell differentiation, angiogenesis, metastasis or other malignant phenotypes [7].

Corresponding author: Xiaofen Sun, MM. Department of the Second Clinic of Internal Medicine, 943 Hospital of the Joint Logistics Support Unit of the Chinese People's Liberation Army (PLA), No.943 Hospital of PLA, 472 Qilian Ave, Liangzhou District, Wuwei, Gansu 733000, China.
Tel: +86 018797389896, Email: 15002610466@163.com
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So far, about 30 miRs have been reported to influence the carcinogenesis, tumor growth and progression of GC [8]. These miRs exert either oncogenic (i.e. miR-21, miR-23a) or anti-cancer effect (i.e. miR-31, miR-34) on GC [9,10]. MiR-21-3p has been previously demonstrated to be involved in inflammatory diseases. Through regulating inflammation signaling, miR-21-3p drives the process of colorectal carcinoma [11]. In addition, overexpression of miR-21-3p stimulates inflammatory response during the process of renal fibrosis [12]. Calsina et al [13] proposed the involvement of miR-21-3p in the prognosis of metastatic pheochromocytoma/paraganglioma. In this paper, we aimed to illustrate the clinical significance of miR-21-3p in influencing clinical features and prognosis of GC patients.

Methods

Clinical data of GC patients

Eligible GC patients (n=100) treated in the 943 Hospital of the Joint Logistics Support Unit of the Chinese People's Liberation Army from June 2017 to May 2019 were included. Inclusion criteria were: (1) Diagnosis of GC by cytology and/or biopsy; (2) GC patients were treated by radical gastrectomy; (3) No preoperative anti-cancer treatment, including chemotherapy and radiotherapy; (4) No distant metastases; (5) Complete clinical data. Exclusion criteria: (1) Combined with mental diseases; (2) Combined with dysfunctions of the heart, lung and other organs; (3) Combined with autoimmune diseases; (4) Patients with severe malnutrition. Their primary GC tissues and paracancer normal mucosa (≥5 cm) were collected during surgery, and pathologically confirmed by experienced pathologists. All samples were quickly frozen in liquid nitrogen and stored at -80°C for use. Clinical data were recorded, including smoking history, tumor size, infiltration depth, TNM staging, etc. TNM staging was defined according to the 7th edition proposed by the American Joint Committee on Cancer [14]. This study was approved by the ethics committee of the 943 Hospital of the Joint Logistics Support Unit of the Chinese People's Liberation Army. Signed written informed consents were obtained from all participants before the study entry.

Quantitative real-time polymerase chain reaction (qRT-PCR)

RNAs were isolated from tissues using TRIzol method (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNAs were determined by spectrophotometry. After reverse transcription, complementary DNAs (cDNAs) were subjected to qRT-PCR at 95°C for 30 s, and 40 cycles at 95°C for 5 s and 60°C for 34 s using ABI 7500 system (Applied Biosystems, Foster City, CA, USA). Dissociation curves of PCR products were plotted with U6 as the internal reference. Relative level was

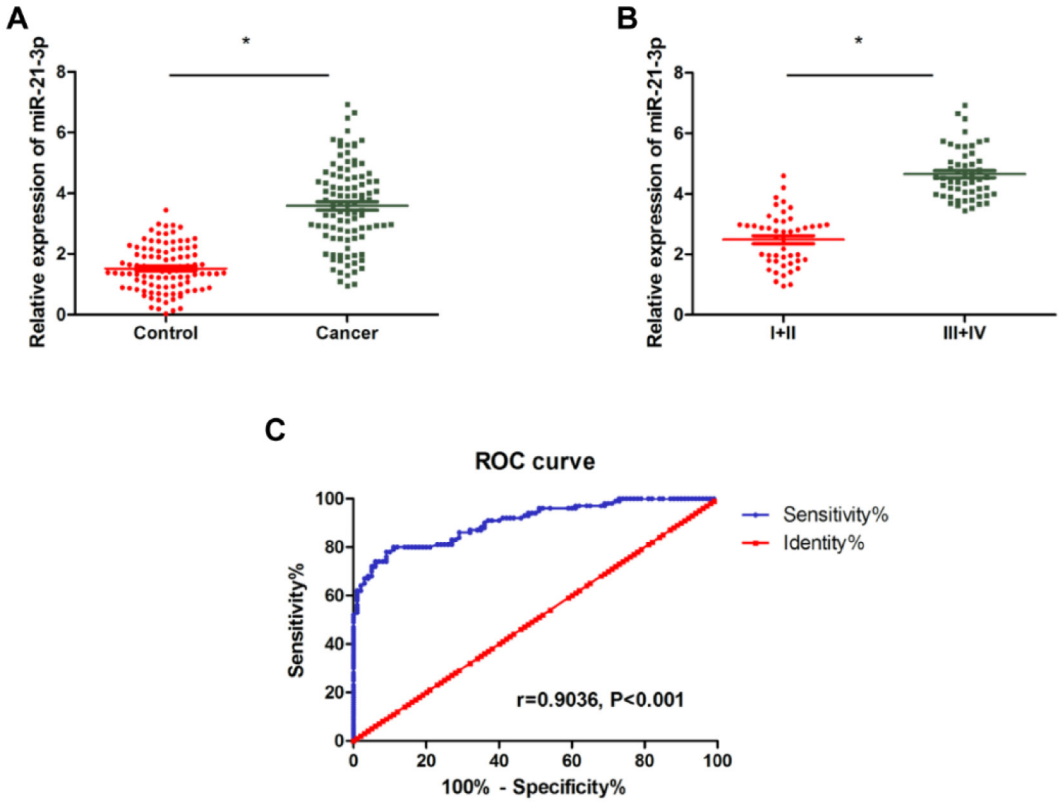

Figure 1. Upregulated miR-21-3p in GC tissues. **A:** Differential level of miR-21-3p in primary GC tissues and paracancerous normal mucosa. **B:** MiR-21-3p level in stage I+II and stage III+IV GC cases. **C:** Diagnostic potential of miR-21-3p in GC (*p<0.05).

calculated by $2^{-\Delta\Delta CT}$ method. Primer sequences were as follows: MiR-21-3p: 5'-GAAATGCCTCACAGCTATCGT-3' (forward) and 5'-CCTCCACAAAGAGCCACC-3' (reverse); U6: 5'-CTCGCTTCGGCAGCACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse).

Follow-up

Follow-up was conducted every three months by telephone or outpatient visit. The last follow-up was in 2019. Overall survival (OS) was defined as the duration from the time of initial diagnosis to the last follow-up or the time of death. Progression-free survival (PFS) was defined as the duration from the time of anti-cancer treatment to the time of disease progression or death of any cause.

Statistics

Statistical analyses were conducted by SPSS 22.0 (IBM, Armonk, NY, USA). All data were expressed as mean ± SD. The t-test was used to compare the differences between groups. Kaplan-Meier survival curves were plotted, followed by comparing differences in OS and PFS using Log-rank test. Potential factors influencing GC prognosis were analyzed by univariate and multivariate Cox regression analyses. P<0.05 was statistically significant.

Results

Upregulated miR-21-3p in GC tissues

QRT-PCR data showed that miR-21-3p was upregulated in primary GC tissues than in paracancer normal mucosa (Figure 1A). Furthermore, miR-21-3p level was detected to be higher in stage III+IV GC cases compared with that of stage I+II cases (Figure 1B). To analyze the diagnostic potential of miR-21-3p in GC, ROC curves were plotted. MiR-21-3p displayed satisfactory specificity (89%) and sensitivity (80%) in diagnosing GC (cut-off value=2.46, AUC=0.9036, Youden index=0.69, p<0.001) (Figure 1C). Therefore, miR-21-3p had a certain potential as a biomarker for GC.

Table 1. Correlation between miR-21-3p level and clinical features in gastric cancer patients

Clinical features	*n*	*miR-21 expression*	*t*	*p*
Gender				
Male	68	3.98±1.87	0.71	0.479
Female	32	4.27±1.98		
Age, years				
<50	47	3.79±1.65	0.204	0.839
≥50	53	3.86±1.77		
Tumor size (cm)				
<5	44	2.68±1.16	4.845	<0.001
≥5	56	4.71±2.58		
Lymph node metastasis				
No	69	3.16±2.07	4.189	<0.001
Yes	31	5.21±2.65		
TNM stage				
I+II	56	2.87±0.75	9.014	<0.001
III+IV	44	5.22±1.76		

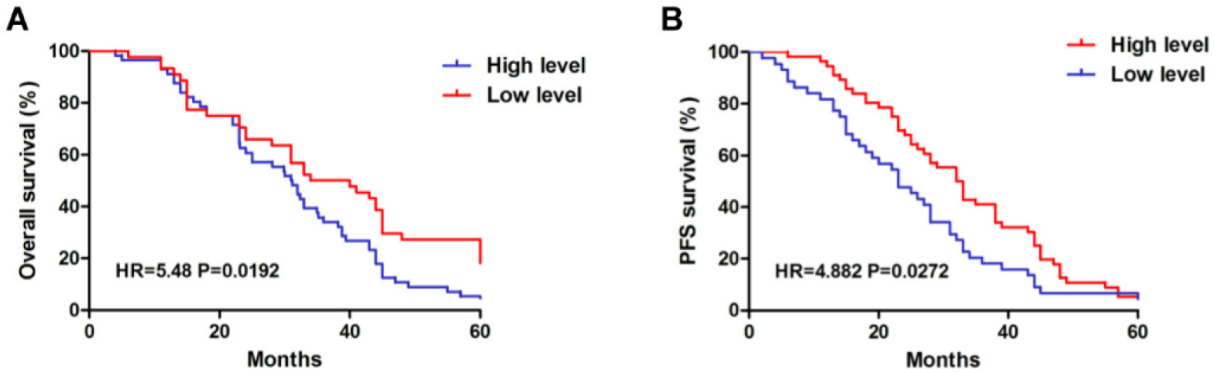

Figure 2. Prognostic potential of miR-21-3p in GC. **A:** Overall survival in GC patients with high or low level of miR-21-3p. **B:** Progression-free survival in GC patients with high or low level of miR-21-3p.

Table 2. Potential influences on the overall survival of gastric cancer analyzed by univariate and multivariate Cox regression analysis.

Variables	*HR*	*95%CI*	*p*
Tumor size (cm) <5, ≥5	2.414	1.760-4.932	0.042
Lymph node metastasis No, Yes	1.547	1.215-3.219	<0.001
TNM stage I+II, III+IV	1.883	1.357-2.668	0.007
miR-21-3p Low, High	2.314	1.759-3.431	0.018

Table 3. Potential influences on the progression-free survival of gastric cancer analyzed by univariate and multivariate Cox regression analyses

Variables	*HR*	*95%CI*	*p*
Lymph node metastasis No, Yes	1.486	1.225-3.851	0.005
TNM stage I+II, III+IV	1.874	1.435-4.602	0.027
miR-21-3p Low, High	1.786	1.053-3.861	<0.001

HR=hazard ratios, CI=confidence interval

Correlation between miR-21-3p and clinical features in GC

Clinical data of the included GC patients were analyzed. No significant differences in age and gender were found between GC patients expressing high or low level of miR-21-3p ($p>0.05$). Nevertheless, GC patients with large tumor size (≥5 cm), lymph node metastasis or advanced stage (III+IV) had high level of miR-21-3p ($p<0.05$) (Table 1). It is concluded that miR-21-3p may influence tumor size, occurrence of lymph node metastasis and TNM staging in GC.

Prognostic potential of miR-21-3p in GC

To further clarify the influence of miR-21-3p on the prognosis of GC, we conducted follow-up in every patient for 5 years. Worse OS (HR=5.48, p=0.0192) and PFS (HR=4.882, p=0.0271) were identified in GC patients expressing high level of miR-21-3p, suggesting that miR-21-3p was unfavorable to the prognosis of GC (Figure 2A, 2B).

Risk factors of GC prognosis

According to the cut-off value of miR-21-3p the patients were classified into high level group (≥2.46) and low level group (<2.46). Age, gender, tumor size, lymph node metastasis status, TNM staging and miR-21-3p level were subjected to univariate and multivariate Cox regression analyses. As the data revealed, tumor size ≥5 cm, presence of lymph node metastasis, stage III+IV and high level of miR-21-3p were the independent factors influencing the OS in GC patients (Table 2). In addition, presence of lymph node metastasis, stage III+IV and high level of miR-21-3p were independent factors influencing the PFS in GC patients (Table 3).

Discussion

As a prevalent tumor in the digestive system, GC is the second lethal tumor in the world. An abnormality in any event in cell activities, such as cell proliferation, differentiation and apoptosis, can lead to tumorigenesis. MiRs have been demonstrated as vital regulators involved in cell functions [15,16]. Differentially expressed miRs in GC profile have been searched, which remarkably influence malignant phenotypes of GC cells [17].

Previous studies have shown that miR-21-3p is upregulated in solid tumors, which are capable of regulating tumor-suppressor genes, including PTEN, and actin-binding proteins [18-20]. Our results showed that miR-21-3p was upregulated in GC tissues than controls, especially in advanced stage GC cases. By analyzing clinical features of

GC patients, it was revealed that miR-213-p level was linked to tumor size, lymph node metastasis and TNM staging in GC. Highly expressed miR-21-3p was an independent risk factor of GC prognosis. Furthermore, ROC curves confirmed the diagnostic potential of miR-21-3p in GC. We believe that miR-21-3p is a promising biomarker that could be utilized in clinical diagnosis and treatment of GC.

Conclusions

MiR-21-3p is upregulated in GC samples, and is closely related to GC progression. MiR-21-3p can be used to predict the prognosis of GC.

Conflict of interests

The authors declare no conflict of interests.

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