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Original Article

Reduced miR-339 expression predicts the poor prognosis of gastric cancer

Haijun Li[1], Weixing Wang[1], Ju Wang[2]

[1]*Department of Hepatobiliary and Laparoscopic Surgery, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, P.R. China;* [2]*Department of Gastrointestinal Surgery, Inner Mongolia Autonomous Region People's Hospital, Hohhot 010017, Inner Mongolia Autonomous, P.R. China*

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Abstract: Gastric cancer (GC) is one of the most deadly malignant diseases and no effective treatment is available. A number of microRNAs (miRNAs) were found to play a central role in tumorigenesis. The goal of this study was to evaluate the prognostic value of miR-339 in GC. Real-time PCR was used to compare miR-339 levels between GC tissues and adjacent normal tissues. Chi-squared test, Kaplan-Meier analysis and Cox regression analysis were performed to evaluate the clinical significance of miR-339 in GC. We found that the expression level of miR-339 was remarkably reduced in GC compared to the controls. MiR-339 expression level was associated with advanced clinical stage and lymph node metastasis. In addition, the overall survival and relapse-free survival of patients with lower miR-339 expression were significantly shorter than patients with higher miR-339 expression. Multivariate analysis showed that low miR-339 level was an independent prognostic factor to predict the unfavorable prognosis. Collectively, miR-339 was reduced in GC and correlated with worse clinical outcome, suggesting that miR-339 might serve as a potential predictor of poor prognosis in GC.

Keywords: MiR-339, gastric cancer, prognosis

Introduction

Despite the incidence of gastric cancer (GC) has been decreasing, it remains globally the fifth most commonly cancer and the second leading cause of cancer-related mortality worldwide, especially in east Asia [1]. Most of patients with GC are not suitable for surgical resection as they are often diagnosed at the advanced stage, which is the major reason responsible for the poor clinical outcome of this deadly malignancy [2]. Prognostic biomarkers are not only useful for risk stratification, but also are important for tailoring the treatment plan for the individual patient. Unfortunately, no ideal biomarker is currently available for predicating the prognosis of GC [3].

MicroRNAs (miRNAs) are a class of evolutionally conserved, small non-coding RNAs of 18-25 nucleotides. MiRNAs regulate gene expression by translation repression or mRNA degradation by binding to their 3'-untranslated regions (3'UTR) [4, 5]. A large number of research studies have shown that miRNAs have a critical role in the initiation and development of cancer [6, 7]. Up to 50% of miRNAs are located in the cancer related genomic regions, might act as oncogenes or tumor suppressor genes [8, 9]. MiR-630 was significantly increased in gastric cancer tissues. In addition, elevated miR-630 levels were associated with poor prognosis of GC, indicating that miR-630 might act as an oncogene to promote the GC carcinogenesis [10]. Both miR-200b and miR-200c were reduced in gastric cancer specimens and cell lines. Overexpression of miR-200b and miR-200c suppressed the proliferation and invasion capacity of GC cells. In addition, downregulation of miR-200b and miR-200c was positively correlated with tumor progression, suggesting these two molecules might function as tumor suppressor genes in GC [11].

Deregulated expression of miR-339 has been reported in many types of cancer such as non-

Table 1. The correlation between miR-339 expression and the clinicopathological characteristics in GC

Parameters	Total patients	MiR-339 level		*P*
		Low	High	
Age		58.3±9.6	58.9±10.3	0.642
Gender				0.254
Male	73	38	35	
Female	37	15	22	
Localization				0.315
Proximal	39	22	17	
Middle	17	7	10	
Distal	54	21	30	
Tumor size				0.080
<5	51	20	31	
≥5	59	33	26	
Depth of invasion				0.073
T1-T2	47	18	29	
T3-T4	63	35	28	
Lymph node metastasis				0.002
No	41	12	29	
Yes	69	41	28	
Distant metastasis				0.927
No	104	50	54	
Yes	6	3	3	
Histological grade				0.383
Well	15	7	8	
Moderate	42	17	25	
Poor	53	29	24	
TNM Stage				<0.001
I-II	45	13	32	
III-IV	65	40	25	

small cell lung cancer, melanoma and gastric cancer [12-14]. However, whether aberrant miR-339 expression has any clinical significance in GC remains unclear. In the present study, we first evaluated the expression levels of miR-339 in GC by real-time PCR. Then its potential prognostic value was examined.

Materials and methods

Patients and tissue specimens

A total of 110 samples of tumor and adjacent normal tissues from GC patients who underwent surgical resection in the Department of Gastrointestinal Surgery, Inner Mongolia Autonomous Region People's Hospital were collected. All the cases were confirmed by histological evaluation. The pathological stage of GC was classified based on the 7th edition of the AJCC TNM classification system. Overall survival was defined as time from randomization to death, irrespective of cause. Relapse-free survival was defined as the time from randomization to relapse of GC or death from any cause. The study was approved by the Ethics Committee of Inner Mongolia Autonomous Region People's Hospital and written informed consents were obtained from all patients for using their specimens and medical records. The clinical characteristics of GC patients were described in **Table 1**.

Quantitative reverse-transcription PCR (qRT-PCR)

Total RNA was extracted from fresh GC/matched normal tissues using Trizol according to the manufacturer's instructions. Then RNA was reverse-transcribed into cDNA using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) for subsequent PCR amplification. QRT-PCR was performed using the TaqMan microRNA Assay® Kit (Applied Biosystems) and an ABI 7500 real-time PCR System (Applied Biosystems). The PCR condition was as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 8 s and 60°C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to quantify relative expression of miR-339 and U6 was used as endogenous control.

Statistical analysis

Statistical analyses were carried out with SPSS version 18.0 and GraphPad Prism 6.0 software. Mann-Whitney U test was used to compare miR-339 expression levels between primary cancerous tissues and the corresponding paired noncancerous tissues. The median value of miR-339 was used as the cutoff point to divide the patient cohort into two groups. Correlation between miR-339 levels and clinicopathological parameters was evaluated by Chi-squared test. Kaplan-Meier method was constructed for survival analyses and differ-

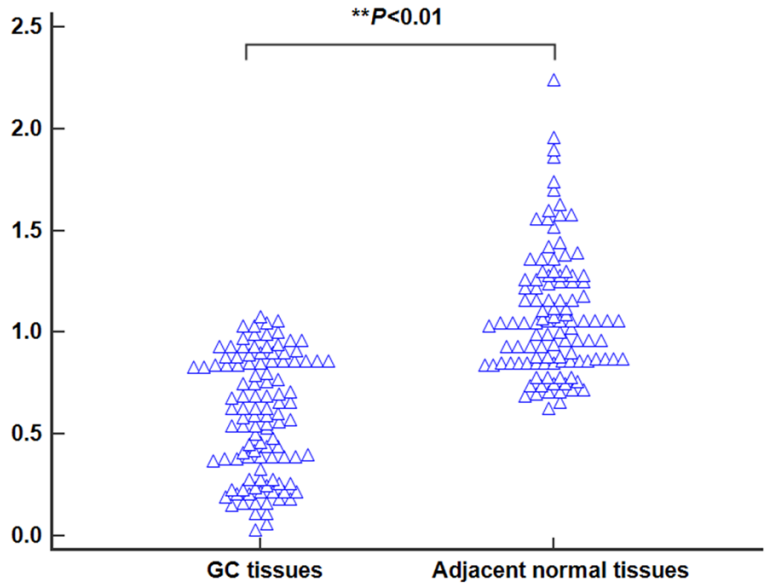

Figure 1. Expression level of miR-339 in GC.

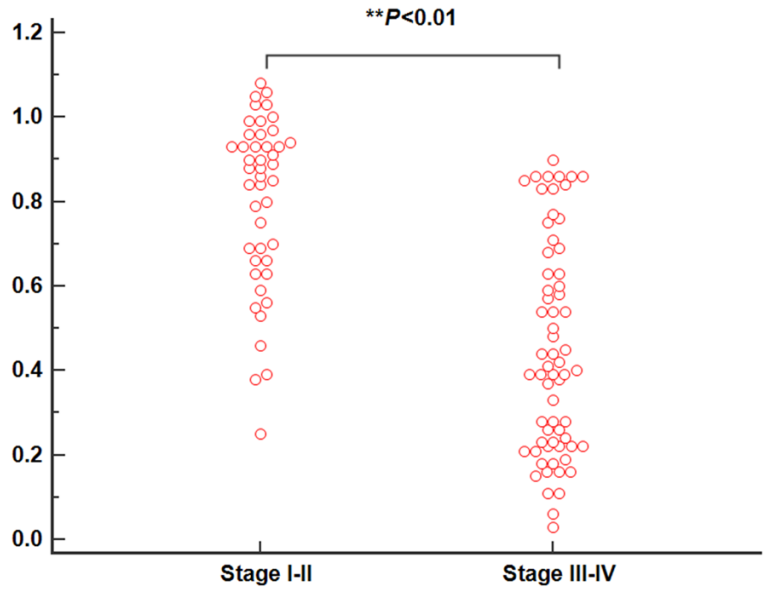

Figure 2. MiR-339 expression level was reduced in GC patients in the advanced stage.

ences were tested by the log-rank test. The Cox proportional hazards regression model was used to find out the significant prognostic indicators for patient survival. Data were considered to be statistically significant when $P<0.05$.

Results

Expression level of miR-339 in GC tissues

Real-time PCR was carried out to compare the expression level of miR-339 between GC tissues and adjacent normal tissues. The results showed that miR-339 levels were significantly downregulated in GC tissues compared with adjacent normal tissues ($P<0.01$) (**Figure 1**). In addition, the expression level of miR-339 was remarkably reduced in advanced stage GC compared to early stage GC ($P<0.01$) (**Figure 2**), indicating miR-339 levels were closely associated with the progression of GC.

Association of miR-339 expression with clinicopathologic characteristics in GC

Table 1 showed a summary of the association between miR-339 expression levels and clinicopathologic variables in patients with GC. Significant association was found between miR-339 levels and TNM stage ($P<0.001$) and lymph node metastasis ($P=0.002$). However, we did not observe correlation between miR-339 expression level and other clinicopathologic parameters including age, gender, localization, tumor size, depth of invasion, distant metastasis and histological grade. For tumor size and depth of invasion, there was a higher percentage of GC patients in the low miR-339 group had larger tumor size and deeper invasion depth. The *P* value did not achieve statistical significance might be due to the limited sample size.

Reduced miR-339 was associated with poor prognosis of GC

Our survival analysis showed patients with reduced miR-339 expression had poorer overall survival ($P<0.01$) and disease-free survival ($P<0.01$) in GC (**Figure 3**). The 5 year overall survival rate was 32.07% in the low miR-339 group and 66.67% in the high miR-339 group. Disease-free survival was 24.53% and 50.88%, respectively.

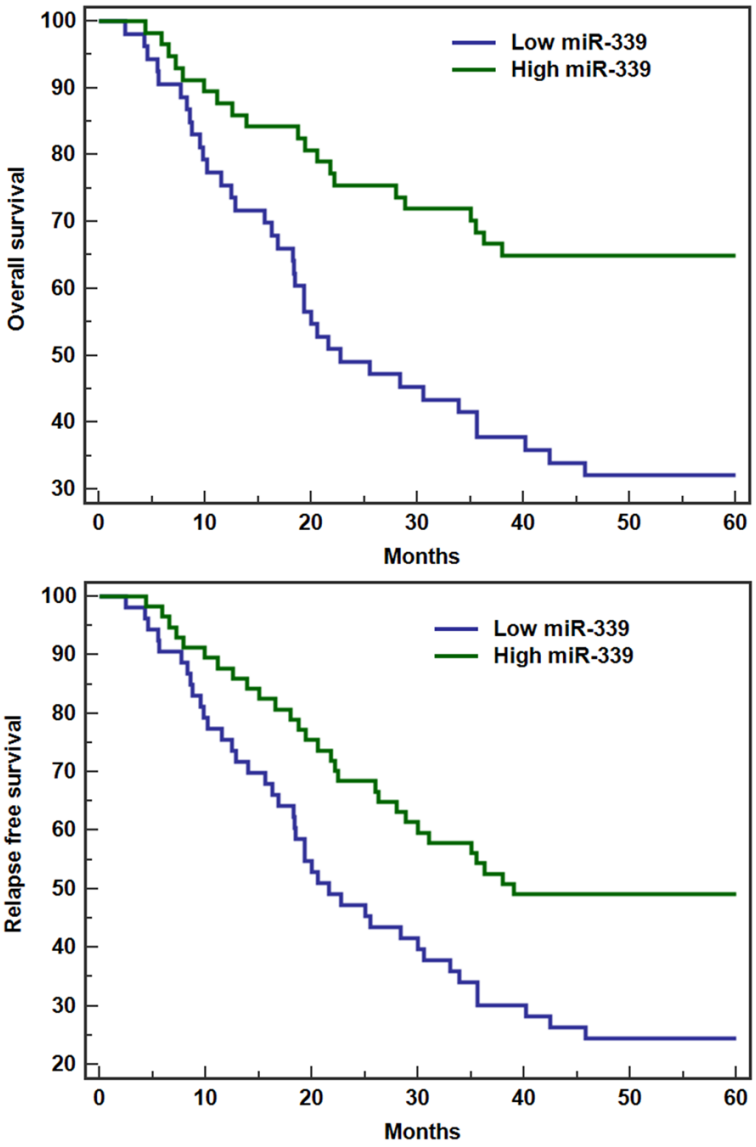

Figure 3. The correlation between miR-339 levels and overall/relapse free survival.

Table 2. Multivariate analysis of prognostic markers for overall/relapse free survival in GC

Variable	HR	95% CI	*P*
Overall survival			
Lymph node metastasis	2.86	1.56-6.21	0.025
TNM stage	4.52	2.03-9.65	0.006
MiR-339	3.63	1.86-7.83	0.013
Relapse free survival			
Lymph node metastasis	2.55	1.48-5.77	0.034
TNM stage	4.83	1.94-9.43	0.008
MiR-339	3.75	1.71-7.09	0.011

Our multivariate analysis showed that miR-339 was an independent prognostic biomarker for overall survival (HR=3.63, 95% CI=1.86-7.83, *P*=0.013) and disease free survival (HR=3.75, 95% CI= 1.71-7.09, *P*=0.011) (**Table 2**).

Discussion

Due to lack of specific symptoms, most patients with GC cannot be detected at the early stage [15]. In some cases, the conventional TNM classification system fails to predict the prognosis of GC because of tumor heterogeneity. Therefore, exploring novel molecular biomarkers are very important. In the current study, our data showed that miR-339 was reduced in GC tissues compared to adjacent normal tissues. A positive correlation was observed between low miR-339 levels and advanced TNM stage and positive lymph node. In addition, GC patients with low miR-339 had shorter median overall and disease free survival than the patients with high miR-339. Moreover, we also found that miR-399 was an independent prognostic biomarker for patients with GC. Our results suggest that downregulation of miR-339 can promote the carcinogenesis of GC and restore its expression might be an effective strategy to improve the clinical outcome of GC. Consistent with what has been reported in previous study, miR-339 was significantly decreased in primary GC tissues. Ectopic expression of miR-339 suppressed the proliferation, migration and invasive capacity *in vitro* and inhibits tumor growth *in vivo.* In addition, NOVA1 was identified as a direct target of miR-339 [14]. These data demonstrated that miR-339 played a tumor suppressive role in the carcinogenesis of GC.

In addition to GC, miR-339 was also found to inhibit progression of many types of cancers. MiR-339-5p was able to target 3'UTR of MDM2 mRNA, leading to degradation of MDM2 protein and promoting the function of p53. MiR-339-5p overexpression decreased p53-control cellular responses, and *vice versa.* This study suggested that miR-339-5p might exert its anti-tumor effects by decreasing MDM2 levels [16]. MiR-339-5p was decreased in colorectal cancer (CRC) tissues and highly invasive cell lines. In addition, overexpression of miR-339-5p suppressed the oncogenic activities both *in vitro* and *in vivo*, and phosphatases of regenerating liver-1 were demonstrated to be a downstream target of miR-339-5p [17]. The expression level of miR-339-5p was downregulated in hepatocellular carcinoma (HCC) tissues. Upregulation of miR-339-5p inhibited the invasive capability of HCC cells and decreased miR-339-5p levels were correlated with worse clinical outcome of HCC [18]. The expression level of plasma miR-339 was significantly downregulated in patients with acute myeloblastic leukemia in comparison with healthy controls [19]. This study suggested that cancer cells might secret miR-339 into the circulation system, thus detecting serum or plasma miR-339 might help monitor the disease progression in real time. Further studies should evaluate the clinical significance of serum/plasma miR-339 in GC.

In conclusion, downregulation of miR-339 was observed in GC tissue specimens. Moreover, reduced miR-339 expression was associated with poor prognosis of GC. We believe that miR-339 might be a promising prognostic biomarker for GC. The molecular basis for tumor suppressive effects of miR-339 in GC should be elucidated in further studies. In addition, the clinical utility of miR-339 in GC requires additional validation with larger cohort of patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Weixing Wang, Department of Hepatobiliary and Laparoscopic Surgery, Renmin Hospital of Wuhan University, No.99 Zhangzhidong Road, Wuchang District, Wuhan 430060, Hubei Province, P.R. China. Tel: +86-27-88041911; E-mail: weixingwangwhu@sina.com

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