

Expression of miR-1294 is downregulated and predicts a poor prognosis in gastric cancer

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) play critical roles in regulating tumor development and progression. The aim of the study is to investigate the clinical significance of miR-1294 expression in gastric cancer (GC).

PATIENTS AND METHODS: The expression of miR-1294 in 82 cases of GC tissues and adjacent normal tissues was determined using quantitative Real Time-PCR (qRT-PCR) analyses. Survival plot was calculated using the Kaplan-Meier methods and log-rank test from the date of operation to the time of death or last follow-up date. The association between miR-1294 expression and clinical categorical data was analyzed using the chi-squared test. Moreover, Univariate and multivariate Cox analysis were performed to assess the risk factors of GC prognosis.

RESULTS: We showed that miR-1294 expression was significantly downregulated in GC tissues compared to adjacent normal tissues. The low expression of miR-1294 in patients with GC was correlated with clinicopathological parameters including larger tumor size, lymph node metastasis, and distant metastasis. Kaplan-Meier survival analysis showed that GC patients with lower miR-1294 expression exhibited a shorter disease-free survival (DFS) and overall survival (OS) time compared to those patients with higher miR-1294 expression. Multivariate Cox analysis showed that lower miR-1294 expression, tumor size, lymph node metastasis, and distant metastasis were identified as independent risk factors of GC prognosis.

CONCLUSION: Our results provided evidence that miR-1294 expression was significantly downregulated in GC and may serve as a predictor of GC prognosis.

Keywords:

Gastric cancer, microRNAs, miR-1294, prognosis.

resection in Asia. However, patients who were diagnosed at late stage present a poor prognosis due to tumor relapse and metastasis. Thus, to explore novel and specific diagnostic and prognostic biomarkers that identify high-risk GC patients are urgently required.

MicroRNAs (miRNAs) are single-stranded RNA molecules (21-23 nucleotides) and could regulate their target gene expression by either interfering with transcription or inhibiting translation. In several tumors, miRNAs have been identified as potentially diagnostic and prognostic biomarkers⁵. For instance, decreased miR-503 expression in gastric cancer is inversely correlated with carcinoembryonic antigen and acts as a potential prognostic and diagnostic biomarker⁶. miR-145-5p is significantly down-expressed in GC and lower miR-145-5p expression can be used as a biological marker of poor prognosis in GC patients⁷. Reduced miR-485-5p is an independent predictor of poor prognosis in GC patients⁸. These above studies indicate that microRNAs show important biological functions in tumor development. However, the role of miR-1294 expression in GC remains unknown.

In the study, we first showed that miR-1294 expression was significantly downregulated in GC tissues compared to adjacent normal tissues. The low expression of miR-1294 in patients with GC was associated with larger tumor size, lymph node metastasis, distant metastasis, and poor prognosis. Moreover, we demonstrated that lower miR-1294 expression was identified as an independent risk factor of GC prognosis.

Patients and Methods

Patient Tissue Samples

A total 82 cases of GC tissues and adjacent normal tissues (distance from the tumor, ≥ 5 cm) used in the study were obtained from the Department of General Surgery, the First Affiliated Hospital of Wenzhou Medical University between

Introduction

Gastric cancer (GC) is the second leading cause of cancer-related mortality worldwide¹. Early GC patients exhibit a good five-year survival rate that is more than 90% after curative

March 2011 and Feb 2013. Patients consist of 48 males and 33 females (mean age, 55.3 years, age range 30-88-year-old). All tissue samples were obtained from patients through surgery. None of the patients had received chemotherapy or radiotherapy prior to surgery. After surgery, the tissue samples were stored at -80°C until further RNA analysis. The clinical data was shown in Table I. The overall survival time was identified from the date of operation to the time of death or last follow-up date. All the participants signed the informed consent. The study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University.

Cell Lines Cultures

Human GC cell lines including SGC-7901, MKN-45, BGC-803, BGC-823, AGS, and the immortalized gastric epithelial cell line GES-1 were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Thermo

Scientific HyClone, Beijing, China) supplemented with 10% fetal bovine serum (FBS; Thermo Scientific HyClone, Beijing, China). Cells were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air.

Quantitative Reverse Transcription-PCR (QRT-PCR)

Total RNA sample was extracted from GC tissue samples and adjacent normal tissues. Total RNA reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Total RNA was reverse-transcribed to cDNA using the TaqMan® Reverse Transcription Reagent Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. QRT-PCR reactions were performed using SYBR-Green Master Mix (Applied Biosystems, Foster City, CA, USA) on the ABI 7500 Real Time-PCR system (Applied Biosystems, Foster City, CA, USA). The reaction cycling condition was as follows: 95°C for 10 min, and then 40 cycles of 95°C for 10 sec and 60°C for 30 sec. The U6

Table I. Association of between miR-1294 expression level and clinicopathological characteristics of patients.

Clinicopathological characteristics	Patients (n=87)	Lower expression (n=38)	Higher (n=44)	p-value
Age (years)				0.335
≤55	16	16	14	
>55	52	22	30	
Gender				0.576
Female	34	17	17	
Male	48	21	27	
Tumor size				0.022*
<3 cm	35	12	25	
≥3 cm	45	26	19	
Differentiation				0.772
High	31	15	16	
Medium and poor	51	23	28	
Distant metastasis				0.006*
Yes	19	14	5	
No	63	24	39	
Lymph node metastasis				0.007*
Negative	39	12	27	
Positive	43	26	17	
Local invasion				0.215
T1-T2	48	25	23	
T3-T4	34	13	21	
TNM stage				0.109
I-II	38	14	24	
III-IV	44	24	20	

*p<0.05.

expression was used as an internal control. The miR-1294 expression was calculated using the $2^{-\Delta\Delta Ct}$ methods. The primer sequence for miR-1294 (forward: 5'-TATGATCTCACCGAGTCCT-3', reverse: 5'-CACCTTCCTAATCCTCAGTT-3') was purchased from Guangzhou Ribo Bio Co., Ltd. (Guangzhou, China).

Statistical Analysis

The SPSS software (version 19.0; SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis. The relationship between the expression of miR-1294 and clinical characteristics was analyzed using the Student's *t*-test or a χ^2 -analysis. The DFS and OS were plotted according to the Kaplan-Meier method and log-rank test. A $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression of miR-1294 is Downregulated in GC Tissues and Cells

In the study, we first detected the expression of miR-1294 in 82 cases of GC tissues and matched adjacent normal tissues by using qRT-PCR analyses. The results demonstrated that the expression of miR-1294 in GC tissues was markedly downregulated than those in matched adjacent normal tissues (Figure 1A, $p < 0.05$). Similarly, miR-1294 expression was significantly downregulated in five human GC cells lines (MKN-45, SGC-7901, MGC-803, AGS, and BGC-823) compared with

the gastric epithelial cell line (GES-1) (Figure 1B, $p < 0.05$). These results indicated that miR-1294 expression was lower in GC tissues and cells, respectively.

Correlation Between miR-1294 Expression and Clinicopathological Factors in GC Patients

To investigate the clinical role of miR-1294 expression in GC, the χ^2 -analysis was used to assess the association between miR-1294 expression and clinicopathological factors in GC patients. As shown in Table I, according to the expression of miR-1294 in GC tissues, the patients were divided into two groups (higher miR-1294 expression and lower miR-1294 expression). The results showed that lower miR-1294 expression was correlated with various clinicopathological parameters, including larger tumor size, lymph node metastasis, and distant metastasis in GC patients (all of $p < 0.05$, Table I). However, no association was found between miR-1294 expression and age, gender, differentiation, and the other factors (all of $p > 0.05$, Table I). Thus, these results first showed that miR-1294 was related to some clinicopathological parameters of GC patients.

Correlation Between miR-1294 Expression and DFS and OS of GC Patients

To investigate the prognostic value of miR-1294 expression in GC patients, a survival analysis was performed. The patients were divided into higher miR-1294 expression ($n=44$) and low

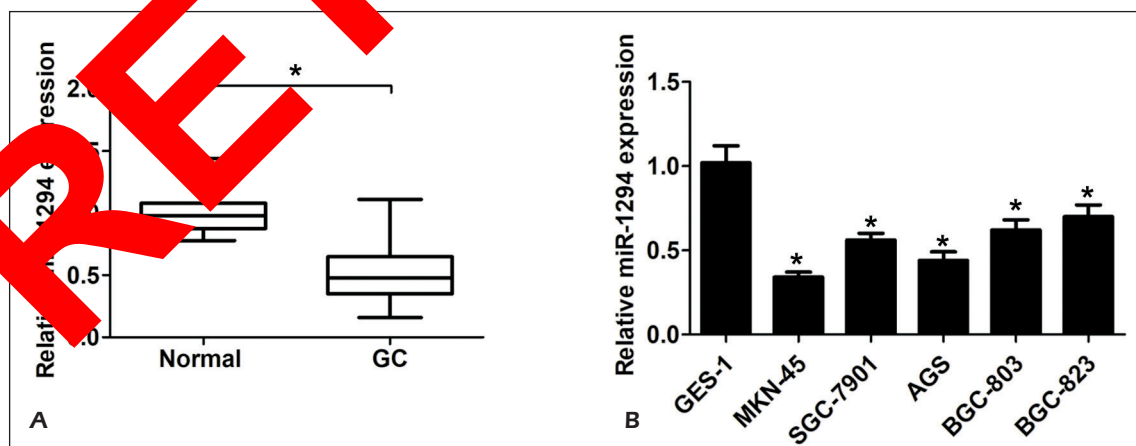


Figure 1. Expression of miR-1294 is downregulated in GC tissues and cells. **A**, The expression of miR-1294 was determined using qRT-PCR analyses in 82 cases of GC tissues and adjacent normal tissues, $*p < 0.05$. **B**, The expression of miR-1294 was determined using qRT-PCR analyses in human GC cell lines SGC-7901, MKN-45, BGC-803, BGC-823, AGS and the immortalized gastric epithelial cell line GES-1, $*p < 0.05$.

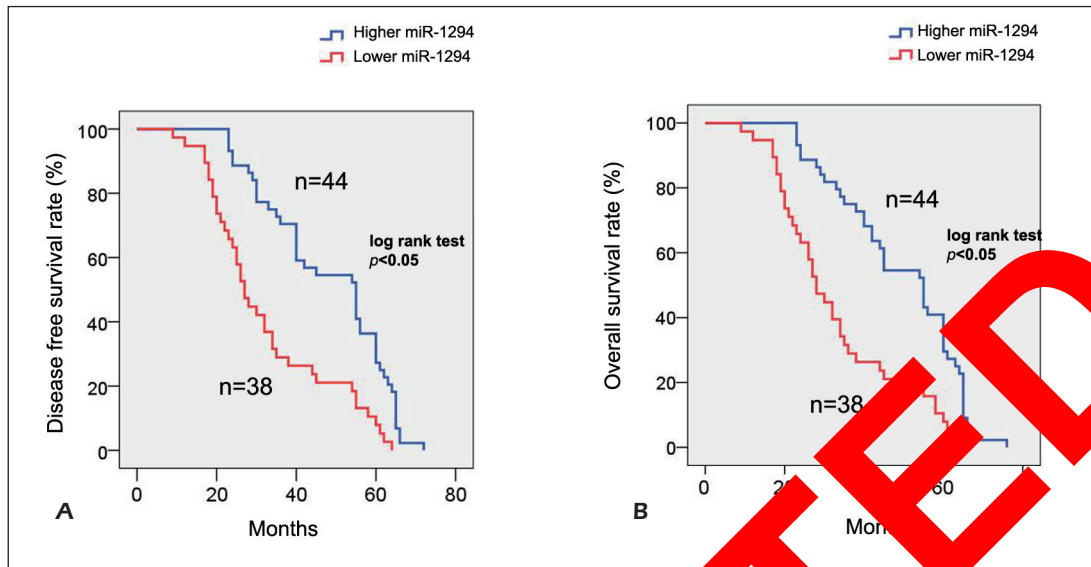


Figure 2. Correlation between miR-1294 expression and DFS and OS of GC patients. **A** Kaplan-Meier method and log-rank test showed that lower miR-1294 expression exhibited a shorter disease free survival (DFS) (log-rank test, $p < 0.05$) and **(B)** overall survival rate (OS) (log-rank test, $p < 0.05$) compared to higher miR-1294 group in GC patients.

miR-1294 expression (n=38) groups. According to the Kaplan-Meier method and log-rank test, the results showed that lower miR-1294 expression exhibited a shorter disease-free survival (DFS) rate (log-rank test, $p < 0.05$, Figure 2A) and overall survival rate (OS) (log-rank test, $p < 0.05$, Figure 2B) compared to those patients with higher miR-1294 expression. Furthermore, univariate and multivariate Cox analysis showed that lower miR-1294 expression (HR, 2.013, 95% CI, 0.645-3.965, $p < 0.05$), large tumor size (HR, 1.866, 95% CI, 0.654-3.346, $p < 0.05$), lymph node metastasis (HR, 1.982, 95% CI, 0.601-3.855, $p < 0.05$) and distant metastasis (HR,

1.982, 95% CI, 0.787-3.565, $p < 0.05$) were identified as independent risk factors of DFS in GC patients (Table II). Similarly, the results showed that lower miR-1294 expression (HR, 2.206, 95% CI, 0.965-4.047, $p < 0.05$), large tumor size (HR, 1.906, 95% CI, 0.709-3.535, $p < 0.05$), lymph node metastasis (HR, 2.113, 95% CI, 0.887-3.866, $p < 0.05$) and distant metastasis (HR, 2.008, 95% CI, 0.966-3.873, $p < 0.05$) were independent prognostic factors of OS in GC patients (Table III). Thus, these results indicated that miR-1298 expression significantly correlated with GC prognosis and serves as a biomarker of GC prognosis.

Table II. Univariate and multivariate analysis of DFS in 82 cases of GC patients.

Factors	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (<65)	0.589 (0.263-1.146)	0.912		
Gender	0.843 (0.411-1.355)	0.698		
Tumor size (≥ 3 cm)	2.126 (0.864-3.845)	0.001*	1.866 (0.654-3.346)	0.004*
Differentiation	1.034 (0.655-1.654)	0.598		
Distant metastasis	2.454 (0.944-3.926)	0.001*	1.988 (0.787-3.565)	0.001*
Lymph node metastasis	2.386 (0.772-4.268)	0.001*	1.982 (0.601-3.855)	0.001*
Local invasion	0.784 (0.441-1.268)	0.832		
TNM stage	1.125 (0.341-1.347)	0.498		
Lower miR-1294	2.480 (0.866-4.414)	0.001*	2.013 (0.645-3.965)	0.001*

* $p < 0.05$.

Table III. Univariate and multivariate analysis of DFS in 82 cases of GC patients.

Factors	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (years)	0.697 (0.326-1.255)	0.887		
Gender	0.955 (0.522-1.861)	0.602		
Tumor size (≥3 cm)	2.246 (0.903-3.766)	0.001*	1.906 (0.709-3.535)	0.002*
Differentiation	0.995 (0.774-1.977)	0.587		
Distant metastasis	2.662 (1.148-4.495)	0.001*	2.008 (0.966-3.873)	0.001*
Lymph node metastasis	2.455 (0.984-4.288)	0.001*	2.113 (0.887-3.866)	0.001*
Local invasion	0.904 (0.611-1.558)	0.733		
TNM stage	1.035 (0.652-1.632)	0.566		
Lower miR-1294	2.746 (1.125-4.899)	0.001*	2.206 (0.965-4.000)	0.001*

* $p < 0.05$.

Discussion

Recently, microRNAs have been identified to participate in tumor genesis and development of gastric cancer⁹. Aberrant expression of different miRNAs could serve as predictors of GC prognosis¹⁰. Such as, high expression of miR-16 and miR-451 predict better prognosis in patients with gastric cancer¹¹. Circulating microRNA-196^{1b} are novel biomarkers associated with metastatic gastric cancer¹². MiR-21-5p is identified as a predictor of recurrence in young gastric cancer patients¹³. MiR-451 showed decreased expression in GC tissues and cell lines. Downregulation of miR-451 is positively correlated with lymphatic metastasis, clinical staging and poor overall survival of patients¹⁴. In previous study, miR-1294 was found to be significantly down-regulated in human ES cells and patients with lower miR-1294 expression exhibited a significantly poorer prognosis than the patients with a higher miR-1294 expression¹⁵. However, the role of miR-1294 expression in GC remains unknown.

In the present work, we first found that miR-1294 expression was significantly downregulated in GC tissues compared to adjacent normal tissues. Furthermore, we observed that the reduced expression of miR-1294 in patients with GC was correlated with larger tumor size, lymph node metastasis, and distant metastasis in GC patients. Kaplan-Meier method and log-rank test showed that GC patients with lower miR-1294 expression exhibited a shorter disease-free survival rate and overall survival rate compared to those patients with higher miR-1294 expression. These results indicated that miR-1294 could serve as a prognostic marker in GC.

Next, univariate and multivariate Cox analysis showed that lower expression of miR-1294, larger

tumor size, lymph node metastasis, and distant metastasis were identified as independent risk factors of GC prognosis. In the previous study¹⁶, cancer antigen 199 (CA199) and carcinoembryonic antigen (CEA) are identified as the common tumor diagnostic markers; however, their specificity and sensitivity are too low for GC diagnosis. Thus, to explore a novel biomarker for diagnosing or predicting GC prognosis is urgently needed. In the future, combination of miRNAs including CEA or CA199 and miRNAs for diagnosing or predicting GC prognosis needs to be investigated.

Conclusions

In the study, we first demonstrated that miR-1294 expression was lower in GC tissues and cells. Lower miR-1294 expression predicted a poor prognosis. MiR-1294 may serve as a potential predictor for GC prognosis.

Competing interests:

The authors declare that they have no competing interests.

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