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Downregulation of miR-145-5p correlates with poor prognosis in gastric cancer

Y. ZHANG[1], X. WEN[2], X.-L. HU[2], L.-Z. CHENG[2], J.-Y. YU[1], Z.-B. WEI[1]

[1]Department of Oncology, Heping Hospital, Changzhi Medical College, Changzhi, Shanxi, P.R. China
[2]Department of Pharmacy, Heping Hospital, Changzhi Medical College, Changzhi, Shanxi, P.R. China

Ying Zhang and Xin Wen contributed equally to this work

Abstract. – OBJECTIVE: The aim of this study was to explore the clinical significance of miR-145-5p expression in gastric cancer (GC).

PATIENTS AND METHODS: Expression of miR-145-5p was evaluated by qRT- PCR in tumor and normal gastric tissues in 145 GC patients. The correlation between the miR-145-5p expression and clinicopathological parameters was investigated. Finally, the survival was assessed by the Kaplan-Meier method and proportional hazards model.

RESULTS: Expression levels of miR-145-5p in GC tissues were significantly lower than those in adjacent normal tissues ($p < 0.001$). MiR-145-5p expression was significantly associated with lymph node metastasis, metastasis stage, and distant metastasis (all $p < 0.05$). Furthermore, patients with low miR-145-5p expression had poorer overall survival time than those with high miR-145-5p expression (p = 0.014).

Moreover, univariate and multivariate Cox analysis showed that miR-145-5p was an independent prognostic indicator for OS ($p = 0.011$).

CONCLUSIONS: MiR-145-5p is down-expressed in GC, and can be used as a marker of poor prognosis in GC patients.

Key Words:
miR-145-5p, Gastric cancer (GC), Prognostic biomarker.

Introduction

Gastric cancer (GC) is one of the most frequent malignancies worldwide accounting for 700,000 deaths annually[1,2]. Though great improvement has been achieved in the diagnosis and the treatment field, in most countries, survival rate from stomach cancer remained in the narrow range of 25-30%[3,4]. Although many studies reported novel prognostic factors in GC patients, truly effective clinical prognostic and diagnostic biomarkers are scarce[5,6]. Therefore, identifying biomarkers which can accurately identify the biological characteristics of gastric cancer is one of the major challenges.

MicroRNAs (miRNAs) are small, endogenous RNA molecules that negatively regulate gene expression at the post-transcriptional level[7,8]. MiRNA has been reported to be involved in many physiological processes such as differentiation, cell proliferation, and others[9,10]. Aberrantly expression of miRNAs has been found in several cancers including GC. Recent studies have found that more than 40 miRNAs may be used as prognostic biomarkers[11]. However, the clinical significance of miR-145-5p in GC has not been elucidated. Here we investigate the clinical significance of miR-145-5p.

Patients and Methods

Patients and Tissue Samples

The human GC tissue samples were obtained from the patients who underwent surgery at the Department of Oncology, Heping Hospital, Changzhi Medical College between May 2008 and April 2014. Patients with GC were diagnosed GC cases, without any preoperative radiotherapy or chemotherapy before. Resected tissue samples were immediately cut and snap-frozen in liquid nitrogen before being stored at –80°C until RNA was extracted. The clinical information is provided in Supplementary Table I. The written informed consent had been obtained from all the patients. All our protocols were approved by the Human Research and Ethics Committees of at Heping Hospital in China.

RNA extraction and real-time RT-PCR

Total RNA was extracted from frozen tissues using the RNeasy mini kit (Invitrogen Corp,

Corresponding Author: Zi-bai Wei, MD; e-mail: weiw5m@sina.com

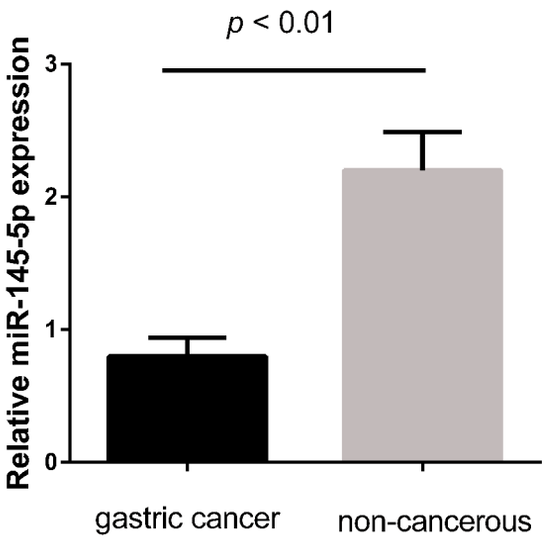

Figure 1. The expression level of miR-145-5p in GC tissues and noncancerous gastric tissues.

Carlsbad, CA, USA) according to the manufacturer instructions. We used an ABI 7500 Fast Real-Time PCR System (Invitrogen, Corp, Carlsbad, CA, USA) to determine the relative expression levels of miR-145-5p and (GADPH, evaluated as control transcript). Gene expression was quantified in relation to the values of the control group following normalization against the internal control using the $2^{-\Delta\Delta Ct}$ method. Each sample was examined in triplicate.

Statistical Analysis

All data about continuous variables were expressed as mean ± SD. All statistical analyses were carried out by SPSS13.0 software (SPSS Inc., Chicago, IL, USA). The expression level of miR-145-5p was compared between GC tissues and matched adjacent normal tissues using the two-sample Student *t*-test. Survival curves were plotted by the Kaplan-Meier method, and the log-rank test evaluated the significance. $p < 0.05$ was considered statistically significant.

Results

MiR-145-5p is Lowly Expressed in GC

To analyze the expression of miR-145-5p in GC, we collected 145 pairs of human GC samples and their adjacent non-tumorous gastric tissues. The expression level of miR-145-5p was detected by qRT-PCR. As shown in Figure 1, miR-145-5p was significantly downregulated in GC tissues compared with the adjacent noncancerous tissues ($p < 0.01$).

Table I. Association of miR-145-5p with clinicopathological characteristics of GC patients.

Variable	Patients, n (n = 145)	miR-145-5p expression (Mean ± SEM)	*p*-value
Age (years)			0.681
≥55	80	1.129 ± 0.0538	
<55	65	1.116 ± 0.0499	
Sex			0.395
Male	90	1.143 ± 0.0733	
Female	55	1.158 ± 0.0693	
Tumor size (cm)			0.458
≥3	76	1.247 ± 0.0351	
<3	69	1.283 ± 0.0419	
Invasion depth			0.749
T1/T2	72	1.277 ± 0.0671	
T3/T4	73	1.293 ± 0.0714	
Lymph node metastasis			0.031
Yes	57	1.035 ± 0.0477	
No	88	1.229 ± 0.0629	
TNM stage			0.000
I-II	75	1.451 ± 0.0677	
III-IV	70	1.016 ± 0.0443	
Distant metastasis			0.022
Positive	81	1.033 ± 0.0518	
Negative	64	1.284 ± 0.0674	

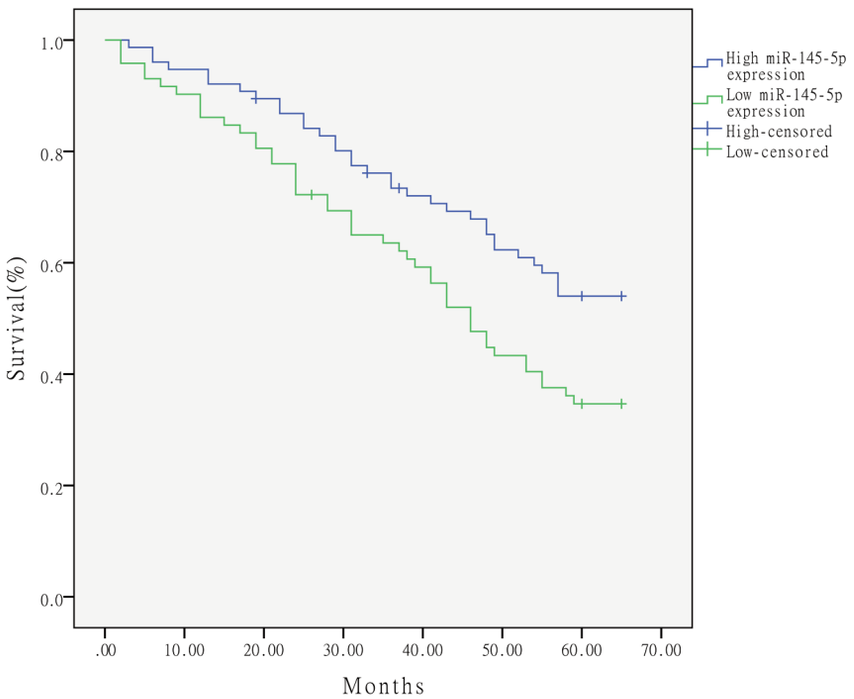

Figure 2. miR-145-5p expression and its association with overall survival in patients with GC.

Down-Regulation of miR-145-5p Associates with Advanced Clinicopathological Features of GC

The relationship between miR-145-5p expression and clinicopathologic parameters was evaluated. As shown in Table I, low miR-145-5p expression was significantly associated with lymph node metastasis, metastasis stage, and distant metastasis (all $p < 0.05$). These results indicated that miR-145-5p might suppress GC progression. However, there was no significant correlation of miR-145-5p expression with other clinical features such as gender and age (all $p > 0.05$, shown in Table I).

Mir-145-5p downregulation associates with poor prognosis in patients with GC

The Kaplan-Meier method was used to plot overall survival according to the expression level of miR-145-5p. As shown in Figure 2, Patients with GC expressing a higher level of miR-145-5p had significantly shorter OS ($p = 0.014$). Furthermore, univariate analysis showed that miR-145-5p expression was statistically significant prognostic factors ($p = 0.012$, Table II). In multivariate analysis, miR-145-5p expression was identified as an independent prognostic factor for OS ($p = 0.001$, Table III).

Discussion

Growing evidence has indicated that miRNAs are implicated in several diseases and cellular functions, including apoptosis, differentiation, as well as proliferation[12-14]. Furthermore, miRNAs have been certified as an important biological RNAs in the post-transcriptional regulation of the target genes[15]. In recent years, more and more studies have identified that miRNAs are correlated with prognosis in tumor patients, In the present study. We focus on miR-145-5p.

Recent studies demonstrated that miR-145 was involved in the progression of cancers. For instance, Xiong et al[16] found that miR-145 inhibited human intrahepatic cholangiocarcinoma cell proliferation by targeting NUAK1. Zhang et al[17] found miR-145 promotes non-small cell lung cancer cell proliferation and invasion through down-regulating FSCN1 expression. Ren et al[18] showed that miR-145-5p inhibited prostate cancer through targeting ZEB2. More importantly, Jiang et al[19] revealed that MicroRNA-145-5p function as a tumor suppressor in gastric cancer by targeting N-cadherin and ZEB2. All those results informed that miR-145-5p may play a suppressive role in the pathogen-

Table II. Univariate analysis of overall survival in GC patients

Variable	No.	Hazard ratio	95% CI	p-value
Age (years)				
≥55	80	0.685	0.355-1.276	0.427
<55	65			
Sex				
Male	90	0.811	0.493-1.552	0.677
Female	55			
Tumor size (cm)				
≥3	76	0.741	0.512-2.349	0.717
<3	69			
Invasion depth				
T1/T2	72	0.344	0.339-1.406	0.413
T3/T4	73			
Lymph node metastasis				
Yes	57	2.336	1.004-6.231	0.013
No	88			
TNM stage				
I-II	75	0.329	0.117-0.829	0.004
III-IV	70			
Distant metastasis				
Positive	81	2.476	1.023-5.441	0.021
Negative	64			
miR-145-5p				
High	76	2.661	1.129-7.772	0.012
Low	69			

Table III. Multivariate analysis of overall survival in GC patients.

Variable	No.	Hazard ratio	95% CI	*p*-value
Lymph node metastasis				
Yes	57	1.231	0.558-2.365	0.663
No	88			
TNM stage				
I-II	75	0.831	0.139-0.993	0.016
III-IV	70			
Distant metastasis				
Positive	81	0.515	0.318-1.553	0.395
Negative	64			
miR-145-5p				
High	76	3.873	1.129-11.443	0.011
Low	69			

esis of tumor. However, until now, the clinical significance of miR-145-5p in gastric cancer patients has not been reported.

In this work, we found that expression of miR-145-5p was commonly down-regulated in GC when compared with that in normal gastric tissues. Also, miR-145-5p expression was associated with lymph node metastasis, metastasis stage, and distant metastasis. Moreover, Kaplan-Meier analysis showed that GC patients with low miR-145-5p expression tend to have shorter overall survival. Finally, univariate and multivariate analysis confirmed that miR-145-5p was an independent prognostic marker for GC.

Conclusions

We indicate that the expression of miR-145-5p is significantly downregulated in GC, and is identified for the first time as an independent poor prognostic factor for patients with GC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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