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Prognostic value of miR-141 downregulation in gastric cancer

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ABSTRACT. Previous research has shown that microRNA-141 (miR-141) expression levels are associated with survival in several types of cancer. In the present study, we investigated the clinical significance and prognostic value of miR-141 in gastric cancer. Paired tissue specimens (tumor and adjacent normal mucosa) from 95 patients with gastric cancer were obtained at the Department of General Surgery, Xiangya Hospital, Central South University from March 2009 to February 2014. The levels of miR-141 in cancerous and corresponding non-cancerous tissues were detected by quantitative reverse transcription-polymerase chain reaction. Associations between clinicopathological parameters and miR-141 expression were evaluated using chi-square tests. Overall survival was calculated and survival curves were plotted using the Kaplan-Meier method; differences between groups were compared using log-rank tests. Compared to the matched normal gastric mucosa, gastric cancer tissues had significantly lower miR-141 expression levels ($P < 0.001$). This decreased miR-141 expression was significantly associated with tumor differentiation ($P = 0.044$), positive lymph node metastasis ($P = 0.010$), distant metastasis ($P < 0.001$), and advanced tumor-node-metastasis (TNM) stage ($P < 0.001$). Furthermore, a significant relationship was found between miR-141 expression and overall survival ($P = 0.012$, log-rank

test). Cox regression analysis revealed that lymph node metastasis (P = 0.003), distant metastasis (P = 0.001), TNM stage (P < 0.001), and miR-141 expression (P = 0.007) were independent prognostic factors in patients with gastric cancer. Our data provide evidence that the downregulation of miR-141 may contribute to the aggressive progression and poor prognosis of human gastric cancer.

Key words: Gastric cancer; MiR-141; Expression; Prognosis

INTRODUCTION

Gastric cancer is the fifth most common cancer and the third leading cause of cancer death in the world (Siegel et al., 2015). Thirty-three percent of gastric cancer cases occur in China (Ye et al., 2011). There is regional variation in worldwide incidence rates, with more than 70% of gastric cancers occurring in developing countries because of high prevalence of *Helicobacter pylori* (Wang et al., 2002). Even with maximal trimodality therapies, the prognosis of gastric cancer patients remains poor, with a 5-year survival rate of 25-35% for loco-regional disease and a median survival rate ranging from 10 to 14 months in advanced disease states (Cervantes et al., 2007). Therefore, it is important to identify novel markers and therapeutic targets for an improvement in the prognosis and mortality of gastric cancer.

Mature microRNAs (miRNAs) are 21-22 nucleotides in size and affect the post-translational expression of genes by interacting with complementary target sites within the 3'-untranslated region of the messenger RNA (Morris et al., 2004). Studies have indicated that miRNAs can regulate a wide range of biological processes such as cell proliferation, differentiation, and apoptosis (Bartel, 2004). MicroRNA-141 (miR-141) is a member of the miR-200 family, which plays an important role in the pathogenesis of various malignant tumors. Previous studies have indicated that the expression pattern of miR-141 varies in different malignant tumors. Furthermore, it has also been shown that the expression level of miR-141 was associated with cancer-specific survival (Wszolek et al., 2011; Brunet Vega et al., 2013). In the present study, we investigated the clinical significance and prognostic value of miR-141 in gastric cancer.

MATERIAL AND METHODS

Patients and specimens

Paired tissue specimens (cancerous and non-cancerous) from 95 patients with gastric cancer were obtained and histologically confirmed by a pathologist at the Department of General Surgery, Xiangya Hospital, Central South University from March 2009 to February 2014. Fresh tissues, including gastric cancer tissues and adjacent normal tissues, were collected, immediately flash-frozen in liquid nitrogen after surgery, and stored at -196°C until use. None of the patients had received chemotherapy or radiotherapy prior to surgery. The clinical and pathological data for the patients is provided in Table 1. Preoperative demographic and clinical data were collected from the patients. This study was approved by the Medical Ethics Committee of Xiangya Hospital, Central South University, and written informed consent was obtained from all patients. The specimens were handled anonymously according to ethical and legal standards.

Table 1. Association of miR-141 expression with clinicopathological features of gastric cancer.

Variables	Cases (N)	miR-141 expression		P value
		Low (N = 46)	High (N = 49)	
Age (years)				
<60	44	17	27	0.100
≥60	51	29	22	
Gender				
Male	57	30	27	0.403
Female	38	16	22	
Differentiation degree				
Well/Moderately	66	27	39	0.044
Poorly	29	19	10	
Depth of invasion				
T1/T2	59	31	28	0.398
T3/T4	36	15	21	
Lymph node metastasis				
N0/N1	70	28	42	0.010
N2/N3	25	18	7	
Distant metastasis				
Yes	9	8	1	<0.001
No	86	38	48	
TNM stage				
I+II	58	19	39	<0.001
III+IV	37	27	10	

Total RNA isolation and quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from frozen tissues using the RNeasy mini kit (QIAGEN, Hilden, Germany) according to the manufacturer instructions. Mature miRNAs were reverse transcribed using the TaqMan® MicroRNA reverse transcription kit (Invitrogen Company, USA), and quantitative PCR was performed using TaqMan microRNA assays with specific primers for miR-141. Quantitative PCR was performed on the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, USA). The cycling conditions were as follows: 95°C for, 15 min, followed by 30 cycles of 60°C for 30 s and 72°C for 30 s. Samples were maintained at 4°C until further analysis. Small nucleolar RNA U6 was used as an internal standard for normalization. The cycle threshold (Ct) value was calculated and the $2^{-\Delta Ct}$ ($^{\Delta}Ct = Ct_{miR-141} - Ct_{U6RNA}$) method was used to quantify the relative amount of miR-141.

Statistical analysis

All statistical analyses were performed using SPSS 18.0 software and graphs were plotted using GraphPad Prism 5.02. Associations between clinicopathological parameters and miR-141 expression were evaluated using chi-square tests. Overall survival was calculated and survival curves were plotted using the Kaplan-Meier method; differences between groups were compared using log-rank tests. Significant variables in univariate models were further analyzed by multivariate Cox proportional hazards regression models to identify the independent prognostic values. A two-tailed P value of less than 0.05 was considered significant.

RESULTS

miR-141 is downregulated in gastric cancer tissues

The levels of miR-141 in 95 cancerous and corresponding non-cancerous tissues were detected by qRT-PCR. Compared with the matched normal gastric mucosa, gastric cancer tissues had significantly lower miR-141 expression levels (P < 0.001; Figure 1). By adopting a cut-off value corresponding to the median miR-141 level, patients were sorted into two categories: 46 patients had low levels of miR-141 and 49 patients had high levels of miR-141.

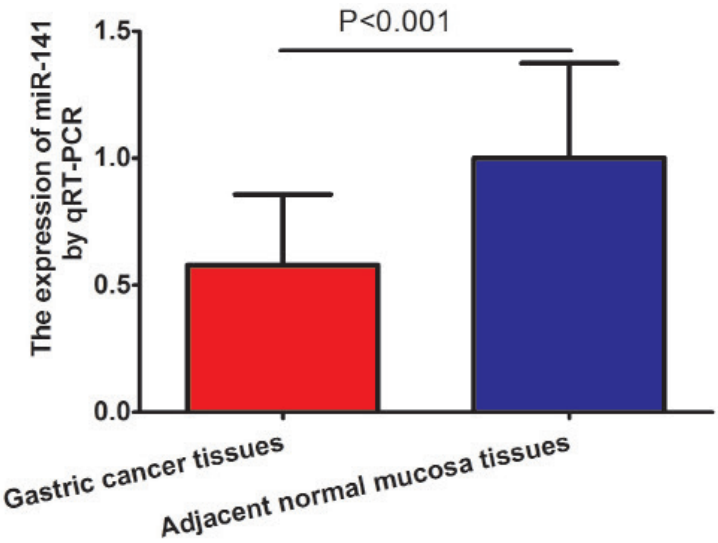

Figure 1. Relative expression of miR-141 in gastric cancer tissues and the matched normal gastric mucosa.

miR-141 downregulation is associated with aggressive progression in gastric cancer

The association between expression level of miR-141 and clinicopathological features of gastric cancer are summarized in Table 1. The results revealed that low miR-141 expression was significantly associated with tumor differentiation (P = 0.044), positive lymph node metastasis (P = 0.010), distant metastasis (P < 0.001), and advanced TNM (tumor-node-metastasis) stage (P < 0.001) in patients with gastric cancer.

miR-141 downregulation predicts poor prognosis in patients with gastric cancer

A Kaplan-Meier plot of overall survival stratified by expression level of miR-141 is shown in Figure 2. A significant relationship was found between miR-141 expression and overall survival (P = 0.012, log-rank test). Table 2 shows the multivariate analyses of factors related to patient prognosis. Factors with possible prognostic effects in gastric cancer were analyzed by Cox regression analysis. The results revealed that lymph node metastasis (P = 0.003), distant metastasis (P = 0.001), TNM

stage (P < 0.001), and miR-141 expression (P = 0.007) were independent prognostic factors in patients with gastric cancer.

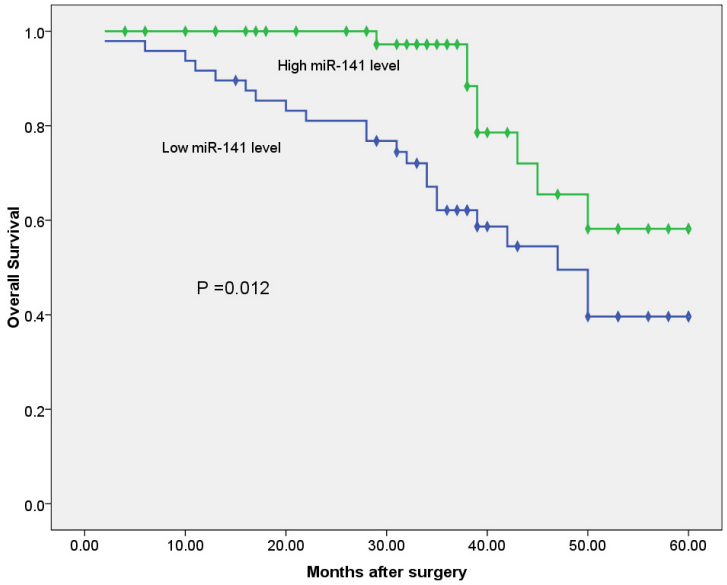

Figure 2. Survival curves in patients with gastric cancer according to miRNA-141 expression levels.

Table 2. Cox regression model for multivariate analysis of prognostic factors for gastric cancer.

Variable	Overall survival		
	HR	95%CI	P value
Age	1.387	0.763-2.993	0.142
Gender	1.212	0.261-3.192	0.579
Differentiation degree	3.192	0.891-12.194	0.051
Depth of invasion	1.979	0.782-7.328	0.091
Lymph node metastasis	3.464	1.834-14.835	0.003
Distant metastasis	3.873	2.013-18.934	0.001
TNM stage	4.829	3.192-19.276	<0.001
miR-141 expression level	2.972	1.297-10.001	0.007

HR = Hazard ratio; CI = confidence interval.

DISCUSSION

Epidemiological data indicate that the current upward trend of gastric cancer worldwide is quite severe, particular in Asia (Koessler et al., 2014). The major reason for this is a lack of specific symptoms for early diagnosis and few treatment options for gastric cancer. This is illustrated by the fact that gastric cancer is diagnosed at an early stage in less than 10% of cases in Europe. The identification of new molecular biomarkers will improve early diagnosis, treatment, and clinical outcome in gastric cancer patients.

Growing evidence shows that miRNAs play important roles in a variety of biological processes related to apoptosis, proliferation, differentiation, metastasis, angiogenesis and immune response, and their dysregulation may be crucial to cancer initiation, progression, and treatment outcome (Palanichamy and Rao, 2014). Thus, miRNAs have been recognized as good candidates for diagnostic, prognostic, and predictive biomarkers for many human cancers, including gastric cancer. A number of miRNAs have been reported to be upregulated and function as oncogenes in gastric cancer. MiR-141 is a member of the miR-200 family, which plays an important role in the pathogenesis of various malignant tumors. Previous studies have indicated that the expression pattern of miR-141 varies in different malignant tumors. It has been observed that miR-141 was downregulated in pancreatic cancer, renal cancer, hepatocellular carcinoma, and breast cancer, whereas it was upregulated in colorectal cancer and prostate cancer (Yaman Agaoglu et al., 2011; Maruyama et al., 2012; Brunet Vega et al., 2013; Luo et al., 2013; Yu et al., 2013; Yeh et al., 2014). Furthermore, it was also shown that the expression level of miR-141 was associated with cancer-specific survival. Tejero et al. (2014) found that high miR-141 expression was associated with shorter overall survival in patients with non-small cell lung cancer (NSCLC). Gao and Wu (2015) found that serum miR-141 levels were significantly elevated in epithelial ovarian cancer patients compared to healthy controls, and patients with low miR-141 levels showed a significantly higher rate of survival. Therefore, expression level of miR-141 may be a predictive biomarker for ovarian cancer prognosis.

Previously, Zuo et al. (2015) found that the expression of miR-141 was significantly reduced in gastric cancer tissues compared with paired adjacent normal tissues. MiR-141 inhibited tumor growth and metastasis in gastric cancer by directly targeting the transcriptional co-activator with PDZ-binding motif, TAZ. In the present study, we investigated the clinical significance and prognostic value of miR-141 in gastric cancer. The levels of miR-141 in cancerous and corresponding non-cancerous tissues collected from 95 gastric cancer patients were detected by qRT-PCR. Compared with the matched normal gastric mucosa, gastric cancer tissues had significantly decreased miR-141 expression levels. Low miR-141 expression was significantly associated with tumor differentiation, positive lymph node metastasis, distant metastasis, and advanced TNM stage of patients with gastric cancer, indicating that miR-141 downregulation was associated with aggressive progression in gastric cancer. Moreover, we found a significant relationship between miR-141 expression and overall survival of gastric cancer patients. Cox regression analysis revealed that lymph node metastasis, distant metastasis, TNM stage, and miR-141 expression were independent prognostic factors in patients with gastric cancer. In conclusion, our data provide evidence that the dysregulation of miR-141 may contribute to the aggressive progression and poor prognosis of human gastric cancer.

Conflicts of interest

The authors declare no conflict of interest.

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