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Original Article

Serum *miR-133a* is down-regulated and associated with the diagnosis of patients with gastric cancer

Yingbin Hu[1*], Junjiang Wang[2*], Yong Han[3], Qun Liu[4], Qiong Niu[1]

[1]Department of Gastroenterology, Binzhou Medical University Hospital, Binzhou 256603, Shandong, China; [2]Department of General Surgery, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China; [3]Department of General Surgery, Binzhou Medical University Hospital, Binzhou 256603, Shandong, China; [4]Department of Health Examination Center, Binzhou Medical University Hospital, Binzhou 256603, Shandong, China. [*]Co-first authors.

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Abstract: Aim: The present study was designed to detect the expression of *miR-133a* and assess the diagnostic value of it in gastric cancer (GC). Methods: The expression of serum *miR-133a* was examined by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Chi-square test was used to delineate the association between *miR-133a* expression and clinical parameters of patients with GC. Receiver operating characteristics (ROC) curve was plotted to describe the diagnostic value of *miR-133a* in GC. Results: *miR-133a* expression was decreased in serum of patients with GC compared with that in healthy controls ($P<0.001$). And low expression of *miR-133a* was significantly related to lymph node metastasis, venous invasion, tumor node metastasis and TNM stage, but shared no association with age, gender and tumor size. ROC curve showed *miR-133a* had a high diagnostic value with an area under the ROC curve (AUC) of 0.873 corresponding with a sensitivity of 72.3% and a specificity of 88.6%, respectively. Conclusion: In conclusion, *miR-133a* was down-regulated and could serve as a potential molecule marker for the diagnosis of GC patients.

Keywords: *MiR-133a*, diagnosis, gastric cancer

Introduction

As the third most common cancer worldwide, gastric cancer is the leading cause of cancer-related deaths [1]. The incidence of GC is obviously different among various countries and frequently occurs in Asia [2, 3]. The carcinogenesis of GC is a multi-step and progressive process which begins with chronic gastritis and involves numerous genetic and epigenetic alterations of tumor-related genes [4-6]. Though significant achievements in the early detection have contributed to the survival improvement of early GC and the developments in therapeutic options such as surgery, chemotherapy and radiotherapy, the long-time survival rate of advanced GC remains quite low and the diagnosis of GC patients is still immature [7-10]. Thereby, it is urgently needed to identify efficient and novel biomarkers for early diagnosis of GC.

MicroRNAs (miRNAs) are a class of small, conserved and non-coding RNAs with a length of about 22 nucleotides [11-13]. miRNAs are considered to function as gene regulators and regulate the expression of target genes at both molecular and protein levels through inducing the mRNA degradation or inhibiting the protein translation of targeted genes by binding to the 3'-untranslated region (UTR) of target miRNAs [14-16]. *miR-133a* belongs to the *miR-133* family and is first known as a muscle-specific miRNA [17, 18]. Recently, many reports have demonstrated that *miR-133a* serves as a tumor suppressor in various type of cancers, including bladder cancer, prostate cancer and head and neck squamous cell carcinoma [19-21]. Besides, *miR-133a* is also found to act as a tumor suppressor and inhibit the proliferation and invasion of cells in GC [22, 23]. However, the diagnostic value of *miR-133a* remains unknown in GC.

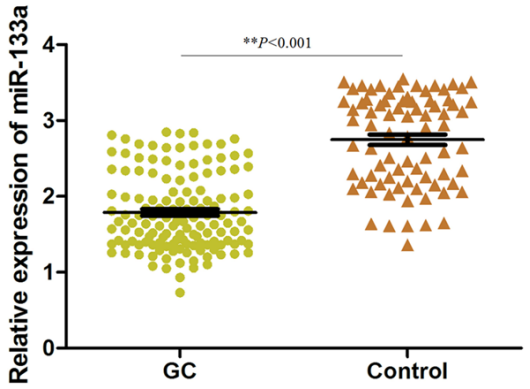

Figure 1. The expression of serum *miR-133a* in GC patients and healthy controls. Serum *miR-133a* expression was significantly lower in GC patients than that in healthy controls ($P<0.001$).

In this study, we detected the expression of *miR-133a* and investigated its relationship with clinical factors of patients with GC. What's more, we estimated the diagnostic value of *miR-133a* in GC via building a ROC curve.

Materials and methods

Patients and specimens

A total of 137 patients, including 73 males and 64 females, who were diagnosed as GC in Binzhou Medical University Hospital were selected in this study. All patients received the same physical examination, blood test and without any therapeutic before sampling. In addition, 79 healthy volunteers were enrolled as healthy controls. The study was authorized by the Ethics Committee of Binzhou Medical University Hospital. All participants had signed written informed consents in advance.

Serum samples from patients with GC and healthy controls were obtained and put into blood collection tubes of EDTA, respectively. Then all samples were severally stored at -80°C for RNA extraction. The clinicopathologic characteristics of GC patients including age, gender, tumor size, lymph node metastasis, venous invasion, tumor node metastasis and TNM stage were recorded in a database.

RNA extraction and qRT-PCR analysis

Total RNA was isolated from all serum specimens using QIAamp blood mini kit (Qiagen, Hilden, Germany) according to the manufacture's instruction, respectively. Reverse transcription was conducted with Tagman MicroRNA Reverse Transcription Kit (Applied Biosystems, CA) to synthesize the first chain of cDNA. Then the RT-PCR was performed in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). U6 small nuclear RNA (U6) was taken as internal controls. The relative expression of *miR-133a* was calculated by the $2^{-\Delta\Delta Ct}$ method. Each sample was in triplicate.

Statistical analysis

Statistical analysis was analyzed using SPSS 18.0 software (SPSS Inc., Chicago, USA) and the figures were designed by Graphpad prism 5. All data were presented as Mean ± standard deviation (SD). The difference of the *miR-133a* expression between GC patients and healthy controls was analyzed via student's t-test. The relationship between *miR-133a* expression and clinical factors was evaluated by chi-square test. ROC curve was established to estimate the diagnostic value of *miR-133a* in GC. The difference was considered to be significant when *P* was less than 0.05.

Results

Down-regulation of miR-133a was observed in GC patients

We determined the expression of miR-133a in 137 GC patients and 79 healthy individuals with qRT-PCR analysis. The relative expression level of miR-133a in GC patients was 1.79±0.51 while that in healthy controls was 2.75±0.60. As shown in **Figure 1**, the expression of miR-133a in GC patients was significantly lower than that in the healthy controls (P<0.001).

Relationship between miR-133a expression and clinical factors of GC patients

The clinical information of GC patients was provided by the hospital. The correlation of *miR-133a* expression and clinical factors was analyzed by Chi-square test. The out- come suggested that the expression of *miR-133a* was tightly related to lymph node metastasis ($P=0.023$), venous invasion ($P=0.012$) and TNM stage ($P=0.008$) (**Table 1**). However, there was no significant relevance between *miR-*

Table 1. Relationship between *miR-133a* expression and clinical factors of patients with GC

Characteristics	Cases (n=137)	*miR-133a* expression		χ^2	*P* value
		Low (n=99)	High (n=38)		
Age				1.058	0.304
≤60	66	45	21		
>60	71	54	17		
Gender				0.449	0.503
Male	73	51	22		
Female	64	48	16		
Tumor size (cm)				1.811	0.178
≤3	56	37	19		
>3	81	62	19		
Lymph node metastasis				5.207	0.023
Absent	72	58	14		
Present	65	41	24		
Venous invasion				6.317	0.012
Yes	67	55	12		
No	70	44	26		
TNM stage				7.128	0.008
I, II	58	35	23		
III, IV	79	64	15		

133a expression and age, gender and tumor size (*P*>0.05, **Table 1**).

Diagnostic value of miR-133a in GC

The diagnostic value of *miR-133a* in GC was estimated by establishing a ROC curve. The result showed that with a AUC of 0.873 combing with a sensitivity of 72.3% and specificity of 88.6%, the diagnostic value of *miR-133a* was high (**Figure 2**). And the optimal cutoff value was 2.04.

Discussion

GC is a kind of malignant tumor which derives from the gastric mucosa epithelial and accounts for the first place of digestive tract malignant tumors. So far, GC has become a common disease that severely threats human's health. The clinical survival of GC patients was significantly unfavorable because of the advanced stage at the diagnosis time. Therefore, early diagnosis is essential for GC patients.

In previous studies, there were some bio-markers for the diagnosis of GC. For instance, OyamaK et al. suggested M30 and M65 were useful biomarkers for diagnosis of GC patients via investigating the serum levels of them with ELISA analysis [24]. *MiR-199a-3p* was proved to be a potential diagnostic marker for GC by Li et al. due to its up-regulation in this disease [25]. Su et al. showed that *miR-18a* could discriminate GC patients from healthy controls with a high AUC of 0.907 as well as a high sensitivity and specificity [26]. *miR-133a* had been reported to played a crucial role in myoblast proliferation and differentiation during embryonic muscle development [27]. Accumulated evidences showed it abnormal expression and related to many processes of cancers. Yuan et al., found that *miR-133a* was down-regulated and involved in the development of breast cancer by regulating the expression of UCP-2 [28]. *miR-133a* was found to repress cell invasion of colorectal cancer by targeting Fascin1 in the study of Zheng et al. [15]. Wang et al., considered that *miR-133a* act as a promising biomarker for the diagnosis of acute myocardial infarction [29]. The down-regulation of *miR-133a* could promote cell proliferation, migration and invasion in esophageal squamous cell carcinoma via targeting by EMT-related transcription factor Sox4 [30].

In the present study, we first detected the expression level of serum *miR-133a* of GC patients and healthy controls. And the result displayed that *miR-133a* was significantly down-regulated in GC patients compared with the healthy controls. This revealed the tumor suppressor role of *miR-133a* in GC which was in accordant with the previous study [23]. Then the relationship between the expression of *miR-133a* and clinical factors of GC patients was performed based on the above result. The resulted demonstrated that *miR-133a* participated in the development of GC.

As there were also some articles had showed the diagnostic or prognostic value of *miR-133a*

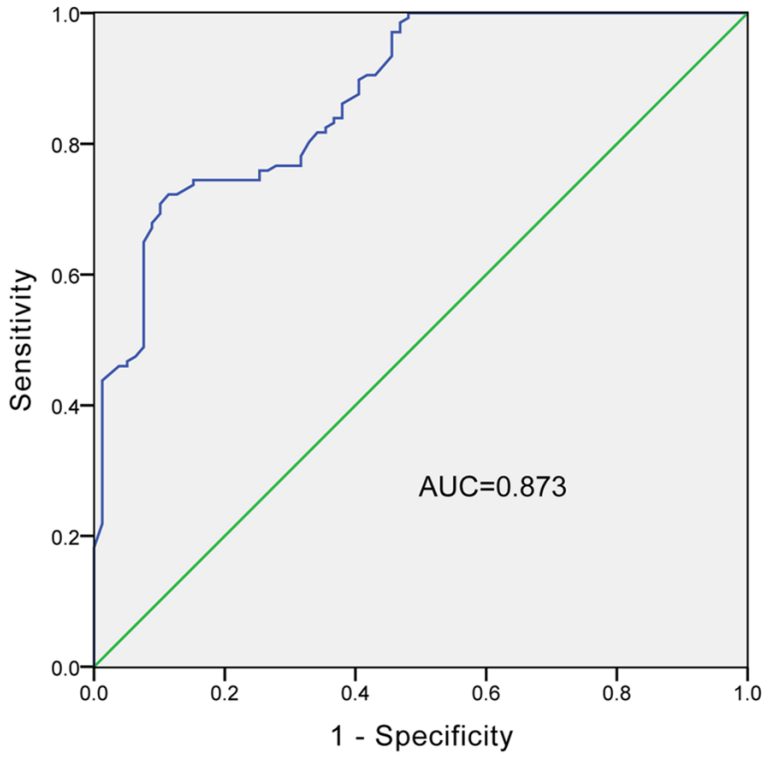

Figure 2. The diagnostic value of *miR-133a* for GC was estimated by ROC curve.

in several cancers such as colorectal cancer, breast cancer, osteosarcoma, and esophageal squamous cell carcinoma [14, 31-33]. Therefore, we investigated its diagnostic value in GC. A high AUC of 0.873 as well as high sensitivity and specificity proved that *miR-133a* cloud be an independent diagnostic marker in the early detection of GC.

Taken together, the expression level of *miR-133a* in GC patients is significantly lower compared to the healthy controls. And it is involved in the progression of GC. What's more, miR-133a can act as a diagnostic marker in patients with GC. However, there are several limitations in the present study. *miR-133a* is expressed in patients with different type of tumors and healthy individuals who lead to it isn't specific for GC. Moreover, the number of patients is small and more patients are needed to better understand the effects of *miR-133a* on GC. Last but not least, all the patients enrolled in this study are from the same hospital and the results may relate to the treatments adopted. Therefore, further studies indicate *miR-133a* was a promising biomarker for early diagnosis of GC patients is still need to be done in future.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qiong Niu, Department of Gastroenterology, Binzhou Medical University Hospital, Binzhou 25-6603, Shandong, China. E-mail: niuqiongsfg@126.com

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