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Original Article

Serum *NDRG2* acts as a novel biomarker for the diagnosis of patients with gastric cancer

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Abstract: Background: Gastric cancer (GC) is one of the most common digestive malignancies worldwide. N-myc downstream-regulated gene 2 (*NDRG2*) is a differentiation-related gene which is considered to be a metastasis suppressor gene. The purpose of this study was to detect the serum expression of *NDRG2* and its clinical significance in the early detection of patients with GC. Methods: Serum *NDRG2* expression were examined in 107 patients with GC, 52 with benign gastric disease patients, and 64 healthy volunteers using reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR) and western blot analysis at mRNA and protein level, respectively. The relationship between *NDRG2* expression and clinicopathologic characteristics was analyzed by chi-square test. The diagnostic value of *NDRG2* was estimated via establishing the receiver operating characteristic (ROC) curve. Results: the serum *NDRG2* expression was lower in GC patients than that in patients with benign disease and healthy volunteers both at mRNA and protein level ($P<0.05$). And the low *NDRG2* expression was significantly associated with tumor size, lymph node metastasis and TNM stage. ROC curve manifested that *NDRG2* had a high diagnostic value with an AUC of 0.896 corresponding with a sensitivity of 85.9% and a specificity of 62.6%. Conclusion: The expression of *NDRG2* was reduced in GC patients. Moreover, serum *NDRG2* could be a potential diagnostic marker for GC.

Keywords: *NDRG2*, diagnosis, gastric cancer

Introduction

Gastric cancer (GC) is one of the most common malignant tumors with a high incidence rate and it is the second most common cause of cancer-related deaths in Asia [1]. Most patients who have been clinically diagnosed are already at middle or advanced stage because there were no obvious symptoms in the early stage of GC which lead to a poor prognosis. The 5-year survival rate is disappointingly less than 10% globally [2]. Moreover, the clinically diagnosed patients may miss the best treatment opportunity [3]. Conventional treatments with surgery, chemotherapies or radiation therapy play minor roles in improving the patients' survival rates [4, 5]. Therefore, it is urgent to find out a specific and sensitive bio-marker for the early diagnosis of GC.

N-myc downstream-regulated gene 2 (*NDRG2*), located on human chromosome 14q11.2, is a member of the NDRG family, a new family of differentiation-related genes which are associated with cell proliferation, differentiation, apoptosis, stress responses, and cell migration/metastasis [6-9]. In previous studies, *NDRG2* is aberrant expressed and often act as a tumor suppressor in various types of cancers such as glioblastoma, colorectal cancer, gallbladder carcinoma, breast cancer, and prostatic carcinoma [10-14]. In addition, previous studies have also demonstrated that *NDRG2* is down-regulated in gastric cancer tissues [15, 16]. However, little is known about the association between *NDRG2* expression and diagnosis in GC.

In this study, we detected the serum expression of *NDRG2* in GC patients, gastric benign disease controls and healthy controls, and analyzed its relationship with clinical factors of patients. What's more, we estimated the diag-

nostic value of *NDRG2* via building a ROC curve in GC.

Materials and methods

Patients and samples

This study was approved by the Ethics Committee of the Chinese PLA General Hospital. All serum specimens and clinical materials were obtained and used after written informed consents from the patients and the Chinese PLA General Hospital was obtained. A total of 107 patients who were diagnosed with GC were enrolled in the study. None of them had received any radiotherapy or chemotherapy before sampling. Tumors were classified according to the TNM cancer staging system set by the Union of International Cancer Control. Besides, 52 patients with benign gastric diseases such as gastritis, gastrohelcosis, and gastric polyps, and 64 healthy volunteers were taken as benign controls and healthy controls, respectively. None of the control patients had formerly been diagnosed with any malignancy.

The blood specimens were severally obtained from 107 patients with GC and 116 controls and lasted for 30-60 min. Then the specimens were centrifuged at 2000 rpm for 15 min at room temperature. The supernatant was transferred to an EP tube and stored at -80°C for further use. The data of clinicopathological features of the GC patients including age, sex, tumor size, histological grade, lymph node metastasis, invasion depth, and TNM stage were recorded in a database.

RNA extraction and qRT-PCR analysis

Total RNA was isolated from all samples by using mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA), respectively. cDNA was generated by reverse transcription using PrimeScript RT (Takara, Dalian, China) according to the manufacturer's instructions. The generated cDNA was pooled and amplified by PCR. Then RT-PCR reaction was conducted at the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). *GAPDH* was taken as the internal controls. The primers sequences of *NDRG2* and *GAPDH* were as follows: *NDRG2*: forward-5'-ATCTCTGGACCAGCTTGCAG-3', reverse-5'-TATCTCGCCAGGATGTAGGC-3'; *GAPDH*: forward-5'-CTGGGCTAC ACTGAGCACC-3', reverse-5'-AAGTGGTCGTTGAGGGCAATG-3'. The relative mRNA expression of *NDRG2* was calculated using the $2^{-\Delta\Delta Ct}$ method. Each sample was in triplicate.

Western blot analysis

Total protein was extracted from the serum of patients with GC, benign controls and healthy controls, respectively. Then the protein was separated by SDS-PAGE and the brands were transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, China). Membranes were blocked in 5% non-fat milk and incubated with primary antibody at 4°C for overnight. After washing for 1 h, the membrane was incubated with HRP-conjugated secondary antibody (Sigma) for 1 h at room temperature. The resulting blot was visualized by ECL-Plus Western detection reagents (Amersham Biosciences).

Statistical analysis

All statistical analyses were carried out using Origin pro 9.0. Each experiment was repeated at least three times, and the data were presented as means ± SD. The differences between two groups were analyzed using Students' t test. The relationship between serum *NDRG2* expression and clinicopathological factors were assessed using x^2 tests or Fisher's exact tests. Receiver operating characteristic (ROC) curve was established to determine the diagnostic performance of serum *NDRG2* levels in distinguishing patients with GC from healthy controls. All P values <0.05 were considered to be statistically significant.

Results

Expression of NDRG2 was decreased in GC patients

QRT-PCR was used to evaluate the mRNA expression of serum *NDRG2* while western blot was taken to measure the protein expression of this gene in 107 GC patients, 52 benign gastric disease patients and 64 healthy volunteers. As shown in **Figure 1**, the serum *NDRG2* expression was significantly lower in patients with GC than that in benign gastric disease controls and healthy controls both at mRNA and protein level (P<0.05).

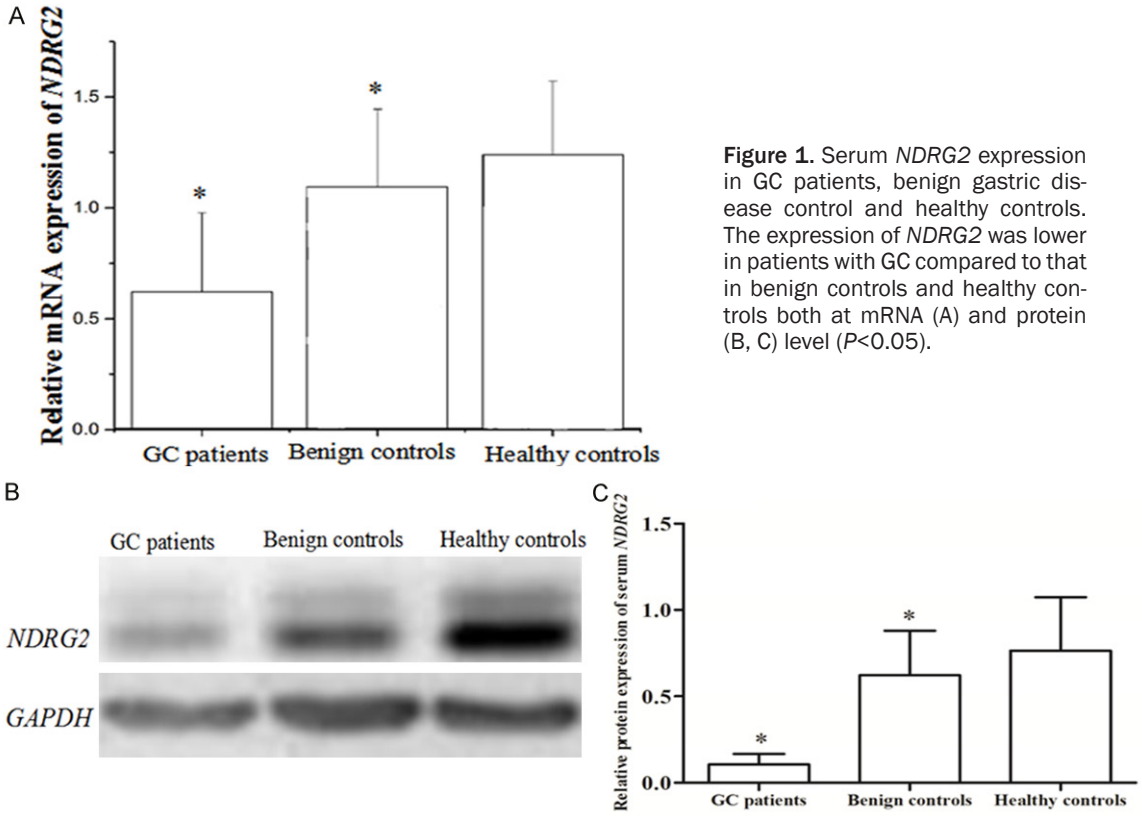

Figure 1. Serum *NDRG2* expression in GC patients, benign gastric disease control and healthy controls. The expression of *NDRG2* was lower in patients with GC compared to that in benign controls and healthy controls both at mRNA (A) and protein (B, C) level ($P<0.05$).

Relationship between NDRG2 expression and clinicopathological characteristics of patients with GC

To further investigate whether *NDRG2* was correlated with the development of GC, we further analyzed the association between it with the clinicopathological data. And all the data of clinicopathological characteristics of the GC patients were showed in **Table 1**. The result showed that the low *NDRG2* expression was closely associated with tumor size ($P=0.003$), lymph node metastasis ($P=0.025$) and TNM stage ($P=002$). However, there was no association between *NDRG2* expression and patients' age, gender, histological grade and invasion depth ($P>0.05$, **Table 1**).

Diagnostic value of NDRG2 in GC

To explore the diagnostic value of *NDRG2* in GC, ROC curve was built. The outcome showed that *NDRG2* had a high diagnostic value with an area under the curve (AUC) value of 0.896 combing with a sensitivity of 85.9% and a specificity of 62.6% (**Figure 2**). And the ideal cutoff value for *NDRG2* expression was 0.8350.

Discussion

GC is one of malignant tumors threatening human health. Despite the application of varieties of techniques of imaging and endoscopic examinations have taken effect on the diagnosis of this tumor which make the survival time of GC patients protracted, it is rather complex and expensive of the examination process, especially difficult to realize the early detection [17, 18]. Therefore, there is limitation in the early diagnosis, judgment of recurrence and evaluation of efficacy for tumors [19].

NDRG2, a newly discovered gene, contains an acyl carrier protein-like domain and can be harvested from human brain tissue by clone technology [20]. An increasing number of studies had revealed that the expression of *NDRG2* was abnormal in variety of tumors. For instance, lorentzen et al., found *NDRG2* was decreased in patients with colorectal carcinoma and related to the progression of the cancer [21]. *NDRG2* was considered to be reduced and involved in the development of liver cancer [22]. In the study of Li et al., *NDRG2* was confirmed to be down-regulated and could predict the progno-

Table 1. Association between serum *NDRG2* expression and clinicopathological features of patients with GC

Variables	Cases (n=107)	*NDRG2* expression		*P* values
		Low (n=64)	High (n=43)	
Age (years)				0.971
<60	50	30	20	
≥60	57	34	23	
Gender				0.273
Male	68	38	30	
Female	39	26	13	
Tumor size				0.003
<5 cm	46	20	26	
≥5 cm	61	44	17	
Histological grade				0.190
Well and moderate	49	26	23	
Poor	58	38	20	
Lymph node metastasis				0.025
Absent	41	19	22	
Present	66	45	21	
Invasion depth				0.087
T1 + T2	37	18	19	
T3 + T4	70	46	24	
TNM stage				0.002
I-II	43	18	25	
II-IV	64	46	18	

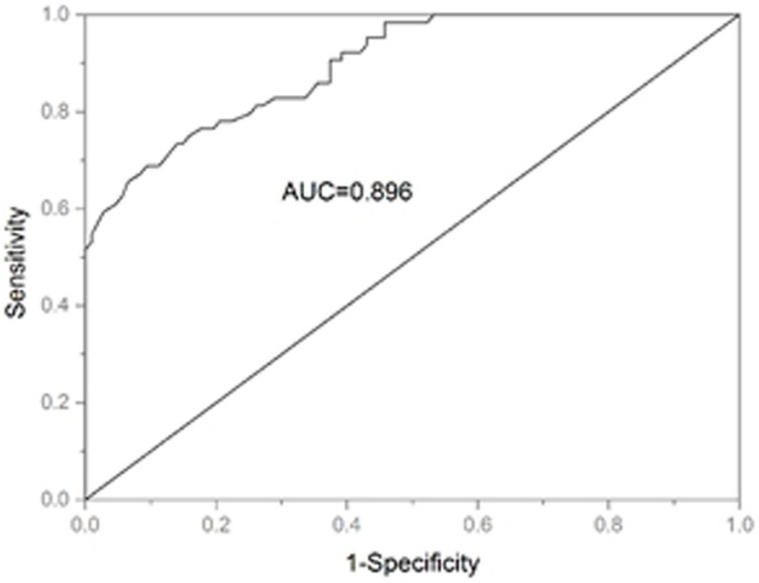

Figure 2. ROC curve for evaluation of the accuracy of serum *NDRG2* to discriminate patients with GC from healthy controls.

sis of patients with astrocytomas [23]. Hu et al. reported that *NDRG2* expression was significantly decreased in human liver and pancreatic cancers, without relationship to mutations in its coding region [24]. In our study, we found that the expression level of *NDRG2* was lower in GC patients than that in gastric benign disease controls and healthy controls. These results are in agreement with previous studies [25]. Our findings revealed *NDRG2* was a tumor suppressor.

NDRG2 was reported to be involved in many physiological and pathophysiological processes of diseases including differentiation, stress injury and organ formation via different ways. It was found that *NDRG2* could suppress cell proliferation possibly by regulating the expression of cyclin D1 and T-cell factor (TCF)/β-catenin activity [26, 27]. Kim et al. showed that *NDRG2* could suppress tumor invasion by inhibiting MMP activities which were regulated by nuclear factor kappa B (NF-kB) signaling [28]. In breast cancer, *NDRG2* could induce BMP-4 and suppress MMP-9 activity, thereby inhibits the metastatic potential of breast cancer cells [29]. Dake et al. reported that decreased expression of *NDRG2* was significantly correlated with differentiation status, lymph node metastasis, and tumor node metastasis stage in patients with colorectal cancer [30]. Ma et al. found that low *NDRG2* expression was significantly correlated with advanced TNM stage in breast carcinoma [31]. Li et al. reported that the expression level of *NDRG2* was decreased in human lung cancer tissues, and positively correlated with tumor grade and tumor size [32]. Therefore, we esti-

mated its relationship with clinical factors to see whether *NDRG2* was involved in the development of GC. The result showed that the expression of *NDRG2* was closely correlated with the clinical factors which indicated it participated in the progression of GC.

The prognostic value of *NDRG2* had been verified in some cancers such as esophageal squamous cell carcinoma, glioblastoma multiforme, prostate cancer and pancreatic cancer [33-36]. However, its diagnostic value was rarely reported. In the present study, we explored its diagnostic value in GC. The outcome exhibited that *NDRG2* could act as an independent diagnostic marker in GC for its high sensitivity and specificity. This was the first study showing the diagnostic value of *NDRG2* expression in GC.

In conclusion, these findings provides the convincing evidence for the first time that the down-regulation of *NDRG2* may serve as a novel molecular marker for the diagnosis of GC, and it is involved in the development and progression of this cancer. However, there are still some limitations. Firstly, the sample size is small. To solve this problem, further studies and more samples will be required to be done. Secondly, the current study has not elucidated the exact molecular mechanisms of *NDRG2* acting on GC, which is also worth to be further investigated.

Disclosure of conflict of interest

None.

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