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Original Article

Serum *WAVE3* is a diagnostic marker for patients with gastric cancer

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Abstract: Objectives: The present study was conducted to investigate the diagnostic significance of Wiskott-Aldrich syndrome verprolin-homologous 3 (*WAVE3*) in patients with gastric cancer (GC). Methods: The serum level of *WAVE3* mRNA was examined by real-time quantity PCR (qRT-PCR). Chi-square test was applied to evaluate the relationship between *WAVE3* expression and clinical characteristics. The diagnostic value of *WAVE3* in GC was assessed via conducting the receiver operating characteristic (ROC) curve. Results: Serum level of *WAVE3* mRNA was significantly higher in GC patients than that in healthy controls ($P<0.001$). Furthermore, high *WAVE3* expression was associated with positive lymph node metastasis ($P=0.036$), deep tumor invasion ($P=0.016$) and high TNM stage ($P=0.041$). The ROC curve revealed that *WAVE3* was a candidate biomarker for the diagnosis of GC, with the sensitivity of 81.5% and the specificity of 92.4%. The optimal cutoff point was 2.34, and the AUC was 0.861. Conclusion: *WAVE3* is up-regulated in GC serum, which is significantly associated with aggressive clinical characteristics. *WAVE3* may be a potential biomarker for GC diagnosis.

Keywords: *WAVE3*, diagnosis, ROC, gastric cancer

Introduction

Gastric cancer (GC) is one of the most frequently observed malignant digestive diseases, which is still a leading reason for cancer-related death in the world [1]. The morbidity of GC is high, especially in developing countries, including China [2, 3]. At the present time, patients with GC are frequently diagnosed at advanced stage, leading to poor outcomes [4, 5]. Thus, early detection is crucial for the prognosis of GC patients. The occurrence and development of GC are a multistep and multifactorial process, which is with the involvement of a variety of environmental and inherited factors [6-8]. Currently, the biomarkers based on genetic factors not only reveal the mechanisms of carcinogenesis in GC, but also exploit novel approaches for diagnosis, therapy and prognosis for the cancer patients. Therefore, in the present study we investigated the clinical significance of Wiskott-Aldrich syndrome verprolin-homologous 3 (*WAVE3*) in GC, which maybe a potential indicator for early detection.

It is known to all that the Wiskott-Aldrich syndrome protein (WASP) family and WASP Verpolin homologous (WAVE) family are structurally related, which are significantly correlated with the motility and invasion of cells [9, 10]. All the WASP/WAVE family members are reported to involve in multiple cellular progresses via their interactions with actin related proteins 2&3, such as regulating cytoskeleton remodeling, actin polymerization and cell shape/morphology [11-13]. As a member of the WAVE family, *WAVE3*, also known as *WASF3*, is located on human chromosome 13, containing two homologous regions: the VCA region and the PRD region [14]. Growing evidences have demonstrated that *WAVE3* is essential for the metastasis and invasion of cells through leading to the formation of lamellipodia extension of invasive cells [15, 16]. The aberrant expression of *WAVE3* has been proved to contribute to various tumor progressions, such as breast cancer, colorectal cancer and so on [12, 17]. A study carried out by Yue et al. demonstrated that *WAVE3* could promote GC cell migration and

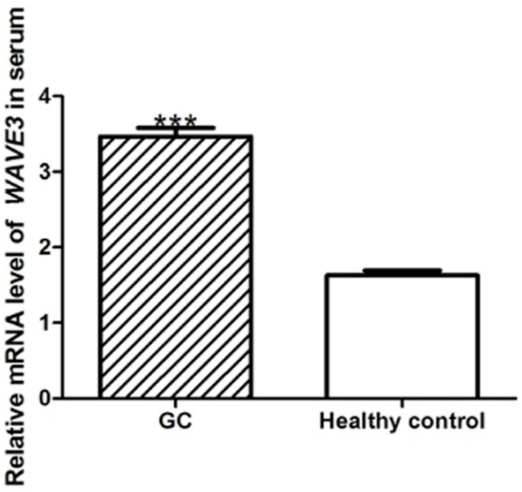

Figure 1. Expression of *WAVE3* mRNA in GC serum and normal controls. *WAVE3* mRNA was highly expressed in GC serum. ***P<0.001.

invasion, which may be a therapeutic target [18]. However, the diagnostic value of *WAVE3* in GC has been rarely reported.

The purpose of the study was to explore the diagnostic value of *WAVE3* in GC. Serum *WAVE3* levels in patients pathologically diagnosed with GC and healthy individuals were compared. In addition, we evaluated the association between *WAVE3* level and clinical characteristics in GC patients, as well as its diagnostic significance. The present study may explore a novel diagnostic marker for GC, which may improve the management of GC.

Materials and methods

Patients and specimens

The present study was carried out in Second Affiliated Hospital. 119 patients pathologically diagnosed with GC were collected in the study, including 67 males and 52 females, with the average age of 52.35±12.65 years. None of them had received chemotherapy or radiotherapy before blood collection. Besides, 105 gender-age matched healthy individuals from the physical examination center of the hospital were recruited as healthy controls. 5 mL fasting blood samples were collected from all the participants and then centrifuged at 2500× rpm for 20 min to collect serum specimens. The serum specimens were stored at -80°C until use. The current study was permitted by the ethic committee of the hospital. All the participants or their family singed the informed consents before specimens' collection.

RNA extraction and quantitative real-time quantity PCR (qRT-PCR)

Total RNA was isolated from all serum samples using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. Then RNA with high quality was adopted to synthesize the first strand of cDNA using the TaqMan microRNA assay protocol (Applied Biosystems, Foster City, CA, USA). QRT-PCR was applied to evaluate the relative expression of *WAVE3* in serum specimens. QRT-PCR reaction was performed with a SYBR green I Master Mix kit (Invitrogen) in Applied Biosystems 7900 Fast Real-time PCR system. *GAPDH* was used as an internal control. Each sample was determined in triple and the expression of *WAVE3* mRNA was measured by the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers used in the study were as followed: *WAVE3* forward: 5'-TTCTAGCTCACTGCTTTCAGG-3', reverse: 5'-TGGCCTTCTCCATTCATTTT-3'; *GAPDH* forward: 5'-TGCACCACCAACTGCTTAGC-3', reverse: 5'-GGCATGCACTGTGGTCATGAG-3'.

In addition, the protein level of *WAVE3* was detected by immunohistochemistry.

Statistical analysis

All statistical analyses were carried out in SPSS 18.0 software. Graphs were plotted by GraphPad Prism 5. The continuous data were presented as mean ± SD. The expression differences of *WAVE3* between GC serum and healthy controls were estimated via students' t-test. The relationship between *WAVE3* expression and clinical characteristics was estimated by Chi-square test. The receiver operating characteristic (ROC) curve was used to assess the diagnostic value of *WAVE3* for GC. Statistical significance existed when P was less than 0.05.

Results

Expression of WAVE3 mRNA was increased in GC serum

QRT-PCR was applied to evaluate the relative mRNA level of *WAVE3* in serum specimens collected from GC patients and healthy controls.

Table 1. Relationship between *WAVE3* expression and clinical features of patients

Clinical characteristics	Case	*WAVE3* expression		X^2	*P* value
	NO.	High (n=83)	Low (n=36)		
Age				0.182	0.670
≤55	81	55	26		
>55	38	28	10		
Gender				1.241	0.265
Male	67	50	17		
Female	52	33	19		
Differentiation				0.608	0.435
Well-moderate	58	38	20		
Poor	61	45	16		
Lymph node metastasis				4.409	0.036
Present	62	49	13		
Absent	57	34	23		
Depth of tumor invasion				5.827	0.016
T1-T2	61	36	25		
T3-T4	58	47	11		
TNM stage				4.183	0.041
I-, II	51	30	21		
III-, IV	68	53	15		

Results suggested that *WAVE3* was significantly up-regulated in GC patients, compared with healthy control ($P<0.001$) (**Figure 1**). Immunohistochemistry analysis indicated that the protein level of *WAVE3* was increased in GC patients, which was consistent with the mRNA level.

Association between WAVE3 expression and clinical features of patients

Chi-square test was performed to assess the relationship between *WAVE3* expression and clinical features. Results listed in **Table 1** indicated that high *WAVE3* expression was significantly associated with positive lymph node metastasis ($P=0.036$), deep tumor invasion ($P=0.016$) and high TNM stage ($P=0.041$). No marked correlation was found between *WAVE3* expression and age ($P=0.670$), gender ($P=0.265$) or differentiation ($P=0.435$).

Diagnostic value of serum WAVE3 in GC

The ROC curve was established to estimate the diagnostic value of serum *WAVE3* in GC. Results shown in **Figure 2** suggested that *WAVE3* could discriminate GC patients from healthy individuals with the optimal cutoff point of 2.34. The AUC value was 2.34, with the sensitivity of 81.5% and the specificity of 92.4%.

Discussion

GC is a malignant tumor originating from the gastric mucosa epithelium, which is an aggressive cancer of the digestive tract. Most of the patients are at advanced stage when firstly diagnosed, because there are no typical characteristics at the early stage of tumor progression [19]. Recently, molecular biomarkers for early detection and progression of cancers have become a research hotspot. And numerous biomarkers have been reported to act as diagnostic or prognostic markers for GC patients. Su et al. showed that *miR-18a* was a candidate biomarker for GC and its overexpression predicted poor prognosis of GC patients [20]. In the study of Yang et al., overexpression of *eEF1A2* was found to be associated with unfavorable outcomes in GC patients [21]. The study scheduled by Chen et al. demonstrated that long non-coding RNA *H19* was significantly associated with GC initiation and development, which can serve as a biomarker for the cancer diagnosis and prognosis [22]. The genetic biomarkers combined with clinical characteristics may accurately confirm the patients with risk of GC, which can significantly improve the outcomes of the patients [23]. Therefore, more novel biomarkers for diagnose of GC are urgently needed.

As we all know, the progression of tumors are the consequences of a number of cellular events, such as deregulated proliferation, enforced migration, resistance to apoptosis and disordered microenvironment [24, 25]. *WAVE3* has been proved to regulate the motility and invasion of cell lines, which is correlated with tumor occurrence [14, 18]. In the present study, we detected the diagnostic significance of *WAVE3* in GC patients. Analysis results indicated that *WAVE3* may be a potential indicator for GC diagnosis.

In the current study, we detected the serum level of *WAVE3 in GC* patients and healthy indi-

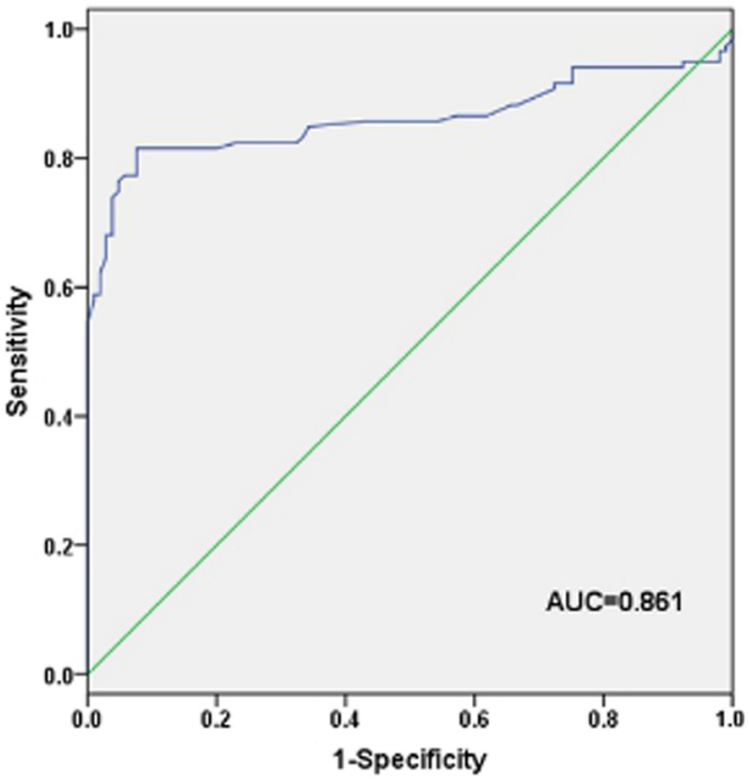

Figure 2. ROC curve was conducted to assess the diagnostic value of *WAVE3* in GC. Analysis results indicated that *WAVE3* was a potential biomarker for GC diagnose, with the sensitivity of 81.5% and the specificity of 92.4%. The cut-off value was 2.34 and the AUC was 0.861.

viduals. The results demonstrated that the expression of *WAVE3* was up-regulated in GC serum, compared to healthy controls. Moreover, the serum level of *WAVE3* was markedly associated with lymph node metastasis, depth of tumor invasion and TNM stage. The conclusion was supported by the previous studies. Yue et al. had reported that the expression level of *WAVE3* was increased in GC cell lines, which could promote proliferation, migration and invasion of the cells in vitro [18]. Similar results were also observed in other tumors. Molly et al. said that the expression of *WAVE3* was higher in metastatic cancer cells than that in the non-metastasis cells, which was correlated with aggressive subtypes of triple-negative breast cancer [26]. In the study of Herman et al., *WAVE3* was positively expressed in prostate cancer cells compared with prostate epithelial cells. Moreover, *WAVE3* regulate the invasiveness of prostate cancer cells [27]. All of the related studies indicated that *WAVE3*, as an oncogene, regulated various tumor aggressiveness.

In addition, ROC curve was conducted to evaluate the diagnostic value of *WAVE3* in GC. Results indicated that *WAVE3* could distinguish the GC patients from the healthy individuals with high sensitivity and specificity. However, the mechanism of *WAVE3* involving in GC initiation has not been identified in the present study. Gangarao et al. suggested that the interaction of *WAVE3* and NFκB played important roles on the invasion and survival of cancer cells [28]. Heather et al. revealed that *WAVE3* was involved in the plasticity and motility of lamellipodial dynamics [29]. These experimental results could provide research directions for our further studies in investigating the mechanism of *WAVE3* in GC.

In a word, serum level of *WAVE3* mRNA is significantly higher in GC patients than that in the healthy controls. Moreover, up-regulated *WAVE3* is markedly associated with positive lymph node metastasis, deep tumor invasion and high TNM stage. *WAVE3* may be a potential biomarker for the diagnosis of GC.

Disclosure of conflict of interest

None.

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