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Serum MALAT1 Assumes Signifying Capacity in Gastric Cancer Diagnosis.

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Abstract

Background: Gastric cancer (GC) represents one of the most serious cancers worldwide with the increasing mortality. Metastasis associated lung adenocarcinoma transcript 1 (MALAT1), a kind of lncRNAs, has been reported to be involved in the progression of cancers. This study aimed to assess serum expression pattern of MALAT1 and its clinical significance in diagnosis of GC.

Methods: Serum specimens were collected from 120 GC patients and 58 healthy individuals. The expression profile of MALAT1 was examined using quantitative real-time polymerase chain reaction (qRT-PCR), and its association with clinical parameters was estimated by chi-square test. The diagnostic value of MALAT1 in GC was evaluated by the receiver operating characteristic (ROC) analysis.

Results: Upregulated expression of MALTA1 was found in GC patients compared with the healthy controls ($P<0.05$). The overexpression of MALAT1 was positively correlated with lymph node metastasis ($P=0.041$) and TNM stage ($P=0.005$). An area under the curve (AUC) was 0.897 in ROC analysis, suggesting the high diagnostic value of MALAT1.

Conclusion: The expression of MALAT1 was upregulated in GC serum samples, and its expression might serve as a potential diagnostic biomarker in patients with GC.

Background

Gastric cancer (GC) is one of the most frequently diagnosed malignant digestive tract tumors, representing a leading cause of malignancy related deaths worldwide [1, 2]. Due to the advances in surgery operation, chemotherapy and radiotherapy, the mortality of GC has been reduced. However, there are abundant patients who are initially diagnosed with GC at the advanced stage, with the 5-year survival rate only 20%-30% [3, 4]. Early diagnosis is of great importance for treatment and outcomes in GC patients. However, until now, there are few effective tools for early detection of GC. Evidences reveal that the clinical symptoms are absent at the early time of GC, which contributes to the difficulty in early diagnosis [5]. Given the natural characteristics of GC, more and more related studies focus on the diagnostic value of molecular biomarkers to improve the early screening [6]. Currently, the commonly used serum biomarkers for early detection of GC included carcinoembryonic antigen (CEA) and carbohydrate antigen 72 – 4 (CA72-4), but their sensitivity and specificity are not satisfactory [7]. Thus, novel and efficient diagnostic biomarkers are in urgent needs for GC patients.

Noncoding RNAs (NcRNAs), a class of RNA molecules without the capacity of protein coding, have been classified into two major types: microRNAs (miRNAs), and long noncoding RNAs (lncRNAs) according to size [8]. As an important member of ncRNAs, IncRNAs play crucial roles in various evens in the tumorigenesis of human cancers, revealing their application values for tumor biomarkers [9, 10]. LncRNA metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*), also named as nuclear-enriched abundant transcript 2 (*NEAT2*), is an extensively studied lncRNA member. The abnormal expression of *MALAT1* has been observed in various human cancers, such as cervical cancer and glioma [11, 12]. In

GC[13], it was reported that the over-expression of *MALAT1* in GC tissue specimens might serve as an indicator for distant metastasis in patients with GC [14]. Given the data in the previous study, we wondered if *MALAT1* could serve as a serum biomarker for early diagnosis of GC.

In the present study, we investigated the expression patterns of *MALAT1* mRNA in GC serum samples. Moreover, its diagnostic value was explored by using the receiver operating characteristic (ROC) analysis.

Methods

Patients and serum collection

The experiment procedures were approved by the Ethics Committee of Southwest Hospital, Army Medical University. All the participators signed the informed consents prior to the sampling. This study included 120 GC patients, who were pathologically diagnosed with GC in Southwest Hospital, Army Medical University. Besides, 58 age and gender matched healthy volunteers were recruited to the control group, and none of them had malignancy history. Before the sampling, none of the patients had ever received any therapies (surgery, chemotherapy or radiotherapy). The venous blood samples were collected from the GC patients and healthy controls and stored in the tubes with EDTA. Serum specimens were isolated from blood through centrifugation, and then stored in the -80°C freezer for the next experiments. The clinicopathological characteristics of GC patients were recorded in **Table 1**, including gender, age, tumor size, tumor invasion, differentiation, lymph node metastasis and TNM stage.

RNA extraction

Total RNAs were obtained from the serum specimens by using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the instruction of manufacturers. The RNA was purified using the RNeasy micro kit and RNase-Free DNase Set (QIAGEN, GmBH, Germany). To evaluate the purity and concentration of RNA, the ratio of OD A260/A280 was calculated. Only the RNA sample with the ratio value of 1.9-2.0 was considered to be used in the further analyses.

Quantitative Real-Time polymerase chain reaction (qRT-PCR)

To estimate the expression patterns of *MALAT1* in serum samples collected from GC patients and healthy controls, we conducted qRT-PCR analysis. Reverse transcription was carried out by using the PrimeScript reverse transcriptase (RT) reagent kit (TaKaRa, Shiga, Japan). The obtained single stranded cDNA was used for qRT-PCR, which was conducted with SYBR Green PCR master mix (Applied Biosystems, USA). β-actin served as the internal control. All of the reactions were performed on the 7300 Real-Time PCR System (Applied Biosystems, USA). Sequences of primers used in the reactions were as follows: *MALAT1* forward: 5’-CTTCCCTAGGGGATTTCAGG-3’, reverse: 5’-GCCCACAGGAACAAGTCCTA-3’; β-actin forward: 5’-TTGTTACAGGAAGTCCCTTGCC-3’, reverse: 5’-ATGCTATCACCTCCCCTGTGTG-3’. $2^{-\Delta\Delta Ct}$ method was performed to calculate the relative expression value of *MALAT1*. Each experiment was repeated in three times.

Statistical analysis

All the statistical analyses were performed in SPSS Version 18.0 statistical software. The data in this study were summarized as mean ± SD, and analyzed using. Student's t-test. Relationship between *MALAT1* expression and clinicopathological features was examined using Chi-square test. To evaluate the diagnostic value of *MALAT1*, receiver operating characteristic (ROC) analysis was applied, and the results were estimated by the area under the curve (AUC), sensitivity and specificity. The difference was considered as statistically significant with the P value less than 0.05.

Table 1
Relationship between *MALAT1* and clinicopathological data of GC patients

Features	No.	*MALAT1* expression		*P* values
	n = 120	Low (n = 54)	High (n = 66)	
Gender				0.549
Female	41	20	21	
Male	79	34	45	
Age (years)				0.969
≤ 60	42	19	23	
> 60	78	35	43	
Tumor size (cm)				0.881
≤ 5	48	22	26	
> 5	72	32	40	
Tumor invasion (T)				0.271
T1 + T2	60	30	30	
T3 + T4	60	24	36	
Differentiation				0.218
Well/Moderate	57	29	28	
Poor	63	25	38	
Lymph node metastasis				**0.041**
Negative	50	28	22	
Positive	70	26	44	
TNM stage				**0.005**
I-II	50	30	20	
III-IV	70	24	46	

Results

Upregulated expression of *MALAT1* in the serum samples of GC patients

The serum levels of *MALAT1* in 120 GC patients and 58 healthy individuals were assessed using qRT-PCR. The results showed that *MALAT1* expression was significantly higher in GC patients than that in the

healthy controls (*P*<0.05, **Figure 1**).

Association of *MALAT1* expression with clinicopathological features of GC

In this study, the relationship between *MALAT1* expression and clinicopathological data of GC was examined using Chi-square test. The GC patients were divided into low expression (n=54) and high expression (n=66) based on their median *MALAT1* expression value. The results of Chi-square test revealed that the over-expression of *MALAT1* was remarkably correlated with positive lymph node metastasis (*P*=0.041) and advanced TNM stage (*P*=0.005), while the significant correlation between *MALAT1* expression and other parameters, such as gender, age, tumor size, tumor invasion or differentiation, was not found (all *P*>0.05) (**Table 1**).

Diagnostic value of *MALAT1* in patients with GC

In addition, we assessed the diagnostic significance of serum *MALAT1* in GC according to ROC analysis. From the ROC curve, we found that *MALAT1* had high diagnostic value to distinguish the GC patients from healthy individuals with an AUC value of 0.897. The cutoff value of serum *MALAT1* level for GC diagnosis was 0.370, and the corresponding sensitivity was 89.2% and the specificity was 77.6% (**Figure 2**).

Discussion

As one of the prevalent malignancy, the mortality of GC is relatively high, posing a great threat to human health worldwide [15]. Early diagnosis following timely treatments can significantly improve the clinical outcomes of GC patients. Unfortunately, early diagnosis remains a great challenge for GC patients, due to the lack of specific symptoms at early stages, and the limited application value of the existing diagnostic tools [16]. Growing evidences have demonstrated that genetic and epigenetic alterations can influence the development of GC, implying their application value as diagnostic biomarkers and therapeutic targets in management of the disease [17]. A variety of molecules have been proved to play crucial roles in GC. For example, Liu et al. demonstrated that serum microRNA-940 was downregulated in GC which could serve as a novel diagnostic biomarker in patients with GC [18]. LncRNA BRAF-activated non-coding RNA (*BANCR*), as another example, was found to be upregulated in GC tissues and cells, moreover, its over-expression could obviously promote GC cell growth and inhibit cell apoptosis [13]. Considering all the data in these previous studies, the identification of novel and efficient cancer related biomarkers is of great importance for early diagnosis and treatment of GC.

Given the functional roles of lncRNA in maintaining normal physiological processes, dysregulation of lncRNA may contribute to oncogenesis. Abnormal expression of lncRNA is an important type of epigenetic modulation in etiology of human cancer. The altered lncRNAs can serve as oncogenes or tumor suppressor genes in initiation and development of malignancies, including GC [19, 20]. As one of the extensively studies lncRNA, *MALAT1* has ever been assessed in diverse cancers, such as pancreatic cancer, nasopharyngeal carcinoma, esophageal squamous cell carcinoma, clear cell renal cell cancer and

ovarian cancer [21–25]. The function of *MALAT1* in GC were also investigated in the previous studies. It was proved that *MALAT1* was associated with the proliferation, metastasis and invasion of GC [14, 26–28]. However, the clinical significance of *MALAT1* in diagnosis of GC has been rarely studies.

In the current study, the serum samples were collected from 120 GC patients and 58 healthy volunteers. QRT-PCR was carried out to estimate the relative expression levels of *MALAT1*. The results showed that the expression of *MALAT1* was significantly upregulated in GC patients compared with the healthy controls. Furthermore, the over-expression of *MALAT1* was correlated with positive lymph node metastasis and advanced TNM stage. Thus, we considered that the *MALAT1*, as an oncogene, was involved in the progression of GC. Qi et al. reported that *MALAT1* could suppress the expression of tumor suppressor *PCDH10*, thus contributing to cell migration and invasion in GC [27]. Deng et al. reported that over-expression of *MALAT1* might enhance the migration and invasion of GC cell line via upregulating the expression of epidermal growth factor-like domain-containing protein 7 (*EGFL7*) [28]. However, the oncogenic mechanisms for *MALAT1* in GC was not investigated in the present study. Further investigations will be required.

In addition, we also focused on the diagnostic value of *MALAT1* in GC patients through ROC analysis. From the ROC curve, we found that the *MALAT1* had high diagnostic accuracy in distinguishing the GC patients from the healthy individuals. However, the results might be limited by the relative small sample size in our article. The application value of serum *MALAT1* in early screening of GC needed further verification with large sample size.

Conclusion

In conclusion, serum *MALAT1* expression is upregulated in GC patients, and its elevated expression shows positive association with metastasis and advanced tumor stages. Serum *MALAT1* may be a candidate diagnostic biomarker for GC patients.

List Of Abbreviations

Gastric cancer (GC)

Metastasis associated lung adenocarcinoma transcript 1 (MALAT1)

quantitative real-time polymerase chain reaction (qRT-PCR)

receiver operating characteristic (ROC)

carcinoembryonic antigen (CEA)

Noncoding RNAs (NcRNAs)

long noncoding RNAs (lncRNAs)

Declarations

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Disclosure

The authors report no conflicts of interest in this work.

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects provided written informed consent for the publication of any associated data and accompanying images.

Data availability

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

K.Z., T.M. design of the work; Z.H., X.W. the acquisition, analysis, Y.P., Y.C. interpretation of data; Y.D., Z.R. the creation of new software used in the work; Z.W. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

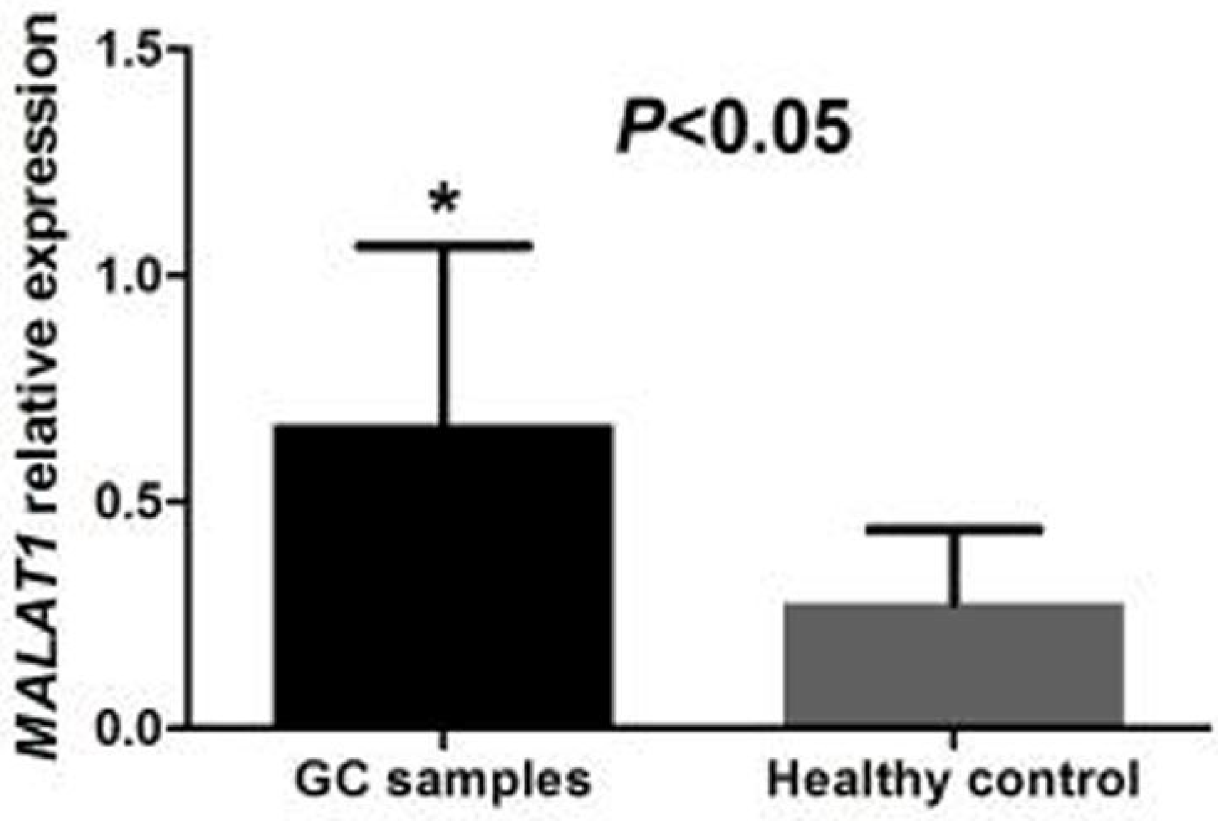

Figure 1

Serum levels of MALAT1 in 120 GC patients and 58 healthy controls. QRT-PCR analysis demonstrated that the expression of MALAT1 was elevated in GC patients compared with the healthy controls (P<0.05).

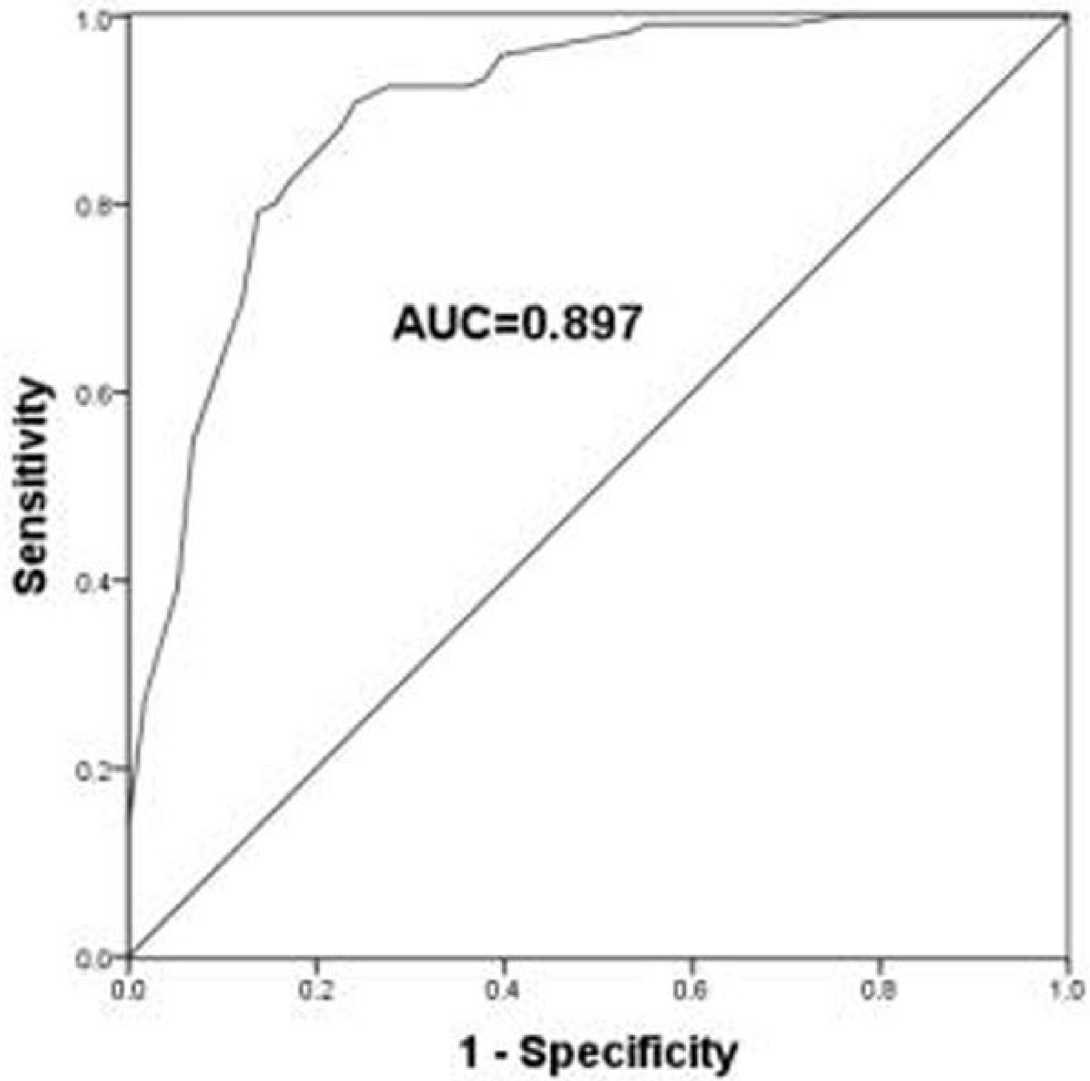

Figure 2

ROC curve based on the serum levels of MALAT1. The AUC value was 0.897, suggesting the high diagnostic value of MALAT1 in GC patients.