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BRIEF ARTICLE

Association between gastric cancer and -1993 polymorphism of *TBX21* gene

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Abstract

AIM: To investigate the association between the polymorphism of *TBX21* gene and the risk of gastric cancer in a Chinese population.

METHODS: The -1993 polymorphism located in *TBX21* gene promoter region was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The risk between *TBX21* gene genotype and gastric cancer was determined by multivariate logistic regression analysis in 220 gastric cancer patients and 262 cancer-free controls matched by age, sex and ethnicity.

RESULTS: Compared with the *TBX21* -1993TT genotype, the -1993CC genotype exhibited a significantly elevated risk for gastric cancer [Odds ratio (OR) = 3.42, 95% confidence interval (CI): 1.41-8.31]. The relationship between the -1993 polymorphic genotype and the invasive status such as lymph node and distant metastasis was found among the gastric cancer patients (OR = 4.02, 95% CI: 1.87-8.66; OR = 7.02, 95% CI: 3.44-14.34, respectively).

CONCLUSION: *TBX21* -1993 polymorphism might contribute to the risk of gastric cancer, especially to the distant metastasis.

Key words: *TBX21* gene; Gastric cancer; Polymorphism; Genetic susceptibility; Association analysis

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INTRODUCTION

Gastric cancer is the fourth most common cancer, which approximately accounts for 12% of all cancer deaths and currently ranks second in global cancer mortality only behind the lung cancer[1]. Until recently, although the global incidence of gastric cancer has been dramatically

decreased in different regions, it still represents a leading cause of death worldwide, especially in China and other Asian countries[2]. It is estimated that more than 930 000 new cases of gastric cancer would be diagnosed each year in the world. Although the different management approaches were performed, a minimum of 700 000 patients would die from this malignant disease[3].

In the previous study, the dietary factors, such as nitrates, smoked fish and salted meats, moldy foods containing aflatoxin, and the infection with *Helicobacter pylori* (*H. pylori*), were thought to be involved in the development of gastric cancer[4], but the detailed mechanisms of gastric carcinogenesis are still unknown. In spite the new insight of the immune surveillance, the molecular elimination mechanism and host susceptibility to malignant gastric cell still remained largely unsolved. The susceptibility of the host genetic factor to malignant disease is a dynamic interactive process, which is thought to be involved in the balance between the host immune response and the malignant cell apoptosis. Moreover, multitudinous cytokines played crucial roles against the malignant transformation of normal cells in this process[5].

Although the genetic basis of host susceptibility to the development of gastric cancer had not been clearly discovered, our previous study reported that the -1661A/G polymorphism located at *cytotoxic T lymphocyte-associated antigen-4* (*CTLA-4*) gene promoter might significantly increase the risk of gastric cancer development[6]. Similarly, in the immune-regulated area, researches demonstrated that the *TBX21* gene (NCBI OMIM nos.*604895), which encoded the transcription factor T-bet (T-box expressed in T cells), was a key transcriptional activator to induce the differentiation from helper T cells to Th1 cells[7]. In the previous experiments, the expression of *TBX21* gene was proved to be associated with breast cancer and the T-bet protein played a key role in natural killer (NK) cell-mediated melanoma metastatic disease[2,8]. These evidences indicated that the expression of *TBX21* gene was closely associated with the development of cancer through the Th1 and NK cells pathway[9]. Moreover, the -1993 polymorphism located in *TBX21* gene promoter region had been verified to alter the transcription factor binding and regulating the *TBX21* gene expression[10]. Hence, in this study, we systematically scrutinized the -1993 genetic polymorphism and assessed the potent susceptibility of this *TBX21* gene (SNP) to gastric cancer in the Chinese population.

MATERIALS AND METHODS

Patients

This population-based case-control study was carried out in two hospitals: Beijing Friendship Hospital, Capital Medical University and Qianfoshan Hospital, Shandong University, China. Followed an identical protocol, each hospital was responsible to recruit the gastric cancer patients consecutively from 2005 through 2010. All patients were histopathologically confirmed by endoscopic biopsy or surgical specimen. At the same time, a comparable group of normal controls without gastric cancer or other disease was included in this study from the two hospitals. Two hundred and twenty eligible patients with gastric cancer (124 males and 96 females) were enrolled in this study. The individuals who possessed secondary, recurrent malignancies, and accepted blood transfusion were excluded. There were eight cases of gastric cancers located in cardia, 117 in non-cardia, six in the upper third, 49 in the middle third, and 40 in the lower third. Two hundred and sixty two controls (143 males and 119 females) proved to be free from any malignant diseases by the physical examination were randomly and simultaneously recruited from the two hospitals. Except for matching the gastric cancer patients by gender and age (within 5 years), control subjects with severe clinical symptoms, previous diagnosis of cancer, and genetic disease were excluded. The mean age of the gastric cancer patients was 41.14 ± 7.10 years (mean ± SD) and the control group was 41.69 ± 8.83 years. There were no statistically significant differences in current smoking or drinking status, age, sex, and race between the two groups. All participants were of Han nationality from Northern China. The minor distinct ethnic groups and migrants from other countries were not included. This study protocol was approved by the Institutional Review Boards of participating institutions, and each participant should provide a signed informed consent before the two mL peripheral blood sample was collected.

DNA extraction and genotyping method

According to the standard protocol as described in a previous study[11], genomic DNA was extracted from peripheral blood leukocytes using sodium dodecyl sulphate lysis and proteinase K digestion followed by the standard phenol-chloroform extraction. DNA samples were quantified and subjected to specific polymerase chain reaction (PCR) amplification as described below. The -1993 polymorphism within *TBX-21* gene promoter region was identified by the reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers which were used in PCR amplification for the promoter specific fragments were described previously[10]. The PCR was performed in a 20 μL total reaction volume containing 2 μL 10 × PCR buffer (Qiagen Inc., Hilden, Germany), 1.5 mmol/L $MgCl_2$, 0.5 μmol/L each primer, 0.2 mmol/L dNTP, 1.2 U Taq polymerase (Qiagen Inc., Hilden, Germany) and 200 ng of genomic DNA. After an initial denaturation at 95 ℃ for 5 min, the DNA was amplified by 35 cycles of 94 ℃ for 30 s, 62.0 ℃ for 40 s, and 72 ℃ for 45 s, with a final elongation at 72 ℃ for 10 min on the Gene-Amp PCR System 9700 (PE Applied Biosystems, Foster City, CA, United States). PCR products were purified using a Multi-Screen-PCR purifying plate (Millipore Company, Billerica, MA, United States). Under the condition recommended by the manufacturer's instruction, the purified PCR products were digested by the restrictive enzyme *Hha* I (New England Biolabs Beverly, MA, United States). The digest-

Table 1 Distribution of *TBX21* -1993T/C allelic frequencies in cases and controls *n* (%)

Variables	Case	Control	Adjusted OR (95% CI)	*P* trend
Age (yr) (mean ± SD)	41.14 ± 7.10	41.69 ± 8.83		0.884[1]
Gender				
Male	124 (56.36)	143 (54.58)		0.695[2]
Female	96 (43.64)	119 (45.42)		
Smoking status				
Never	160 (72.73)	201 (76.72)		0.314[2]
Always	60 (27.27)	61 (23.28)		
Drinking status				
Never	149 (67.73)	188 (71.76)		0.337[2]
Always	71 (32.27)	74 (28.24)		
Family history of cancer				
No	181 (82.27)	240 (91.60)		0.002[2]
Yes	39 (17.73)	22 (8.40)		
TBX21 -1993				
T	356 (80.91)	473 (90.27)	1.00	-
C	84 (19.09)	51 (9.73)	2.06 (1.44-2.96)	< 0.001[2]
T/T	154 (70.00)	219 (83.59)	1.00	-
T/C	48 (21.82)	35 (13.36)	1.84 (1.13-3.01)	0.015[3]
C/C	18 (8.18)	8 (3.05)	3.42 (1.41-8.31)	0.007[3]

[1]Mann-Whitney *U* test; [2]χ^2 test; [3]Multivariate unconditional logistic regression model. SD: Standard deviation; CI: Confidence interval; OR: Odds ratio.

ed PCR products were fractionated on 4% agarose Tris-borate-EDTA gel (Agarose 1000; Gibco BRL, Rockville, MD, United States) and stained with ethidium bromide. All assays were conducted blindly by two researchers without the knowledge of case or control status. Additionally, about 10% of the samples were randomly selected and retested, and the results were 100% concordant.

Statistical analysis

The frequency distributions of demographic variables and putative risk factors to gastric cancer, including age, gender, smoking or drinking status, and family history of cancer were examined in case and control groups by Mann-Whitney *U* test or Chi-square test. Multivariate unconditional logistic regression was used to determine the statistical significance of interaction between the certain variables and gastric cancer. Odds ratios (ORs) were adjusted by matching the variables. The Chi-square was used to compare the observed genotype distributions with the expected genotype by the Hardy-Weinberg equilibrium. All calculations were performed using the SPSS/Win statistical program (version 11.5.1 for Windows; SPSS Inc, Chicago, IL, United States). The statistical tests were two-sided, and the values of $P < 0.05$ were considered statistically significant.

RESULTS

Study population characteristics

In our experiment, *TBX21* gene -1993 loci within its promoter region were found in 482 Chinese individuals consisting of 220 gastric cancer patients and 262 controls, and the substitution of nucleotide from T to C was detected by PCR-RFLP method. The observed -1993 T/C genotypes of *TBX21* gene were in Hardy–Weinberg equilibrium ($P > 0.05$). There were no statistically significant differences in age ($P = 0.884$) or gender distribution ($P = 0.695$) between patients and controls by the Mann-Whitney *U* test or Chi-square test (Table 1). The family cancer history in case group showed statistically significant difference from the control group ($P = 0.002$). The amounts of alcohol intake and tobacco smoking were similar between patients and controls.

Association between TBX21 -1993 polymorphism and gastric cancer

Under the subgroup analysis stratified by the confounding factors or as a whole group analysis in the gastric cancer cases, the association between the genotype frequencies of *TBX21* gene -1993T/C polymorphism and gastric cancer is shown in Tables 1 and 2. The distribution of the T allele at the -1993 loci was significantly different between the control and case groups (OR = 2.06, 95% CI: 1.44-2.96, Table 1). The -1993TC and -1993CC genotypes were associated with the risk of gastric cancer (OR = 1.84, 95% CI: 1.13 -3.01; OR = 3.42, 95% CI: 1.41-8.31; respectively). When the data were stratified by gender, age, smoking or drinking status and the family history of cancer, the stronger association was revealed in the male patients aged less than 65 years with frequent smoking, especially in the patients who had family histories of cancer (OR = 3.99, 95% CI: 1.06-15.03, Table 2).

Clinicopathological association between patients and TBX21 -1993 polymorphism

The association between the clinicopathological characteristics of the gastric cancer and the genotype distributions is shown in Table 3. In the multivariate unconditional logistic regression analysis, the clinicopathological parameters did not show any significant relations to -1993TC genotype in age ($P = 0.218$), gender ($P = 0.388$), tumor size ($P = 0.369$), degree of differentiation ($P =$

Table 2 Multivariate logistic regression analysis of *TBX21* -1993T/C stratified by the selected variables

Variables	Cases/controls		Adjusted OR (95% CI)	*P*
	T/T	T/C + C/C		
Gender				
Male	82/118	42/25	2.37 (1.31-4.31)	0.005
Female	72/101	24/18	1.89 (0.95-3.76)	0.071
Age (yr)				
< 55	94/114	50/27	2.79 (1.61-4.81)	< 0.001
55–65	56/59	15/11	2.13 (1.36-3.31)	0.001
> 65	4/16	1/5	0.58 (0.04-7.92)	0.683
Smoking status				
Never	118/167	42/34	1.77 (1.06-2.97)	0.029
Always	36/52	24/9	3.38 (1.31-8.71)	0.012
Drinking status				
Never	105/155	44/33	2.00 (1.18-3.89)	0.010
Always	49/64	22/10	2.48 (1.04-5.90)	0.040
Family history of cancer				
No	131/201	50/39	1.99 (1.23-3.21)	0.005
Yes	23/18	16/4	3.99 (1.06-15.03)	0.041

CI: Confidence interval; OR: Odds ratio.

Table 3 Clinicopathological characteristics analysis of 220 gastric cancer patients and *TBX21* -1993T/C polymorphism *n* (%)

Variables	All	T/T	T/C	C/C	Adjusted OR (95% CI)	*P*
Age (yr)						
⩾ 60	26 (11.82)	21 (80.77)	3 (11.54)	2 (7.69)	1.93 (0.68-5.47)	0.218
< 60	194 (88.18)	133 (68.55)	45 (23.20)	16 (8.25)		
Gender						
Male	124 (56.36)	82 (66.13)	29 (23.39)	13 (10.48)	0.70 (0.32-1.56)	0.388
Female	96 (43.64)	72 (75.00)	19 (19.79)	5 (5.21)		
Tumor size						
⩾ 5 cm	123 (55.91)	84 (68.29)	30 (24.39)	9 (7.32)	0.75 (0.40-1.41)	0.369
< 5 cm	97 (44.09)	70 (72.16)	18 (18.56)	9 (9.28)		
Degree of differentiation						
G1-2	107 (48.64)	70 (65.42)	23 (21.50)	14 (13.08)	1.64 (0.87-3.07)	0.124
G3-4	113 (51.36)	84 (74.34)	25 (22.12)	4 (3.54)		
TNM stage						
Stage Ⅰ / Ⅱ	67 (30.45)	47 (70.15)	13 (19.40)	7 (10.45)	0.73 (0.36-1.46)	0.370
Stage Ⅲ / Ⅳ	153 (69.55)	107 (69.93)	35 (22.88)	11 (7.19)		
Lymph node status						
N_0	78 (35.45)	66 (84.62)	6 (7.69)	6 (7.69)	4.02 (1.87-8.66)	< 0.001
N_{1-3}	142 (64.55)	88 (61.97)	42 (29.58)	12 (8.45)		
Distant metastasis						
M0	148 (67.27)	121 (81.76)	18 (12.16)	9 (6.08)	7.02 (3.44-14.34)	< 0.001
M1	72 (32.73)	33 (45.83)	30 (41.67)	9 (12.50)		

CI: Confidence interval; OR: Odds ratio.

0.124), and the TNM stage ($P = 0.370$). In contrast, the patients were dichotomized in TC + CC and TT genotypes, a significantly higher rate of pN_{1-3}-category was detected in TC + CC allele carriers (38.03%). It showed that the -1993 SNP was significantly associated with the lymph node metastasis ($P < 0.001$, OR = 4.02, 95% CI: 1.87-8.66; Table 3). Moreover, the eventual statistical results revealed that the TC+CC genotype in gastric cancer patients was more closely associated with the tumor distant metastasis with respect to the frequency of wild genotype TT ($P < 0.001$, OR = 7.02, 95% CI: 3.44-14.34).

DISCUSSION

In antitumor immunity, the local progression or distant metastasis of primary tumors was extrinsically controlled by type 1 immune responses, particularly *via* the cytokine interferon (IFN)-γ pathway[12-14]. The secretion of IFN-γ was highly dependent on helper T cells, and then the helper T cell-dependent type 1 (IFN-γ)-related responses promoted the tumor immunogenicity and inhibited the tumor cell progression. The transcription factor T-bet (*TBX21*; GenBank database accession number: AC003665) gene is a member of the T-box family, which is located on chromosome17q21.32, and plays a critical role in the development of type 1 helper T cells[15]. In the *T-bet* gene knockout mice, it exhibited a dramatic difference from the wild-type mice in the expression of IFN-γ, TNF-α, IL-1β, IL-12, and IL-13[16,17]. The levels of these cytokines significantly decreased in T-bet knockout mice

compared with the wild-type mice. Because the infection of *H. pylori* is one of the major risk factors for gastric cancer, T-bet seemed to be important for *H. pylori*-induced gastric cancer in mice[18]. In mouse T-bet transgenic adenocarcinoma model, another study demonstrated that T-bet could affect the primary tumor incidence and lead to a moderate decrease in the rate of tumor progression. Meanwhile, T-bet not only possessed a moderate effect on the primary cancers but primarily exerted a significant suppressor capability in the metastasis of cancer[19]. These findings strongly supported that the T-bet regulation role in immune response was related to cancer risk and could account for a portion of this risk in cancer metastasis[20].

This is the first epidemiologic study to evaluate the association between -1993 variant of *TBX21* gene and gastric cancer. A positive association was observed between -1993T/C and gastric cancer. When analyzed by genotypes, the CC genotype was strongly associated with the gastric cancer development after adjusting for potential confounders (OR = 3.42). When stratified by gender, age, smoking or drinking status, and the family history of cancer, the adjusted OR was 3.38 (95% CI: 1.31-8.71) for smokers and the adjusted OR was 3.99 (95% CI: 1.06-15.03) for the individuals with cancer family histories.

In our present experiment, when stratified by age and gender among gastric cancer patients, no positive association was found between the -1993 polymorphism and gastric cancer. Similarly, no clear difference was noticed between this polymorphic site and clinicopathological characteristics such as tumor size, degree of differentiation, and TNM stage status. Although no association was observed between -1993TC and the progression of gastric cancer, the invasive status of lymph node was related to the distant metastasis rate of tumor (OR = 4.02 and 7.02). The results revealed that the *TBX21* -1993T/C polymorphism played a crucial role in the process of gastric cancer metastasis. To our best knowledge, *TBX21* -1993T/C polymorphism has not been investigated in gastric cancer. However, the SNPs have often been observed to be amplified in virus infection[21-22], systemic sclerosis[23], asthma[24], rheumatoid arthritis[25], and systemic lupus erythematosus[26], suggesting that our results are not by chance.

In summary, this population-based case-control study indicated that the host *TBX21* -1993T/C polymorphism was associated with an increased risk of gastric cancer, especially with the distant metastasis. The findings suggested that host *TBX21* -1993 polymorphism might be a potential marker to identify the individuals who were in the risk of gastric cancer, especially in China. Because of the limited sample size and single race for these genotypes, further replicated studies or pooled analyses are needed to confirm our results and the interaction study between the *H. pylori* infection and the genetic factors should be conducted as well.

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COMMENTS

Background

TBX21, which played a critical role in modulating the development of naive T lymphocyte, has been thought to be a susceptibility gene in the progress of cancer. In this study, the authors investigated the association between the potentially functional polymorphism of *TBX21* gene and the risk of gastric cancer in the Chinese population.

Research frontiers

Gastric cancer is the one of the most common cancers in the Chinese population, many studies have reported that the expression of *TBX21* gene is closely associated with the development of cancer through the Th1 and NK cells pathway. In this study the authors reported that the -1993 genetic polymorphism located in *TBX21* gene promoter region and assessed the potent susceptibility of this *TBX21* gene to gastric cancer in the Chinese population.

Innovations and breakthroughs

This is one of the first studies of its kinds to investigate whether the host *TBX21* -1993T/C polymorphism was associated with an increased risk of gastric cancer, especially the distant metastasis. The findings suggested that host *TBX21* -1993 polymorphism might be a potential marker to identify the individuals who were at the risk of gastric cancer.

Applications

This study provides the evidence of the association between gastric cancer and the -1993 polymorphism of *TBX21* gene. Because of the limited sample size and single race for these genotypes, further studies are needed to confirm the findings.

Terminology

The *TBX21* gene (NCBI OMIM nos.*604895), which encoded the transcription factor T-bet (T-box expressed in T cells), is a key transcriptional activator to induce the differentiation from helper T cells to Th1 cells. The gene polymorphism represents the different base substitution or replacement in the DNA sequence.

Peer review

In the present study, the TBX21-1993 polymorphism was suggested to be associated with the risk of gastric cancer, in particular, the distant metastasis. The finding overall is interesting.

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