Association between the *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms and gastric cancer: A case-control study

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Abstract. The transforming growth factor-β (TGFβ) pathway plays an important role in various types of human cancer. However, the role of *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms in gastric cancer is controversial. We aimed to investigate the associations between these polymorphisms and gastric cancer susceptibility, clinicopathological parameters and survival. A case-control study was conducted in 1,010 gastric cancer patients and 1,500 healthy controls. Genotypes were determined by PCR-restriction fragment length polymorphism and DNA sequencing. Compared with the TT genotype, the *TGFB1* -509 C allele (CT/CC) was significantly associated with a reduced risk of gastric cancer (OR, 0.71; 95% CI, 0.58-0.87; P=0.001) and certain subtypes of gastric cancer including intestinal type (OR, 0.70; 95% CI, 0.57-0.87; P=0.001), poorly differentiated (OR, 0.67; 95% CI, 0.54-0.85; P=0.001) and stage TNM III+IV (OR, 0.73; 95% CI, 0.58-0.92; P=0.008). Compared with the *TGFBR2* -875 GG genotype, carriers of the A allele (AA/AG) had a significantly decreased gastric cancer risk (OR, 0.58; 95% CI, 0.62-0.91; P<0.001). A combination of the *TGFB1* -509 C and *TGFBR2* -875 A alleles was associated with a further decreased risk of gastric cancer (OR, 0.42; 95% CI, 0.32-0.57, P<0.001). No significant correlation was observed between polymorphisms and survival of gastric cancer patients. Our results suggest that both the *TGFB1* -509 and *TGFBR2* -875 polymorphisms contribute to a decreased gastric cancer risk. The *TGFB1* -509 polymorphism affects certain subtypes of gastric cancer according to clinicopathological parameters. A combination of the *TGFB1* -509 C and *TGFBR2* -875 A alleles conferred a further decreased gastric cancer risk. These findings provide clues to the biological mechanisms that underline tumor heterogeneity.

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Introduction

Gastric cancer is one of the most common malignancies worldwide and remains a leading cause of death in Asia (1). The association between gastric cancer and chronic inflammation has generally been recognized, and chronic *Helicobacter pylori (H. pylori)* infection has been found to be a significant risk factor for gastric cancer (2,3). Only a small proportion of chronic *H. pylori*-infected individuals develop malignancies (2), suggesting that other factors, particularly host genetic factors, modulate the effects of inflammation in carcinogenesis.

The transforming growth factor-β (TGFβ) pathway plays an important role in inflammatory responses in the tumor microenvironment (4). TGFβ uniquely promotes the differentiation of human naive $CD4^+$ T cells into TH17 cells, thereby becoming involved in the modulation of complicated inflammation pathways (5,6). As a multifunctional cytokine, TGFβ1 regulates a number of important cellular responses, in addition to its role in cell differentiation, including apoptosis, cell migration, immune responses and angiogenesis. TGFβ1 initiates downstream signaling events by binding to the transforming growth factor β receptor 2 (TGFβR2), a constitutively active kinase which activates type 1 receptors by the phosphorylation of serine and threonine residues in the GS box and downstream signaling (7).

TGFB1 -509C/T (rs1800469) and *TGFBR2* -875A/G polymorphisms (rs3087465) have been reported to be associated with gastric cancer risk (8-10). However, the results of studies from different geographical regions, ethnic groups and study groups are controversial (8-12). For example, certain studies found that carriers of *TGFB1* -509T were associated with a decreased (8) or increased risk of gastric cancer (9). On the other hand, other studies have reported that the *TGFB1* -509 C/T polymorphism has no association with the risk of gastric cancer or patient survival (11,12). The limitations of these studies should be considered when interpreting their findings. The majority of studies dismissed potential confounding

factors such as age, gender, *H. pylori* status, tumor location and histological subtype, which may result in the bias and inaccuracy of results. In addition, it is known that *TGFB1* and *TGFBR2* map to different chromosomes, i.e., 19q13.2 and 3p24.1, respectively, indicating that *TGFB1* and *TGFBR2* are independently inherited. Subsequently, variants of *TGFB1* -509C/T and *TGFBR2* -875A/G exhibit a potential interaction with respect to gastric cancer risk.

This study aimed to investigate whether the *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms are correlated to the susceptibility of gastric cancer with respect to clinical and pathologic parameters. The combination of the genotypes of *TGFB1* -509 and *TGFBR2* -875 was also evaluated for their association with the risk of gastric cancer.

Materials and methods

Subjects. A population-based case-control study was conducted between 1999 and 2006 in Guangdong Province, China. The case series included 1,010 Chinese gastric cancer patients, newly diagnosed at the First Affiliated Hospital and Cancer Center of Sun Yat-Sen University, Guangzhou, China, from January 1999 to December 2006. The control series included 1,500 Cantonese healthy subjects from the general population of Guangzhou in southern China. Diagnosis was confirmed histologically as gastric cancer. The median duration of follow-up was 14 months (ranging from 0 to 98 months). Clinical data of the patients were collected from medical records and structured interviews were conducted using questionnaires. Patients diagnosed with gastric cancer received surgical resection of the tumors. Patients and control subjects provided written informed consent for participation in this study, and the study protocol was approved by the Clinical Research Ethics Committee of the Sun Yat-Sen University of Medical Sciences.

DNA extraction. Genomic DNA was extracted from paraffin-embedded tissues of 1,010 gastric cancer patients using QIAamp DNA Mini kit (Qiagen, Hilden, Germany). Of these patients, 963 were included as the case group due to good DNA quality. Genomic DNA was also extracted from 2 ml of peripheral blood of 1,500 healthy subjects using the Gentra Puregene Blood kit (Gentra Systems, Minneapolis, MN, USA). Of these, 787 subjects were selected as healthy controls frequency matched to the case group by age and gender. There were 655 males (68%) and 308 females (32%) in the gastric cancer case group, whereas the control group included 512 males (65%) and 275 females (35%). Frequency matching by age and gender was not complete, and thus these factors were further regulated in the multivariate data analysis stage. Extracted DNA was resuspended in UltraPure RNAse/DNAse-free distilled water (Invitrogen, Hong Kong, China).

Genotyping analysis. *TGFB1* -509C/T and *TGFBR2* -875A/G genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primer sequences for *TGFB1* -509C/T and *TGFBR2* -875A/G were: sense 5'-GAGACGCCTTGAAGTAACTG-3' and anti-sense 5'-AACCAAAGATGTTCTGAACTGA-3' for *TGFB1* -509C/T; and sense 5'-GCAAGAAAGGAAATTTGAAAGTT TGT-3' and anti-sense 5'-TCACCTGAATGCTTGTGTTTT-3' for *TGFBR2* -875A/G. PCR was performed under the conditions: denaturation at 94˚C for 10 min, followed by 94˚C (30 sec), 65˚C (30 sec), and 72˚C (30 sec) for 38 cycles, followed by a final extension at 72˚C for 7 min. PCR products were digested overnight at 37˚C with *Bsu*36I (New England BioLabs, Ipswich, MA, USA) for *TGFB1* -509C/T and *Rsa*I (New England BioLabs) for *TGFBR2* -875A/G genotypes, and then separated by electrophoresis on 3% agarose gel with ethidium bromide staining. PCR products were shown to be digested into 3 types of fragments (Fig. 1). To confirm the genotyping results, randomly selected PCR samples were examined by DNA sequencing (Fig. 2).

Statistical analysis. Hardy-Weinberg equilibrium in the controls was assessed using the χ^2 test. The effects of the genotypes on the risk of gastric cancer were represented as odds ratios (OR) with 95% confidence interval (CI) by logistic regression. Relationships between the two polymorphisms and clinicopathological characteristics of the gastric cancer patients were compared using contingency tables and the Pearson's χ^2 test. Since frequency matching was not complete, age and gender were included in all of the multiple logistic regression models. Kaplan-Meier survival curves and the log-rank test for trend were used to evaluate the relationship between the two polymorphisms and the prognosis from the date of primary diagnosis to the end of follow-up. The multivariate Cox regression analysis was performed to assess the prognostic value of the two polymorphisms with adjustment for age, gender, tumor size, grade, lymph node status and distant metastasis. Hazard ratios (HR) and 95% confidence interval (CI) for each factor in the multivariate analysis were calculated from the Cox regression model. Differences were regarded as significant at $P<0.05$. The above statistical analyses were performed using SPSS 13.0 software package (SPSS Inc, Chicago, IL, USA).

Results

Association of TGFB1 -509C/T and TGFBR2 -875A/G polymorphisms with gastric cancer susceptibility. Among the controls, the genotype distribution for each SNP was in Hardy-Weinberg equilibrium ($P>0.05$). The frequencies of the polymorphisms in the cases and controls are shown in Table I. Compared with the *TGFB1* -509 TT genotype, the CC and CT genotypes significantly decreased gastric cancer risk with odds ratios (ORs) of 0.70 (95% CI, 0.53-0.94) and 0.72 (95% CI, 0.58-0.89), respectively. Overall, the C carriers (CT or CC) were also associated with a lower gastric cancer risk when compared with the TT carriers (OR, 0.71; 95% CI, 0.58-0.87; $P=0.001$). Analysis of the *TGFBR2* -875A/G genotype polymorphisms showed a significant difference between the gastric cancer patients and controls ($P<0.001$). The AG genotype was associated with a lower gastric cancer risk when compared with the GG genotype (OR, 0.56; 95% CI, 0.45-0.70; $P<0.001$) (Table I).

Association of the TGFB1 -509C/T and TGFBR2 -875A/G polymorphisms with clinicopathological parameters. Stratified by the Lauren classification, the presence of the *TGFB1* -509 C allele genotypes (CC or CT) was significantly associated with intestinal-type gastric cancer with an OR of 0.70 (95%

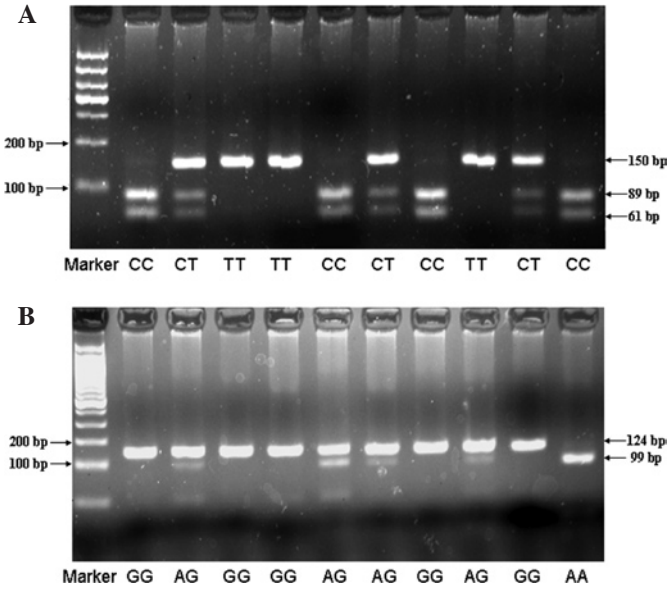

Figure 1. Genotyping of the *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms. (A) PCR-RFLP agarose gel electrophoresis of the *TGFB1* -509 polymorphism showing the CC (89 and 61 bp), CT (150, 89 and 61 bp), and TT (150 bp) genotypes. (B) PCR-RFLP agarose gel electrophoresis of the *TGFBR2* -875 polymorphism showing the AA (99 and 25 bp), AG (124, 99 and 25 bp), and GG (124 bp) genotypes, while 25 bp was too short to be detected.

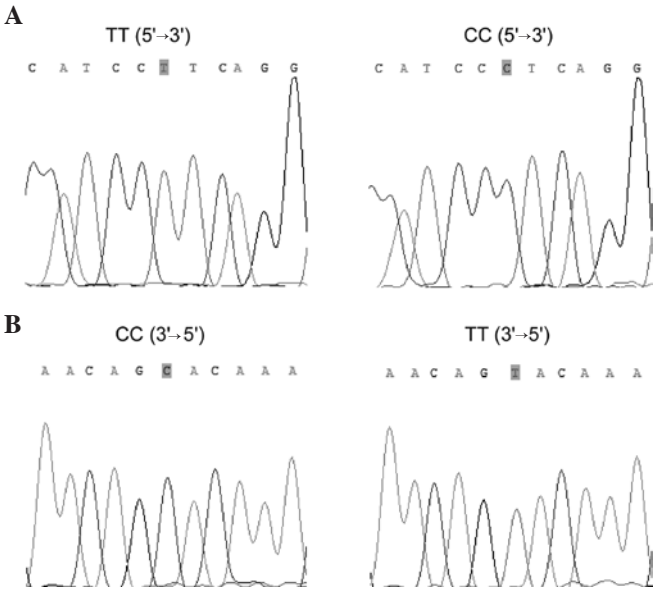

Figure 2. Sequencing analysis for genotypes of the *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms. (A) Sequencing analysis for genotypes of *TGFB1* -509: TT and CC. (B) Reverse sequencing analysis for genotypes of *TGFBR2* -875: GG and AA.

CI, 0.57-0.87; P=0.001), but not with diffuse- or mixed-type gastric cancer, as compared with the TT genotype. In contrast, the association between the *TGFBR2* -875 polymorphism and gastric cancer was not modified by the Lauren classification. Carriers of the AG genotype had a significantly decreased risk of intestinal-, diffuse- and mixed-type gastric cancer, compared to carriers of the GG genotype (Table II).

When the gastric cancers were stratified according to tumor differentiation, the effect of the *TGFB1* -509 CC and CT genotypes remained significant on poorly differentiated gastric cancer with ORs of 0.63 (95% CI, 0.45-0.88; P=0.006) and 0.69 (95% CI, 0.54-0.88; P=0.002), respectively, but not on moderately or well-differentiated gastric cancer. However, the association between the *TGFBR2* -875 polymorphism and gastric cancer was not modified by tumor differentiation. The *TGFBR2* -875AG genotype was found to be associated with both poorly differentiated and moderately or well-differentiated gastric cancer (Table III).

When the gastric cancer patients were divided into two subgroups according to TNM stage, the *TGFB1* -509 CT genotype was found to be associated with TNM III/IV stage gastric cancer with an OR of 0.71 (95% CI, 0.56-0.91; P=0.007), but not with TNM I/II stage gastric cancer, when compared with the TT genotype. In addition, the association between the *TGFBR2* -875 polymorphism and gastric cancer was not modified by TNM stage. The *TGFBR2* -875AG genotype was associated with both TNM I/II and TNM III/IV stage gastric cancer when compared to the GG genotype (Table IV).

Table I. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer in relation to the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes.

Genotype	Control (%)	Cases (%)	OR[a] (95% CI)	P-value
TGFB1 -509C/T				
CT/CC	536 (69)	578 (61)	0.71 (0.58-0.87)	0.001
CC	133 (17)	142 (15)	0.70 (0.53-0.94)	0.016
CT	403 (52)	436 (46)	0.72 (0.58-0.89)	0.002
TT	245 (31)	367 (39)	1	
TGFBR2 -875A/G				
AG/AA	305 (39)	261 (27)	0.58 (0.62-0.91)	<0.001
AA	38 (5)	39 (4)	0.69 (0.43-1.09)	0.114
AG	268 (34)	222 (23)	0.56 (0.45-0.70)	<0.001
GG	478 (61)	702 (73)	1	

[a]Adjusted for age and gender.

Table II. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer, stratified by Lauren classification, in relation to the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes.

		Intestinal			Diffuse			Mixed		
Genotype	Control	Case	OR[a] (95% CI)	P-value	Case	OR[a] (95% CI)	P-value	Case	OR[a] (95% CI)	P-value
TGFB1-509C/T										
CT/CC	536	448	0.70 (0.57-0.87)	0.001	30	0.62 (0.35-1.09)	0.099	90	0.78 (0.54-1.13)	0.192
CC	133	106	0.66 (0.49-0.90)	0.009	8	0.67 (0.29-1.56)	0.353	26	0.89 (0.53-1.49)	0.665
CT	403	342	0.72 (0.57-0.90)	0.004	22	0.60 (0.32-1.11)	0.104	64	0.74 (0.49-1.11)	0.142
TT	245	285	1		22	1		54	1	
TGFBR2 -875A/G										
AG/AA	305	203	0.58 (0.46-0.72)	<0.001	15	0.64 (0.34-1.19)	0.158	36	0.53 (0.35-0.79)	0.002
AA	38	26	0.59 (0.35-0.99)	0.049	5	1.81 (0.67-4.93)	0.244	6	0.61 (0.23-1.58)	0.304
AG	267	177	0.57 (0.46-0.72)	<0.001	10	0.48 (0.24-0.99)	0.047	30	0.51 (0.33-0.79)	0.003
GG	478	552	1		36	1		105	1	

[a]Adjusted for age and gender.

Table III. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer, stratified by tumor differentiation, in relation to the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes.

		Poor			Moderate or well-differentiated		
Genotype	Control	Cases	OR[a] (95% CI)	P-value	Cases	OR[a] (95% CI)	P-value
TGFB1 -509C/T							
CT/CC	536	335	0.67 (0.54-0.85)	0.001	147	0.79 (0.58-1.09)	0.156
CC	133	78	0.63 (0.45-0.88)	0.006	40	0.86 (0.55-1.35)	0.512
CT	403	257	0.69 (0.54-0.88)	0.002	107	0.77 (0.55-1.08)	0.133
TT	245	225	1		83	1	
TGFBR2 -875A/G							
AG/AA	305	167	0.63 (0.50-0.79)	<0.001	53	0.44 (0.31-0.63)	<0.001
AA	38	27	0.83 (0.49-1.38)	0.470	5	0.36 (0.14-0.94)	0.037
AG	267	140	0.61 (0.47-0.77)	<0.001	48	0.46 (0.32-0.65)	<0.001
GG	478	410	1		180	1	

[a]Adjusted for age and gender.

Association of the TGFB1 -509C/T and TGFBR2 -875A/G polymorphisms with the survival of gastric cancer patients. Overall survival of the gastric cancer patients was analyzed using Kaplan-Meier survival curves for dependence on the TGFB1 -509C/T (P=0.859) and TGFBR2 -875A/G genotypes (P=0.652), respectively. In the multivariate Cox regression analysis, the survival of gastric cancer patients was significantly associated with age (P=0.03), tumor staging (P=0.013), lymph node status (P<0.001) and distant metastasis (P<0.001). However, the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes were not associated with outcome in gastric cancer patients (Table V).

Combination of the TGFB1 -509C/T and TGFBR2 -875A/G polymorphisms. We further investigated the effect of the combination of the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes. As shown in Table VI, compared with the *TGFB1* -509 TT and *TGFBR2* -875 GG genotypes, the combination of the *TGFB1* -509CC/CT and *TGFBR2* -875AA/AG genotypes was significantly associated with a decreased risk of gastric cancer (OR, 0.42; 95% CI, 0.32-0.57; P<0.001) (Table VI).

Overall survival of the gastric cancer patients was analyzed using Kaplan-Meier survival curves. No statistical difference was noted between the variant combination of the *TGFB1* -509 and *TGFBR2* -875 genotypes (P=0.459). In the multivariate Cox regression analysis, age, tumor size, tumor grade, lymph node status and distant metastasis were significantly associated with the survival of gastric cancer patients. However, the combination of the *TGFB1* -509 and *TGFBR2* -875 genotypes was not associated with the outcome of the gastric cancer patients (P=0.766) (data not shown).

Table IV. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for gastric cancer, stratified by TNM stage, in relation to the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes.

		TNM I+II			TNM III+IV		
Genotype	Control	Cases	OR[a] (95% CI)	P-value	Cases	OR[a] (95% CI)	P-value
TGFB1 -509C/T							
CT/CC	536	143	0.83 (0.61-1.15)	0.260	339	0.73 (0.58-0.92)	0.008
CC	133	30	0.70 (0.44-1.13)	0.146	91	0.78 (0.56-1.08)	0.138
CT	403	113	0.88 (0.63-1.22)	0.436	248	0.71 (0.56-0.91)	0.007
TT	245	77	1		210	1	
TGFBR2 -875A/G							
AG/AA	305	64	0.59 (0.43-0.82)	0.002	141	0.54 (0.43-0.69)	<0.001
AA	38	7	0.53 (0.23-1.22)	0.137	21	0.66 (0.38-1.14)	0.134
AG	267	57	0.60 (0.43-0.84)	0.003	120	0.53 (0.41-0.68)	<0.001
GG	478	61	1		409	1	

[a]Adjusted for age and gender.

Table V. The Cox multivariate regression analysis of potential factors for overall survival in the gastric cancer patients.

Variable	HR	(95% CI)	P-value
Age	1.02	(1.00-1.03)	0.030
Gender			
Female	1		
Male	1.12	(0.81-1.53)	0.430
Tumor staging			
T1	1		
T2	2.11	(0.40-10.16)	0.350
T3	4.26	(0.995-18.26)	0.050
T4	6.52	(1.49-28.50)	0.013
Lymph node			
Negative	1		
Positive	2.60	(1.59-4.26)	<0.001
Metastasis			
Negative	1		
Positive	3.55	(2.50-5.02)	<0.001
Lauren classification			
Intestinal	1		
Diffuse or mixed	1.14	(0.71-1.82)	0.600
Differentiation			
Poor	1		
Moderate or well	0.90	(0.62-1.31)	0.590
TGFB1 -509C/T			
TT	1		
CT	0.93	(0.67-1.23)	0.630
CC	0.79	(0.47-1.31)	0.360
TGFBR2 -875A/G			
GG	1		
AG	0.88	(0.39-1.98)	0.750
AA	0.87	(0.40-1.90)	0.730

Discussion

In the present study, we investigated the relationship between polymorphisms of the *TGFB1* and *TGFBR2* genes and the risk of gastric cancer in a low-risk Chinese population. *TGFB1* -509 C (heterozygote or homozygote) and *TGFBR2* AG genotypes were found to be associated with a reduced risk of gastric cancer, and this risk was particularly lower among subjects who carried the *TGFB1* -509 C and *TGFBR2* -875 A allele genotypes, suggesting that the *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms were significant host protective biological factors that affected the risk of gastric carcinogenesis in southern China.

Previous studies on the association of the *TGFB1* -509C/T polymorphism and gastric cancer are controversial. Li *et al* found that carriers of *TGFB1* -509T were more susceptible to gastric cancer in northern China (9), which was consistent with our results (Table I). Jin *et al* reported that among a high-risk Chinese population in Nanjing, carriers of *TGFB1* -509 T or *TGFBR2* -875 A had a decreased risk of gastric cancer (8). On the other hand, no association was found between the *TGFB1* -509C/T polymorphism and gastric cancer in a US population (12). Allele frequency patterns of the *TGFB1* -509C/T polymorphism vary greatly between different geographical regions and ethnic groups. For example, the frequencies of the -509 T allele among healthy controls were 0.468, 0.461 and 0.212 in Nanjing, Shandong and the US population, respectively (8,9,12), while the -509 T allele frequency in our healthy controls was 0.572. As a result, the -509 T allele acted as a major allele in our region, but as a minor allele in the above-mentioned regions. Ethnic differences in *TGFB1* allele frequencies may be an explanation for the discrepancies in the findings between the present study and the study in the US population. However, findings of the latter study cannot explain the inconsistency in our study and that by Jin *et al*, where only minor differences in the -509 T allele frequencies were noted in the control subjects. The discrepancy may be

Table VI. Combined distribution of the *TGFB1* -509C/T and *TGFBR2* -875A/G genotypes in the controls and gastric cancer patients.

Genotype[a]	Control (%)	Cases (%)	OR[b] (95% CI)	P-value
3 and 4	213 (57)	158 (43)	0.42 (0.32-0.57)	<0.001
1 and 2	153 (37)	266 (63)	1	

[a]'1', the *TGFB1* -509 TT genotype; '2', the *TGFBR2* -875 GG genotype; '3', the *TGFB1* -509CT/CC genotype and '4', the *TGFBR2* -875 AG/AA genotype. [b]Adjusted for age and gender.

explained due to the different compositions of patients with various clinicopathological characteristics in the two studies. For the allele frequency of *TGFBR2* -875A, similar frequencies were observed between Nanjing (0.197 in controls) and our population group (0.155 in controls) (8).

We further demonstrated that the *TGFB1* -509 C allele was associated with a decreased risk of intestinal-type cancer. It has been widely accepted that intestinal- and diffuse-type gastric cancer differ with regards to age, gender and the process of carcinogenesis (13). The development of intestinal-type malignancy is a longer multistep process via gastric atrophy, intestinal metaplasia, dysplasia and ultimately intestinal-type carcinoma, which is initiated by *H. pylori* infection and by exposure to environmental toxins (14). *TGFB1*, as a regulator of inflammation, may be involved in this multistep process. Messa *et al* reported that patients with *H. pylori* infection had elevated TGFβ1 levels in the gastric antrum, while TGFβ1 expression levels decreased following eradicating treatment (15). Kandulski *et al* reported that *H. pylori*-induced gastritis is associated with a recruitment of naturally occurring FOXP3(+) Treg cells that correlate with higher bacterial colonization and increased mucosal TGFβ1 expression, suggesting that *H. pylori* infection suppresses the maintenance and aggravation of gastric inflammation by activating the TGFβ1-FoxP-$CD4^{+}CD25^{high}$Treg pathway (16). A similar phenomenon was observed in other animal studies (17,18). Based on the above studies, we inferred that TGFβ1 plays an important role in the inhibition of *H. pylori*-induced gastritis. It has been reported that presence of the *TGFB1* -509 C allele is related to a higher production of TGFβ1 in the Chinese (19). Elevated TGFβ1 may in turn affect the multistep process of gastric cancer initiated by *H. pylori* infection by activating $CD4^{+}CD25^{high}$Tregs, which contribute to a reduced incidence of intestinal-type gastric cancer.

This study showed that the *TGFB1* -509 C allele was associated with poorly differentiated gastric cancer, but not with moderately or well-differentiated gastric cancer. Studies have shown that the p38 MAPK pathway is involved in the development of poorly differentiated gastric cancer (20-22). A specific p38 inhibitor was found to reduce the irregular dense membranous accumulation of F-actin in poorly differentiated gastric cancer cell lines (22). Moreover, activation of the phosphatidylinostiol 3-kinase (PI3K) via the p38 MAPK cascade caused the loss of cell-cell contact in poorly differentiated signet-ring cell carcinoma (20,21). *TGFB1* together with the IL1B gene are involved in a complex signal network. Therefore, the *TGFB1* polymorphism may be partially associated with poorly differentiated gastric cancer through inhibition of the p38 MAPK pathway.

In contrast, the *TGFBR2* -875A/G polymorphism was found to be associated with decreased gastric cancer risk, and the association was not modified by pathological types, degrees of differentiation or TNM stages. A possible explanation for this phenomenon is that TGFβ suppresses carcinogenesis by suppressing tumorigenic inflammation and stroma-derived mitogens (23). In the TGFβ pathway, seven type I and five type II receptors paired in different combinations provide the receptor system for the entire TGFβ family. However, among the type II receptors, only TGFβR2 is able to bind to TGFβ to activate downstream signaling (23). Thus, TGFβR2 acts as a key regulator in the TGFβ pathway. The *TGFBR2* -875A/G polymorphism may have an extensive effect in reducing the risk of gastric cancer by altering the protein expression of the *TGFBR2* gene to affect signal transduction.

Another noteworthy finding of this study was that *TGFB1* -509C/T and *TGFBR2* -875A/G gene polymorphisms acted synergistically on the decreased risk of gastric cancer. The risk of gastric cancer was particularly lower among subjects who carried both the *TGFB1* -509 C and *TGFBR2* -875 A allele genotypes. *TGFB1* and *TGFBR2* map to different chromosomes but are essential components of the same signaling pathway. TGFβ signaling initiates carcinoma-immune cell cross-talk within the carcinoma cell and is involved in carcinogenesis, invasion and metastasis (24). TGFβ activation is induced by a number of mechanisms, including the expression of calpain and cathepsin D (24). Once activated, TGFβ binds to TGFβR2 to activate downstream signaling (24). Thus, it is feasible that the combination of the *TGFB1* -509C/T and *TGFBR2* -875A/G gene polymorphisms confer a synergistic effect on gastric cancer initiation and progression. This result confirmed the hypothesis that a combined assessment of functionally relevant common variants may aid in the characterization of cancer risk and prediction of disease aggressiveness (25).

Although studies have shown that TGFβ1 expression is closely linked to the prognosis of gastric cancer (26,27), neither the polymorphisms of *TGFB1* -509C/T and *TGFBR2* -875A/G nor the combination of the two SNPs revealed a correlation with patient survival in this study, consistent with the study in the US population (11). A possible explanation is that overall survival is determined, not only by factors involved in gastric cancer disease activity, but also by underlying diseases and other complications. Thus, SNPs alone may not be enough to affect the complex outcome.

In conclusion, our study provides evidence that the *TGFB1* -509 CC, CT and *TGFBR2* AG genotypes are significantly associated with a decreased risk of gastric carcinoma. The *TGFB1* -509 C allele was found to be associated with a reduced risk of intestinal-type, poorly differentiated and TNM III/IV stage gastric cancer. Moreover, the combination of the *TGFB1* -509C/T and *TGFBR2* -875A/G polymorphisms exhibited a synergistically decreased risk of gastric cancer in a southern Chinese population. These findings may provide clues to the biological mechanisms that underline tumor heterogeneity; thus, further studies are warranted.

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