Int J Clin Exp Med 2015;8(7):11524-11528
www.ijcem.com /ISSN:1940-5901/IJCEM0008219

Original Article

TGF-β1-509C/T polymorphism and the risk of ESCC in a Chinese Han population

Hong-Jian Liu, Qing-Guang Zhang, Yu-Bo Wang, Hai-Tao Xu, Jing-Jing Zhang

Department of Thoracic Surgery, Binzhou Medical College Hospital, Binzhou 256603, Shandong, China

Received March 19, 2015; Accepted June 3, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: Background: The studies investigating whether transforming growth factor (TGF)-β1-509C/T polymorphism is associated with the risk of ESCC is inconsistent. Methods: The TGF-β1-509C/T genotypes were determined by using a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay and DNA sequencing analysis. The differences in demographic variables and genotype distributions of TGF-β1-509C/T polymorphism between ESCC patients and controls were calculated by Pearson's Chi square test. Associations between TGF-β1-509C/T polymorphism genotypes and ESCC risk were estimated by OR and their 95% CIs computed using unconditional logistic regression model. Results: There was a significant difference of TGF-β1-509C/T polymorphism genotype distribution between ESCC group and control group (P<0.001). With the CC genotype as reference, the adjusted OR for CT genotype reached to 0.78 (95% CI: 0.65-0.89; P=0.041), and the adjusted OR for TT homozygous carriers was 0.52 (95% CI: 0.33-0.78; P=0.017). The T allele carriage also presented a lower risk for ESCC (adjusted OR=0.43; 95% CI, 0.29-0.71; P=0.009). Conclusion: TGF-β1-509C/T polymorphism may contributes to ESCC susceptibility in Chinese population.

Keywords: TGF-β1, polymorphism, ESCC, risk

Introduction

Esophageal cancer is the eighth most common malignancy and the sixth most common cause of cancer-related deaths worldwide [1]. Approximately more than 450,000 people are afflicted with esophageal cancer, which shows a rapidly increasing incidence rate [2]. Esophageal cancer involves the malignancies that arise from the epithelial surface of the esophagus, with two major subtypes i.e., Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EADC). It has been suggested that esophageal cancer is a combined effect of multiple factors, which contains both environmental factors and genetic defects.

Transforming growth factor (TGF)-β is a multiplicity factor mediating cellular processes, including cell growth, cell differentiation, apoptosis, and cellular homeostasis [3, 4]. TGF-β is known as low penetrance gene in cancer [5]. There are three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3), of which TGF-β1 is most widely expressed [6]. TGF-β1 gene is located on chromosome 19q13.1 [7]. So far, several polymorphisms in the TGF-β1 gene have been reported and found to affect TGF-β1 protein expression [8].

The association between TGF-β1 polymorphisms and ESCC has been investigated in several studies. The study by Wei et al showed that TGF-β1 gene +869T/C but not -509C/T polymorphism might contribute to a genetic risk factor for ESCC in a Chinese population [9]. In the case-control study by Jin et al, the variant genotypes (-509CT/TT) of TGF-β1 were associated with a 63% significantly decreased risk of ESCC (adjusted OR=0.37, 95% CI=0.27-0.50) compared with -509CC wild-type homozygote [10]. Therefore, the studies investigating whether transforming growth factor (TGF)-β1-509C/T polymorphism is associated with the risk of ESCC is inconsistent.

Materials and methods

Sample collection

This study was approved by the institutional Review Board of Binzhou Medical College Hospital. All participants have provided their

Table 1. Characteristics of ESCC cases and controls

Variable	Cases (n=231)		Controls (n=230)		*P* value
	N	%	N	%	
Age					
≤65	97	41.99	90	39.13	0.570
>65	134	58.01	140	60.87	
Sex					
Male	153	66.23	150	65.22	0.845
Female	78	33.77	80	34.78	
Smoking status					
Smokers	145	62.77	124	53.91	0.693
Nonsmokers	86	37.23	106	46.09	
Drinking status					
Drinkers	178	77.06	161	70.00	0.092
Nondrinkers	53	22.94	69	30.00	

Table 2. Genotype and allele frequencies of TGF-β1-509C/T polymorphism in ESCC patients and healthy controls

	Cases (n=231)		Controls (n=230)		
	N	%	N	%	*P* value
Genotypes					
CC	108	46.75	67	29.13	<0.001
CT	49	21.21	64	27.83	
TT	74	32.03	99	43.04	
Allele					
C	265	57.36	198	43.04	<0.001
T	197	42.64	262	56.96	

written informed consents to participate in this study. A total of 231 ESCC cases from the Department of Thoracic Surgery, Binzhou Medical College Hospital and 230 sex- and age-matched controls were included in this study. Patients were consecutively recruited between March 2008 and May 2014 at Binzhou Medical College Hospital. The diagnosis of all patients was histologically confirmed. Individuals who smoked one cigarette per day for over 1 year were considered as smokers. Subjects were considered as alcohol drinkers if they drank at least once per week. All individuals were ethnic Han Chinese. At recruitment, the informed consent was obtained from each subject. The 5 mL venous blood samples obtained from the subjects were collected in an EDTA tube and stored at -70°C for extraction of DNA genome.

DNA isolation and genotyping

Genomic DNA was extracted from whole EDTA-treated peripheral blood using a QIAamp Blood Kit according to the manufacturer's instructions. The TGF-β1-509C/T genotypes were determined by using a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay and DNA sequencing analysis. The PCR primer for the -509C/T polymorphism was 5'-CAGACTCTAGAGACTGTCAG-3' (forward) and 5'-GTCACCAGAGAAAGAGGAC-3' (reverse). The PCR cycle conditions consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of 30 s at 94°C; 45 s at 59°C; 55 s at 72°C; and a final elongation at 72°C for 8 min. The PCR products were digested 3 h at 37°C with the appropriate restriction enzymes.

Statistical analysis

All calculations were performed with SPSS software package (Version 18.0, SPSS Inc., Chicago, IL). The differences in demographic variables and genotype distributions of TGF-β1-509C/T polymorphism between ESCC patients and controls were calculated by Pearson's Chi square test. Associations between TGF-β1-509C/T polymorphism genotypes and ESCC risk were estimated by OR and their 95% CIs computed using unconditional logistic regression model. All ORs and 95% CIs were adjusted for age, sex, drinking and smoking status. All statistical tests were two-sided. A *P* value of less than 0.05 was used as the criterion of statistical significance.

Results

Characteristics of ESCC cases and controls

A total of 231 ESCC patients (153 males and 78 females) and 230 unrelated healthy individuals (150 males and 80 females) were analyzed in this case-control study. No statistically significant differences were found between ESCC cases and healthy controls in terms of age (P=0.570), sex (P=0.845), smoking status (P=0.693), and drinking status distributions (P=0.092), indicating that the frequency matching was adequate (**Table 1**).

Genotype and allele frequencies of TGF-β1-509C/T polymorphism in ESCC patients and healthy controls

The genotype distribution of TGF-β1-509C/T polymorphism deviated from Hardy-Weinberg equilibrium in both ESCC patients and controls

Table 3. The genotype and allele distribution of TGF-β1 -509C/T polymorphism and the risk of ESCC

	Cases (n=231)	Controls (n=230)	OR	95% CI	*P* value
Genotypes					
CC	108	67	1.000 (reference)		
CT	49	64	0.78	0.65-0.89	0.041
TT	74	99	0.52	0.33-0.78	0.017
Allele					
C	265	198	1.000 (reference)		
T	197	262	0.43	0.29-0.71	0.009

(P>0.05), indicating that it was plausible that selective forces are operating in the population. The allele and genotype frequencies of TGF-β1-509C/T polymorphism for the ESCC cases and controls are presented in **Table 2**. The T allele revealed significantly decreased frequency in ESCC patients compared to healthy controls (42.64% vs. 56.96%, P<0.001). Among 231 ESCC patients, 108 (46.75%) displayed a CC genotype, 49 (21.21%) with a CT genotype and 74 (32.03%) with a TT genotype. Among 230 healthy controls, 67 (29.13%) displayed a CC genotype, 64 (27.83%) with a CT genotype and 99 (43.04%) with a TT genotype. Therefore, there was a significant difference of TGF-β1-509C/T polymorphism genotype distribution between ESCC group and control group (P<0.001).

The genotype and allele distribution of TGF-β1-509C/T polymorphism and the risk of ESCC

We performed the multivariate logistic regression to determine the independent risk factors for ESCC. With the CC genotype as reference, the adjusted OR for CT genotype reached to 0.78 (95% CI: 0.65-0.89; P=0.041) after adjustment for age, gender, smoking status, and alcohol drink, and the adjusted OR for TT homozygous carriers was 0.52 (95% CI: 0.33-0.78; P=0.017). The T allele carriage also presented a lower risk for ESCC (adjusted OR=0.43; 95% CI, 0.29-0.71; P=0.009, **Table 3**).

Discussion

Among human cancers, esophageal cancer appears to be a complex multistep process with multifunctional etiologies in which environmental, geographical and genetic factors have been attributed to play critical roles in the development of cancer. Minimal disease residuals which are not detectable by conventional diagnostic tools may remain present but unnoticed after complete surgical resection, this makes the prediction of the clinical course of esophageal cancer patients difficult [11-13]. Ideal prognostic markers should be easy to determine, be independent of tumor tissue availability and harbor genomic stability that remains unbiased by the type of specific tumor therapy. Unfortunately clinically useful markers are missing in esophageal cancer yet. Important prognostic indicators of clinical outcome in cancer patients might be genetic germline variations [14-16].

TGF-β is a homodimeric, multifunctional, and pleiotropic cytokine with a molecular weight of approximately 25 kDa secreted in a latent form by several cell types such as peripheral blood mononuclear cells, Treg cells, platelets, and endothelial cells and plays pivotal roles in modulation of cellular growth, maturation and differentiation, extracellular matrix formation, homeostasis, endothelial cell plasticity, immunoregulation, apoptosis, angiogenesis, and cancer progression [17]. TGF-β signaling is one of the most commonly altered cellular pathways in human cancers [18, 19]. TGF-β1 is a multi-functional cytokine that plays an important role in carcinogenesis. TGF-β1 is a potent inhibitor of proliferation of epithelial, endothelial and hematopoietic cells, and it acts as a tumor suppressor. TGF-β1 has dual role in carcinogenesis with tumor suppressive effects in epithelial cells, but tumor invasion and metastasis promoting effects during later stages of carcinoma progression [20, 21].

Five polymorphisms have been identified: two in the promoter region at positions -800 and -509, one at position +72 in a untranslated region, and two in the signal sequence at positions +869 and +915. Many studies have investigated the associations between the TGF-β1 polymorphisms and susceptibility of cancers. In the meta-analysis by Alqumber et al, no significant association between TGF-β1 29T/C polymorphism and breast cancer risk was demonstrated overall or on subgroup (Caucasian and Asian) analysis, and it could be concluded that TGF-β1 29T/C polymorphism did not play a role in breast cancer susceptibility in overall or ethnicity-specific manner [22]. In the meta-analy-

sis by Guo et al, TGF-β1 +869C/T polymorphism was significantly associated with HCC risk (OR=1.74, 95% CI: 1.22-2.47, P=0.002). In addition, a significant association between -509C/T polymorphism and HCC risk was observed (OR=1.40, 95% CI: 1.15-1.70, P= 0.0007). Furthermore, significant associations between these polymorphisms and HCC risk were found in Asians and population-based studies [23]. The meta-analysis by Wang et al suggested that TGF-β1 gene promoter -509C allele variant was a possible risk factor for developing colorectal cancer [24]. The meta-analysis by Li et al supported the TGF-β1-509T polymorphism as a susceptibility factor for gastric cancer [25].

The association between TGF-β1 polymorphisms and ESCC has also been investigated in several studies. The study by Wei et al showed that TGF-β1 gene +869T/C but not -509C/T polymorphism might contribute to a genetic risk factor for ESCC in a Chinese population [9]. In the case-control study by Jin et al, the variant genotypes (-509CT/TT) of TGF-β1 were associated with a 63% significantly decreased risk of ESCC (adjusted OR=0.37, 95% CI=0.27-0.50) compared with -509CC wild-type homozygote [10]. Therefore, the studies investigating whether transforming growth factor (TGF)-β1-509C/T polymorphism is associated with the risk of ESCC is inconsistent.

In the present study, the TGF-β1-509C/T genotypes were determined by using a PCR-restriction fragment length polymorphism assay and DNA sequencing analysis. The differences in genotype distributions of TGF-β1-509C/T polymorphism between ESCC patients and controls were calculated by Pearson's Chi square test. We found that there was a significant difference of TGF-β1-509C/T polymorphism genotype distribution between ESCC group and control group. Associations between TGF-β1-509C/T polymorphism genotypes and ESCC risk were estimated by OR and their 95% CIs computed using unconditional logistic regression model. We found that the CC genotype, CT genotype, and the T allele carriage presented a significantly lower risk for ESCC. In conclusion, TGF-β1-509C/T polymorphism may contribute to ESCC susceptibility in Chinese population.

Disclosure of conflict of interest

None.

Address correspondence to: Qing-Guang Zhang, Department of Thoracic Surgery, Binzhou Medical College Hospital, No. 661 The second Yellow River Road, Bincheng District, Binzhou 256603, Shandong, China. Tel: 086-0543-3256544; Fax: 086-0543-3256544; E-mail: drzhangqg@126.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[3] Verrecchia F, Mauviel A. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. J Invest Dermatol. 2002; 118: 211-215.

[4] Castilla A, Prieto J, Fausto N. Transforming growth factors beta 1 and alpha in chronic liver disease. Effects of interferon alfa therapy. N Engl J Med 1991; 324: 933-940.

[5] Gu D, Zhuang L, Huang H, Cao P, Wang D, Tang J, Chen J. TGFB1 T29C polymorphism and breast cancer risk: a meta-analysis based on 10,417 cases and 11,455 controls. Breast Cancer Res Treat 2010; 123: 857-861.

[6] Zheng W. Genetic polymorphisms in the transforming growth factor-beta signaling pathways and breast cancer risk and survival. Methods Mol Biol 2009; 472: 265-277.

[7] Fujii D, Brissenden JE, Derynck R, Francke U. Transforming growth factor beta gene maps to human chromosome 19 long arm and to mouse chromosome 7. Somat Cell Mol Genet 1986; 12: 281-288.

[8] Watanabe Y, Kinoshita A, Yamada T, Ohta T, Kishino T, Matsumoto N, Ishikawa M, Niikawa N, Yoshiura K. A catalog of 106 single-nucleotide polymorphisms (SNPs) and 11 other types of variations in genes for transforming growth factor-beta1 (TGF-beta1) and its signaling pathway. J Hum Genet 2002; 47: 478-483.

[9] Wei YS, Xu QQ, Wang CF, Pan Y, Liang F, Long XK. Genetic variation in transforming growth factor-beta1 gene associated with increased risk of esophageal squamous cell carcinoma. Tissue antigens 2007; 70: 464-469.

[10] Jin G, Deng Y, Miao R, Hu Z, Zhou Y, Tan Y, Wang J, Hua Z, Ding W, Wang L, Chen W, Shen J, Wang X, Xu Y, Shen H. TGFB1 and TGFBR2

functional polymorphisms and risk of esophageal squamous cell carcinoma: a case-control analysis in a Chinese population. J Cancer Res Clin Oncol 2008; 134: 345-351.

[11] Larentzakis A, Theodorou D. A multicenter study of survival after neoadjuvant radiotherapy/chemotherapy and esophagectomy for ypT-0N0M0R0 esophageal cancer. Ann Surg 2014; 259: e67.

[12] Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. Nat Rev Cancer 2008; 8: 329-340.

[13] Smith BR, Chang KJ, Lee JG, Nguyen NT. Staging accuracy of endoscopic ultrasound based on pathologic analysis after minimally invasive esophagectomy. Am Surg 2010; 76: 1228-1231.

[14] Adjadj E, Schlumberger M, de Vathaire F. Germ-line DNA polymorphisms and susceptibility to differentiated thyroid cancer. Lancet Oncol 2009; 10: 181-190.

[15] Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R; SEARCH collaborators, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schürmann P, Dörk T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X; kConFab; AOCS Management Group, Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; 447: 1087-1093.

[16] Hunter KW. Host genetics and tumour metastasis. Br J Cancer 2004; 90: 752-755.

[17] Ling E, Robinson DS. Transforming growth factor-beta1: its anti-inflammatory and pro-fibrotic effects. Clin Exp Allergy 2002 32: 175-178.

[18] Massague J. TGFbeta in Cancer. Cell 2008; 134: 215-230.

[19] Padua D, Massague J. Roles of TGFbeta in metastasis. Cell Res 2009; 19: 89-102.

[20] Pardali K, Moustakas A. Actions of TGF-beta as tumor suppressor and pro-metastatic factor in human cancer. Biochim Biophys Acta 2007; 1775: 21-62.

[21] Bierie B, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 2006; 6: 506-520.

[22] Alqumber MA, Dar SA, Haque S, Wahid M, Singh R, Akhter N. No association of the TGF-beta1 29T/C polymorphism with breast cancer risk in Caucasian and Asian populations: evidence from a meta-analysis involving 55,841 subjects. Asian Pac J Cancer Prev 2014; 15: 8725-8734.

[23] Guo Y, Zang C, Li Y, Yuan L, Liu Q, Zhang L, Li S. Association between TGF-beta1 polymorphisms and hepatocellular carcinoma risk: a meta-analysis. Genet Test Mol Biomarkers 2013; 17: 814-820.

[24] Wang Y, Yang H, Li L, Xia X. An updated meta-analysis on the association of TGF-beta1 gene promoter -509C/T polymorphism with colorectal cancer risk. Cytokine 2013; 61: 181-187.

[25] Li K, Xia F, Zhang K, Mo A, Liu L. Association of a tgf-b1-509c/t polymorphism with gastric cancer risk: a meta-analysis. Ann Hum Genet 2013; 77: 1-8.