Gene 512 (2013) 1–5

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

# Polymorphisms of transforming growth factor beta 1 (RS#1800468 and RS#1800471) and esophageal squamous cell carcinoma among Zhuangese population, China

Ren-Guang Tang [a,*], Yong-Zhi Huang [b], Li-Min Yao [c], Jian Xiao [c], Chuan Lu [c], Qian Yu [c]

[a] Department of Laboratory Science, the Affiliated Hospital of Youjiang Medical College for Nationalities, Baise 533000, P.R. China
[b] Department of Pathology, the Affiliated Hospital of Youjiang Medical College for Nationalities, Baise 533000, P.R. China
[c] Department of Medicine (Grade 2010), Youjiang Medical College for Nationalities, Baise 533000, P.R. China

ARTICLE INFO

*Article history:*
Accepted 9 October 2012
Available online 16 October 2012

*Keywords:*
ESCC
TGF-β1
Polymorphism

ABSTRACT

Epidemiological evidence has shown two polymorphisms (namely RS#1800468G>A and RS#1800471G>C) of transforming growth factor-beta 1 (TGF-β1) gene may be involved in the cancer development. However, their role in the carcinogenic process of esophageal squamous cell carcinoma (ESCC) has been less well elaborated. We conducted a hospital-based case-control study including 391 ESCC cases and 508 controls without any evidence of tumors to evaluate the association between these two polymorphisms and ESCC risk and prognosis for Zhuangese population by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system (ARMS)-PCR techniques. We found that individuals with the genotypes with RS#1800471 C allele (namely RS#1800471-GC or -CC) had an increased risk of ESCC than those without above genotypes (namely RS#1800471-GG, adjusted odds ratio 3.26 and 5.65, respectively). Further stratification analysis showed that this polymorphism was correlated with tumor histological grades and TNM (tumor, node, and metastasis) stage, and modified the serum levels of TGF-β1. Additionally, RS#1800471 polymorphism affected ESCC prognosis (hazard ratio, 3.40), especially under high serum levels of TGF-β1 conditions. However, RS#1800468 polymorphism was not significantly related to ESCC risk. These findings indicated that TGF-β1 RS#1800471G>C polymorphism may be a genetic modifier for developing ESCC in Zhuangese population.



## 1. Introduction

Esophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of death from cancer (Parkin et al., 2005). The predominant type of esophageal cancer in Guangxi is esophageal squamous cell carcinoma (ESCC) (Hongo et al., 2009). Epidemiological studies have shown that many different environmental factors, such as tobacco smoking and alcohol consumption, are responsible for the development of ESCC (Hongo et al., 2009; Morita et al., 2010; Toh et al., 2010). Additionally, increasing evidence has shown genetic factors might play an important role in the ESCC carcinogenesis (Fukai et al., 2003; Fukuchi et al., 2004; Koliopanos et al., 2002; Wei et al., 2007; Yang et al., 2006; Zhang et al., 2010); however, despite considerable efforts in the past decades, ESCC-related genes have less well elaborated.

While transforming growth factor beta 1 (TGF-β1), an important cytokine, plays complex effects on organ development, cell growth and differentiation, expression of extracellular matrix, angiogenesis, and tissue repair (Ignotz and Massague, 1986; Mantel and Schmidt-Weber, 2011). More than one hundred polymorphisms have found in TGF-β1 gene and part of these polymorphisms might affect gene expression and tumors risk (Guan et al., 2009; Peters et al., 2008). Recently, some studies have shown that two polymorphisms of this gene (namely RS#1800468G>A and RS#1800471G>C) may be associated with dysfunction of TGF-β1 and increased tumors risk (Howell et al., 2003; Mazur et al., 2006; Nikolova et al., 2007; Peters et al., 2008; Stanczuk et al., 2002). However, the association between them and ESCC risk and prognosis has not yet been investigated. Therefore, we specifically conducted a hospital-based case-control study to examine whether these two polymorphisms influence the risk and the outcome of ESCC among Zhuangese population, an important minority from China.

*Abbreviations:* ARMS-PCR, amplification refractory mutation system- polymerase chain reaction; CI, confidence interval; ESCC, esophageal squamous cell carcinoma; OR, odds ratio; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; RS#1800471-GC/CC, genotypes with TGF-β1 RS#1800471 C alleles; RS#1800471-GG, TGF-β1 RS#1800471 GG genotype; TGF-β1, transforming growth factor-beta 1.

* Corresponding author at: Department of Laboratory Science, the Affiliated Hospital of Youjiang Medical College for Nationalities, NO. 18, Zhongshan'er Road, Baise City, Guangxi Zhuang Autonomous Region 533000, P.R. China. Tel./fax: +86 776 2848653.
*E-mail address:* trga@sina.com (R.-G. Tang).

0378-1119/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2012.10.009

## 2. Methods

### 2.1. Study population

In this hospital-based case-control analysis, we included 391 patients with newly diagnosed and histologically confirmed ESCC recruited at the Affiliated Hospital of Youjiang Medical College for Nationalities from 2008 to 2010. During the same period, the 508 controls without clinical evidence of any tumors or esophageal diseases were randomly selected from a pool of healthy volunteers who visited the general health check-up center of the same hospital. Controls were individually matched to cases based on sex and age (±5 years). All cases and controls were from Zhuang Nationality, a major minority living in Guangxi Zhuang Autonomous Region, P.R. China (Long et al., 2010). One hundred percent of people asked to participate in this study who did enroll agreed to participate in the investigate study, and no one dropped out. Each eligible participant was interviewed to obtain data regarding age, sex, ethnicity, and smoking and drinking status. The tumor, node, and metastasis (TNM) stages of Tumor were determined according to international union against cancer (UICC) standard; while the histological grades of tumor were determined by the differentiated type of tumor cells. After giving informed consent, each participant donated 2-mL peripheral blood for TGF-β1 genotypes and serum expression analysis. All ESCC patients had been followed until July 31, 2012 for survival analysis. The survival status was confirmed by patients or family contacts. In this study, the duration of survival was defined as the time from surgical resection to death or to the date on which the patient was last known to be alive. The study was approved by the hospital ethics review board, and all subjects were given informed consent before providing blood samples for DNA analysis. The protocol of the study was approved by the Ethics Committees of the hospital involved in the study.

### 2.2. DNA extraction

DNA was extracted from peripheral blood leukocytes from all cancer patients and control subjects through BloodGen Mini Kit (catalog # CW0540, Beijing CoWin Biotech Co., Ltd., Beijing) according to the manufacturer's instructions (Protocol # Version 04202010-2.2, Beijing CoWin Biotech Co., Ltd.). DNA was stored at −20 °C until additional analysis.

### 2.3. Serum expression of TGF-β1 assay

Serum expression levels of TGF- β1 were tested through MaxDiscovery™ Human/Mouse TGF-β1 ELISA Test Kit (catalog#R6831-02, Bioo Scientific Corp., TX, USA) according to the standard procedure (protocol 2129-01, Bioo Scientific Corp.). The quality control for ELISA assays was administered with negative and positive controls.

### 2.4. Genotyping

The genotypes of TGF-β1 RS#1800468G > A and RS#1800471G > C were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system (ARMS)-PCR assay (Liu et al., 2010), respectively. PCR amplified primers were as follows, (1) RS#1800468G > A, 5′-ACAGT TGGCA CGGGC TTTCG-3′ (sense) and 5′-GTCAC CAGAG AAAGA GGAC-3′ (antisense, annealing temperature, 56.2 °C); (2) RS#1800471G > C, 5′-GTGCT GACGC CTGGC CG-3′ (G sense), 5′-GTGCT GACGC CTGGC CC-3′ (C sense), and 5′-GGCTC CGGTT CTGCA CTC-3′ (antisense, annealing temperature, 58.3 °C). All primers (catalog#NSO-306597-001 to -005) were synthesized by Invitrogen Biotech (Shanghai) Ltd., Shanghai, P.R. China. PCR reactions were run in a 25-mL final volume containing 50–100 ng genomic DNA, 0.2 μM of each primer, 200 μM of each dNTP, and 0.625 unit of GoTaq® DNA polymerase (catalog#M3008, Promega Co., WI) in the GoTaq® Reaction Buffer provided by the manufacturer. PCR program initiated by 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s of annealing at 56.2 or 58.3 °C based on the primers and 1 min at 72 °C, and a final elongation step of 5 min at 72 °C. For PCR analysis of RS#1800471 genotypes, control primers that amplify β-actin were also included in each reaction to confirm the presence of amplifiable DNA in the samples and to exclude possible pseudo-negative reactions (Garcia-Closas et al., 1999).

For the genotyping analysis of RS#1800468G > A, PCR products were digested overnight with 5 units of HpyCh4IV (catalog#R0619, New England Biolabs (Beijing) LTD., Beijing, P.R. China). The digestion products were then analyzed by electrophoresis in 1% agarose gels containing ethidium bromide. For RS#1800471G > C genotyping analysis, PCR products were directly tested by electrophoresis in 2% agarose gels.

### 2.5. Statistical analysis

The Pearson's $\chi^2$ test was used to test for differences between the cases and the control subjects in the distribution of gender, age, smoking and alcohol status, and the genotypes of TGF-β1. As the study was an individually matched design, the odds ratios (ORs) with the 95% confidence intervals (CIs) were calculated as an estimate of the relative risk (by conditional logistic regression with matching factors) to evaluate the association between the risk factors and ESCC risk. Kaplan–Meier survival analysis with the log-rank test was used to evaluate the relationship between TGF-β1 polymorphisms and ESCC prognosis. Hazard ratios (HRs) and 95% CIs for risk factors were calculated with the multivariate Cox regression model with stepwise forward selection based on the likelihood ratio test. A $P$ value < 0.05 was considered statistically significant in this study. All statistical analyses were performed with SPSS version 18.0 (SPSS Institute, Chicago, IL).

## 3. Results

The clinical–pathological characteristics of the 508 controls and 391 ESCC patients included in the analysis were summarized in Table 1. There were no statistically significant differences between cases and

**Table 1**
Characteristics of study population.

| Variable | ESCC ($n$ = 391) | Controls ($n$ = 508) | $P$[a] |
|---|---|---|---|
| Age[b] | | | 0.689 |
| ≤56 years | 191 (48.85%) | 255 (50.20%) | |
| >56 years | 200 (51.15%) | 253 (49.80%) | |
| Sex | | | 0.913 |
| Male | 319 (81.59%) | 413 (81.30%) | |
| Female | 72 (18.41%) | 95 (18.70%) | |
| Smoking status | | | 0.230 |
| No | 105 (26.85%) | 155 (30.51%) | |
| Yes | 286 (73.15%) | 353 (69.49%) | |
| Drinking status | | | 0.697 |
| No | 152 (38.87%) | 204 (40.16%) | |
| Yes | 239 (61.17%) | 304 (59.84%) | |
| TNM stage[c] | | | |
| I–II | 210 (53.71%) | | |
| III–IV | 181 (46.29%) | | |
| Histological grading | | | |
| Low grade[d] | 265 (67.77%) | | |
| High grade[e] | 126 (32.23%) | | |

[a] Two-sided $\chi^2$ test.
[b] The mean ± SD ages are 55.92 ± 10.01 years and 56.48 ± 9.05 years for controls and cases, respectively.
[c] TNM stage represents tumor, node, and metastasis stage of tumor.
[d] Low grade is defined as better differentiated type of tumor cells, including well differentiated type and moderately differentiated type.
[e] High grade is defined as worse differentiated type of tumor cells, including poorly differentiated type and undifferentiated type.