



# Role of *TGFB1* polymorphism in the development of metastatic brain tumors in non-small cell lung cancer patients

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**ABSTRACT.** We conducted a case-control study to investigate the role of 3 single-nucleotide polymorphisms of the gene encoding transforming growth factor- $\beta$ 1 (*TGFB1*) in the development of metastatic brain tumors in non-small cell lung cancer patients. The polymorphisms in *TGFB1* rs4803455, rs1800469, and rs1800470 were evaluated by polymerase chain reaction-restriction fragment length polymorphism. Odds ratios and their corresponding 95% confidence intervals were used to assess the influence of *TGFB1* rs4803455, rs1800469, and rs1800470 on metastatic brain tumors. We found that cases were more likely to have a later disease stage when compared with control subjects, without brain metastasis. Individuals carrying the *TGFB1* rs1800469 TT and CT+TT genotypes had an increased risk of developing brain metastasis compared with the rs1800469 CC genotype. Moreover, a significant interaction was observed between the rs1800469 polymorphism and disease stage. However, no significant association between polymorphisms rs4803455 and rs1800470 and the risk of developing brain metastasis were observed. We found that the *TGFB1* rs1800469 polymorphism may be predictive biomarker for the risk of developing brain metastasis in non-small cell

lung cancer patients.

**Key words:** Brain metastasis; Non-small cell lung cancer; Polymorphism; *TGFBI*

## INTRODUCTION

An estimated 150,000 patients are diagnosed with brain metastases every year, and non-small cell lung cancer (NSCLC) is the most common primary site for secondary brain metastasis (Subramanian et al., 2002; Nathoo et al., 2005; Bovi and White, 2012). With advanced therapy and increased use of combined-modality therapy, the distant metastases of lung cancer have been reduced and overall survival has improved. However, overall brain failure is increasing (Gore et al., 2011). Even in patients with good performance status and controlled extra-cranial disease, the median survival time is approximately 7 months (Gaspar et al., 2000). Therefore, identifying patients at risk of developing metastatic brain tumors can lead to more effective prevention measures for these patients.

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is one type of TGF, and signaling pathways of TGF- $\beta$  can influence cell proliferation, morphogenesis, migration, extracellular matrix production, and apoptosis. Previous studies have shown that TGF- $\beta$  can suppress early-stage tumor development through its potent growth inhibitory effects, but that it is a pro-oncogenic factor that stimulates tumor cell growth and invasiveness during end-stage tumorigenesis (Javelaud et al., 2008; Massagué, 2008). Tumor cells can escape the antiproliferative action of TGF- $\beta$  by developing mutations in components of relevant signaling pathways or by disrupting TGF- $\beta$  signaling. Therefore, TGF- $\beta$  expression may play a role in the metastatic process.

In our study, we examined whether common genetic polymorphisms of TGF- $\beta$  pathways play a role in maintaining cellular function and their effect on distant metastasis. We conducted a case-control study to investigate the role of 3 single-nucleotide polymorphisms (SNPs) of *TGFBI* in the development of metastatic brain tumors in NSCLC patients.

## MATERIAL AND METHODS

### Patient population

A total of 176 subjects in this study were selected from Tangshan Gongren Hospital between January 2007 and December 2009. All cases with NSCLC were pathologically diagnosed. All patients had at least a single brain metastasis of NSCLC and subsequently underwent either brain radiotherapy or local brain radiotherapy. A total of 198 patients with NSCLC and without brain metastasis were included as a control group. Patients with any other malignant tumor history within the past 5 years, pregnancy or lactation, cardiopulmonary insufficiency, serious cardiovascular disease, and serious infection as well as severe malnutrition were excluded from the study.

Demographic and clinical data were obtained from medical records, including gender, age, disease stage, tumor histology, and smoking and drinking habits. The disease stage of patients was classified based on the tumor/node/metastasis (TNM) system according to the American Joint Committee on Cancer staging manual. The diagnosis of brain metastasis was conducted based on computed tomography and magnetic resonance imaging records. Smok-

ing habit was classified into never and ever smokers. Individuals who smoked 20-50 packs of cigarettes per year, or smoked more than 1 cigarette per day continuously for 6 months were regarded as smokers. The drinking habit was classified as never and ever drinkers. Subjects who drank more than 50 g alcohol per week continuously for 6 months were regarded as drinkers.

### Genotype analysis

Each case and control subject provided 5 mL venous blood. Ethylenediaminetetraacetic acid (1.5-2.2 mg/mL) was used as an anticoagulant during blood sample storage. DNA was extracted using the TIANamp blood DNA kit (Tiangen Biotech; Beijing, China) according to manufacturer instructions. The *TGFB1* polymorphisms rs4803455, rs1800469, and rs1800470 were analyzed by polymerase chain reaction (PCR)-restriction fragment length polymorphism. The primers used for *TGFB1* rs4803455, rs1800469, and rs1800470 were designed using the Sequenom Assay Design 3.1 software (San Diego, CA, USA). Each PCR mix was conducted in a total volume of 20  $\mu$ L and included 50 ng genomic DNA, 200  $\mu$ M dNTPs, and 2.5 U *Taq* DNA polymerase as well as 200  $\mu$ M primers. The PCR amplification was started with preliminary denaturation at 95°C for 10 min to activate the *Taq* polymerase, and consisted of 45 cycles (95°C for 5 s and 60°C for 30 s), with a final extension at 72°C for 3 min.

### Statistical analysis

All statistical analyses were conducted using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows. Continuous variables are expressed as means  $\pm$  SD, while categorical variables are expressed as N of subjects (%). Continuous variables and categorical variables were analyzed using the independent sample *t*-test or  $\chi^2$ -test, respectively. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to assess the influence of *TGFB1* rs4803455, rs1800469, and rs1800470 on metastatic brain tumors. The homozygous genotypes for the 3 SNPs were used as references. All comparisons were 2-sided, and  $P < 0.05$  was considered to be statistically significant.

## RESULTS

A total of 176 cases and 198 control subjects were enrolled in our study. The general characteristics of all subjects are summarized in Table 1. No significant difference was found between cases and controls in terms of gender, age, smoking, and drinking habits ( $P > 0.05$ ). Cases were more likely to have a higher disease stage compared with control subjects.

In the conditional regression analysis, we found that individuals carrying the *TGFB1* rs1800469 TT genotype had an increased risk of developing brain metastasis compared with the rs1800469 CC genotype, with adjusted ORs (95%CI) of 1.76 (1.12-3.15) (Table 2). Similarly, we found that subjects carrying the rs1800469 CT+TT genotypes had a slightly increased risk of developing brain metastasis, and the adjusted OR (95%CI) was 1.46 (95%CI = 1.03-2.53) after adjusting for gender, age, smoking and drinking habit, and disease stage. However, no significant association between polymorphisms in rs4803455 and rs1800470 and the risk of developing brain metastasis was observed.

We further analyzed the interaction between the *TGFB1* rs1800469 polymorphism and disease stage on the risk of developing brain metastasis (Table 3). Individuals with rs1800469

TT showed a significant association with a greatly increased risk of developing brain metastasis among patients with disease stage at IIIA-IV (OR = 2.58, 95%CI = 1.19-5.67). Subjects carrying the rs1800469 CT+TT genotypes were associated with a moderately increased risk of developing brain metastasis. Moreover, a significant interaction was found between the rs1800469 polymorphism and disease stage (P = 0.03).

**Table 1.** Demographic and clinical characteristics of cases and controls.

Characteristics	Patients	%	Control	%	$\chi^2$	P value
Gender						
Male	45	25.57	57	28.79		
Female	131	74.43	141	71.21	0.49	0.48
Age						
<60	73	41.48	86	43.43		
≥60	103	58.52	112	56.57	0.15	0.71
Smoking habit						
No	80	45.45	96	48.48		
Yes	96	54.55	102	51.52	0.34	0.56
Drinking habit						
No	107	60.80	126	63.64		
Yes	69	39.20	73	36.87	0.25	0.62
Disease stage at diagnosis						
I-II	84	47.73	115	58.08		
IIIA-IV	92	52.27	83	41.92	4.01	0.05

**Table 2.** Association between *TGFBI* genotypes and brain metastasis.

TGFBI	Case	%	Control	%	OR (95%CI) <sup>1</sup>	P value	OR (95%CI) <sup>2</sup>	P value
rs4803455								
AA	75	42.5	90	45.3	1.0 (Ref.)	-	1.0 (Ref.)	-
AC	67	37.9	74	37.5	1.09 (0.68-1.75)	0.72	1.27 (0.74-1.95)	0.53
CC	34	19.6	34	17.2	1.2 (0.65-2.20)	0.53	1.32 (0.81-2.45)	0.43
AC+CC	101	57.5	108	54.7	1.12 (0.73-1.73)	0.58	1.26 (0.91-2.05)	0.26
rs1800469								
CC	69	39.1	92	46.3	1.0 (Ref.)	-	1.0 (Ref.)	-
CT	59	33.7	64	32.5	1.23 (0.75-2.02)	0.39	1.36 (0.89-2.27)	0.21
TT	48	27.2	42	21.2	1.53 (0.89-2.65)	0.11	1.76 (1.12-3.15)	0.04
CT+TT	107	60.9	106	53.7	1.35 (0.87-2.08)	0.16	1.46 (1.03-2.53)	0.09
rs1800470								
CC	68	38.5	84	42.3	1.0 (Ref.)	-	1.0 (Ref.)	-
CT	73	41.2	80	40.4	1.13 (0.70-1.81)	0.60	1.37 (0.87-2.02)	0.27
TT	36	20.3	34	17.3	1.31 (0.71-2.40)	0.35	1.47 (0.84-2.83)	0.15
CT+TT	109	61.5	114	57.7	1.18 (0.76-1.83)	0.43	1.26 (0.84-2.07)	0.26

<sup>1</sup>Not adjusted. <sup>2</sup>Adjusted for gender, age, smoking and drinking habit and disease stage.

**Table 3.** Interaction between *TGFBI* rs1800469 polymorphism and disease stage on the risk of developing brain metastasis.

<i>TGFBI</i>	I-II		OR (95%CI) <sup>1</sup>	P value	IIIA-IV		OR (95%CI) <sup>1</sup>	P value
	Case	Control			Case	Control		
rs1800469								
CC	36	53	1.0 (Ref.)	-	33	39	1.0 (Ref.)	-
CT	35	38	1.52 (0.74-2.78)	0.25	24	26	1.17 (0.62-2.55)	0.56
TT	14	24	1.06 (0.46-2.36)	0.70	34	18	2.58 (1.19-5.67)	0.01
CT+TT	49	62	1.24 (0.71-2.15)	0.62	58	44	1.73 (1.02-3.46)	0.03

<sup>1</sup>Not adjusted.

## DISCUSSION

Characterization and identification of genes involved in the genetic predisposition or progression of cancer are critical for both clinical practice and basic medical research. Because the expression of *TGFBI* may have a role in the metastatic process, numerous studies have shown that *TGFBI* polymorphisms were associated with the prognosis and metastasis of various cancers such as breast, lung, oral, and rectal cancers (Gaur et al., 2011; Slattery et al., 2011; Yuan et al., 2013; Wendt et al., 2014). Only 2 studies reported an association between *TGFBI* polymorphisms and risk of brain metastasis (Li et al., 2012; Yuan et al., 2013). However, the results were inconsistent. Therefore, we investigated the influence of *TGFBI* polymorphisms on the risk of developing metastasis.

In the present study, we found that the *TGFBI* rs1800469 polymorphism was associated with the risk of developing brain metastasis in Chinese NSCLC patients. However, the *TGFBI* rs4803455 and rs1800470 polymorphisms were not associated with the risk of brain metastasis. In addition, in stratified analyses based on disease stage, a significant association was found between the *TGFBI* rs1800469 polymorphism and disease stage.

It is well-known that members of the TGF- $\beta$  cytokine superfamily showed a diverse range of cellular responses, such as proliferation, migration, fibrosis, apoptosis, tissue inflammation, wound repair, and angiogenesis (Massagué, 1998; Roberts, 1999; Massagué and Gomis, 2006). Changes in TGF- $\beta$  signaling have been reported to have an effect on the prognosis of NSCLC (Kang et al., 2000; Finger et al., 2008). For TGF- $\beta$  isoforms, *TGFBI* signaling downregulates the migration of tumor cells and suppresses the development of metastasis (Saunier and Akhurst, 2006; Tsuchida et al., 2006). Thus, TGFBI may play a crucial role in cancer development and the suppression of tumor growth during the initial phases of carcinogenesis, while it plays a role in promoting tumor progression and metastasis during more advanced stages (Derynck et al., 2001; Wakefield and Roberts, 2002). *TGFBI* polymorphisms have been used as predictive biomarkers in several cancers (Gaur et al., 2011; Slattery et al., 2011; Li et al., 2012; Yuan et al., 2013; Wendt et al., 2014). Yuan et al. (2013) conducted a study in China and found that *TGFBI* genotypes at rs1800469 and rs1982073 can be used as predictive markers for the effectiveness of chemotherapy and prognosis of NSCLC. Gaur et al. (2011) reported that the *TGFBI* functional genetic polymorphism at 869 T>C was associated with risk of oral cancer in Asian Indians. For brain metastasis in patients with NSCLC, Li et al. (2012) reported a study conducted in China and found no significant association between *TGFBI* polymorphisms and brain metastasis. The discrepancy in these results may be explained by the differences in ethnicities, source of control subjects, and sample sizes. Further studies are needed to confirm the association between *TGFBI* polymorphisms and risk of brain metastasis.

In conclusion, we found that the *TGFBI* rs1800469 polymorphism was statistically associated with the risk of developing brain metastasis in NSCLC patients, and has an interaction with disease stage. However, no association was found between polymorphisms in rs4803455 and rs1800470 and the risk of brain metastasis. The rs4803455 and rs1800470 polymorphisms may be used as predictive markers for the development of brain metastasis. A larger sample study is necessary to confirm the role of *TGFBI* polymorphisms in the development of brain metastasis.

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