DNA methyltransferase 3B -149C/T polymorphism and the risk of laryngeal squamous cell carcinoma: a case-control study

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ABSTRACT. A variety of molecular epidemiological studies have been conducted to examine the association between the *DNMT3B* -149C/T polymorphism and cancer susceptibility; however, there has been no study investigating the association between the *DNMT3B* -149C/T polymorphism and the risk of laryngeal squamous cell carcinoma (LSCC) until now. To determine the role of the *DNMT3B* -149C/T polymorphism in LSCC, we genotyped 113 patients with LSCC and 110 controls from a Chinese population using polymerase chain reaction-restriction fragment length polymorphism analysis. The chi-square test was used to examine differences in the distributions of genotypes studied between patients and controls. The association between the *DNMT3B* -149C/T polymorphism and the risk of LSCC was estimated using ORs and their 95%CIs. Genotypic frequencies in the patients with LSCC were not similar to those of the controls, with the differences being statistically significant (P =

0.001). When the *DNMT3B* -149 CC genotype was used as the reference group, the CT genotype was not associated with LSCC risk (adjusted OR, 2.12; 95%CI = 0.89-5.19; P = 0.07), but the TT genotype was associated with significantly increased risk for LSCC (adjusted OR = 3.27; 95%CI = 1.79-10.66; P = 0.009). Under the recessive model of inheritance, the TT genotype was associated with significantly increased risk for LSCC (adjusted OR = 1.98; 95%CI = 1.12-5.95; P = 0.012), compared with other genotypes. These results suggested that the *DNMT3B* -149C/T polymorphism is associated with a genetic susceptibility for developing LSCC in a Chinese population.

Key words: *DNMT3B* -149C/T polymorphism; Genetic susceptibility; Risk; LSCC

INTRODUCTION

Laryngeal squamous cell carcinoma (LSCC) is the most frequent type of head and neck cancer. The risk of LSCC results from complex interactions between numerous genetic and environmental factors (Hashibe et al., 2007). Effective treatments include radiotherapy and chemotherapy, although surgery is currently the only treatment that consistently prolongs survival (Moyer et al., 2004). The most effective approaches to achieving an improved prognosis in patients with LSCC are prevention and early diagnosis. Furthermore, accumulating evidence indicates that genetic polymorphisms are associated with laryngeal carcinoma (Boccia et al., 2008).

The *DNMT3B* gene is located on chromosome 20q11.2 and contains a number of single-nucleotide polymorphisms (SNPs) that have been described in the literature, including a single C to T transition polymorphism (C46359T) in the promoter region, 149 base pairs upstream of the transcription start site (-149C/T, rs2424913). This SNP has been shown to result in greatly increased promoter activity of the gene (Shen et al., 2002; Zhu et al., 2015). Recently, a variety of molecular epidemiological studies have been conducted to examine the association between the *DNMT3B* -149C/T polymorphism and cancer susceptibility (Meng et al., 2014); however, there has been no study investigating the association between the *DNMT3B* -149C/T polymorphism and the risk of LSCC until now.

MATERIAL AND METHODS

Patients and controls

The study protocol was approved by the Medical Research Council of Union Hospital, Huazhong University of Science and Technology. Informed consent was obtained from each participating patient and healthy individual. Between June 2009 and May 2014, 113 patients with LSCC were enrolled in the study at the Department of Otorhinolaryngology, Union Hospital, Huazhong University of Science and Technology. All patients were of Han Chinese origin and were recruited from various geographical regions of China. Healthy volunteers of equivalent ethnicity, gender, and age were enrolled as the control group (N = 110). The clinicopathological findings of the cancer group were collected. All the cell type pathologies in the cancer group were of squamous cell carcinoma. The characteristics of the various subgroups are shown in Table 1.

DNA extraction and *DNMT3B* genotyping

A 5 mL sample of venous blood was drawn from each subject into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at 4°C. Genomic DNA was extracted within one week after sampling using proteinase K digestion followed by a salting-out procedure. The C/T transition of the *DNMT3B* SNP creates a *Bln*I restriction site, which can be exploited for genotyping by polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP) analysis. PCR was performed in a 25 µL volume containing 100 ng DNA template, 10X PCR master mix (Promega, Madison, WI, USA), and 10 pM each sense (5' - TGC TGT GAC AGG CAG ATG CAG - 3') and antisense (5' - GGT AGC CGG GAA CTC CAC GG - 3') primers. For PCR amplification, an initial denaturation step at 94°C for 5 min was followed by 30 cycles at 94°C for 30 s, 57°C for 30 s, and 72°C for 30 s, with a final extension step at 72°C for 7 min. Subsequently, the PCR products were digested with BlnI overnight at 37°C. RFLP bands were visualized by ethidium bromide staining under UV light. Assessment of the -149C>T polymorphism was dependent upon the existence of the *Bln*I recognition site; thus the *DNMT3B* T/T genotype was expected to show two DNA bands at the positions of 207 and 173 bp, the C/C genotype was expected to show a single band (380 bp), and the heterozygote was expected to have three bands (380, 207, and 173 bp).

Statistical analysis

A chi-square test was used to examine differences in the distributions of genotypes studied between patients and controls. The association between the *DNMT3B* -149C/T polymorphism and the risk of LSCC was estimated using ORs and their 95%CIs. ORs were adjusted for age, sex, and drinking and smoking status, as appropriate. A P value of less than 0.05 was used as the criterion of statistical significance, and all statistical tests were two-sided. All analyses were performed with the SPSS software package (version 18.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

The baseline clinical characteristics of the patients with LSCC and the control subjects are summarized in Table 1. No statistically significant differences were observed between patients and controls in terms of age (P = 0.473) or sex distributions (P = 0.679). There were more smokers among patients with LSCC than among healthy controls (92.04 *vs* 68.18%; P < 0.001). Similarly, more drinkers were found among patients with LSCC compared with control subjects (87.61 *vs* 70.91%; P = 0.003).

Genotypic frequencies of *DNMT3B* −149C/T in patients with LSCC and controls

The observed genotype distributions in the controls did not differ from those expected from Hardy-Weinberg equilibrium (P > 0.05). Compared to healthy controls, patients with LSCC had a lower frequency of the CC genotype (35.40 *vs* 60.91%) and a higher frequency of CT (33.63 *vs* 20.00%). The homozygous TT genotype was found in 35 patients with LSCC and in 21 controls. Thus, genotypic frequencies in the patients with LSCC were not similar to those of the controls, with the differences being statistically significant (P = 0.001, shown in Table 2).

Table 1. Baseline clinical characteristics of patients with LSCC and control subjects.

Variables	Cases [N = 113) (%)]	Controls [N = 110 (%)]	P value
Gender			
Female	45 (39.82)	40 (36.36)	0.679
Male	68 (60.18)	70 (63.64)	
Age (years)			
≥60	71 (62.83)	77 (70.00)	0.473
<60	39 (34.51)	33 (30.00)	
Smoking			
No	9 (7.96)	35 (31.82)	<0.001
Yes	104 (92.04)	75 (68.18)	
Alcohol consumption			
No	14 (12.39)	32 (29.09)	0.003
Yes	99 (87.61)	78 (70.91)	
Tumor stage at diagnosis			
I	25 (22.12)		
II	37 (32.74)		
III	40 (35.40)		
IV	11 (9.73)		
Degree of differentiation			
Well differentiated	38 (33.63)		
Moderate differentiated	44 (38.94)		
Poor differentiated	31 (27.43)		

Table 2. Frequency distribution of DNMT3b-149C>T genotypes.

DNMT3b-149C>T	Cases	%	Controls	%	P value
CC	40	35.40	67	60.91	0.001
CT	38	33.63	22	20.00	
TT	35	30.97	21	19.09	

DNMT3B −149C/T polymorphism and the risk of LSCC

When the *DNMT3B* -149 CC genotype was used as the reference group, the CT genotype was not associated with LSCC risk (adjusted OR, 2.12; 95%CI = 0.89-5.19; P = 0.07), but the TT genotype was associated with significantly increased risk for LSCC (adjusted OR = 3.27; 95%CI = 1.79-10.66; P = 0.009). Under the recessive model of inheritance, the TT genotype was associated with significantly increased risk for LSCC (adjusted OR = 1.98; 95%CI = 1.12-5.95; P = 0.012, shown in Table 3) compared with other genotypes after adjustment for age, sex, smoking, and alcohol use in the multivariate logistic regression analysis.

Table 3. The association of DNMT3b-149C>T polymorphism with LSCC risk.

DNMT3b-149C>T polymorphism	LSCC patients	Controls	OR (95%CI)[1]	P value
General genotype				
CC	40	67	1.00 (Reference)	
CT	38	22	2.12 (0.89-5.19)	0.076
TT	35	21	3.27 (1.79-10.66)	0.009
Dominant genotype				
CC	40	67	1.00 (Reference)	
CT+TT	73	43	3.19 (0.92-9.27)	0.043
Recessive genotype				
CC+CT	78	89	1.00 (Reference)	
TT	35	21	1.98 (1.12-5.95)	0.012
Allele frequency				
C	118	156	1.00 (Reference)	
T	108	64	2.08 (0.92-8.26)	0.048

[1]Adjusted for sex, age, smoking status, and drinking status.

DISCUSSION

SNPs are the most common form of human genetic variation, and might contribute to an individual's susceptibility to cancer. Some studies have suggested that certain variants in the promoter regions of genes might affect either their expression or activity levels of DNA modification enzymes, and therefore might be mechanistically associated with cancer risk (Skoog et al., 1999; Momparler and Bovenzi, 2000; Shen et al., 2002). DNA methylation is a major epigenetic modification involving the addition of a methyl group to the 5' position of a cytosine in a CpG dinucleotide. A number of studies have suggested that aberrant DNA cytosine methylation might play an important role in carcinogenesis (Cooper and Youssoufian, 1988). DNMT3A and DNMT3B are required for the establishment and maintenance of genomic methylation patterns and for proper murine development (Bachman et al., 2001; Robertson, 2001; Gowher and Jeltsch, 2002). Recently, several studies have shown that certain SNPs in the *DNMT3B* gene might influence the activity of DNMT3B with respect to DNA methylation, thereby modulating the susceptibility to cancer.

The *DNMT3B* gene contains a single C to T transition polymorphism within the promoter region, 149 base pairs upstream from the transcription start site, which might result in greatly increased promoter activity of the gene (Shen et al., 2002). Recently, a variety of molecular epidemiological studies have been conducted to examine the association between the *DNMT3B* -149C/T polymorphism and cancer susceptibility. For example, Montgomery et al. (2004) investigated the *DNMT3B* -149C/T promoter polymorphism and the risk of breast cancer in a British population. They found that the C allele was more common in patients than in control subjects (patients, 0.59; controls, 0.54). Their study also suggested that there might be an association between the C allele and women with early-onset breast cancer, bilateral breast cancer, or with a family history of the disease. In a study by Lee et al. (2005), the *DNMT3B* genotype was determined in 432 patients with lung cancer and 432 healthy controls that were frequency-matched for age and sex. Individuals with at least one T allele were found to be at a significantly decreased risk of adenocarcinoma and small cell carcinoma (OR = 0.48; 95%CI = 0.28-0.82; P = 0.007; and adjusted OR = 0.47; 95%CI = 0.24-0.93; P = 0.03, respectively) compared with those harboring a CC genotype. Wang et al. (2005) investigated the SNP in the promoter of the *DNMT3B* gene and the risk for development and lymphatic metastasis of gastric cardiac adenocarcinoma (GCA). The C/C genotype was not detected in either patients with GCA or in controls. In control subjects, the frequencies of the T/T and C/T genotypes were 94.9 and 5.1% respectively, and those of the T and C alleles were 97.4 and 2.6%, respectively. The genotype and allelic distributions in the patients with GCA were not significantly different from those in the controls (P = 0.34 and 0.33, respectively). When stratified by smoking status and family history of upper gastrointestinal cancer, significant differences in the genotype distributions were not observed between patients with GCA and controls. In addition, the distributions of *DNMT3B* genotypes in patients with GCA with or without lymphatic metastasis did not show significant differences (P = 0.42). However, there has been no study investigating the association between the *DNMT3B* -149C/T polymorphism and the risk of LSCC until now.

In the present study, to determine the role of the *DNMT3B* -149C/T polymorphism in LSCC, we genotyped 113 patients and 110 controls from a Chinese population by PCR-RFLP. Compared to healthy controls, patients with LSCC had a lower frequency of the CC genotype and a higher frequency of CT. The homozygous TT genotype was found in 35 patients with LSCC and in 21 controls. Thus, genotypic frequencies in the patients with LSCC were not similar to those of the controls, with the differences being statistically significant. When the *DNMT3B* -149 CC genotype was used as the reference group, the CT genotype was not associated with LSCC risk, but the TT

genotype was associated with significantly increased risk for LSCC. Under the recessive model of inheritance, the TT genotype was associated with significantly increased risk for LSCC compared with other genotypes after adjustment for age, sex, smoking, and alcohol use in the multivariate logistic regression analysis.

In conclusion, the *DNMT3B* -149C/T polymorphism was found to be associated with a genetic susceptibility of developing LSCC in a Chinese population. More studies are needed to confirm this association.

Conflicts of interest

The authors declare no conflict of interest

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