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Functional polymorphisms in *FAS* and *FASL* contribute to risk of squamous cell carcinoma of the larynx and hypopharynx in a Chinese population

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ABSTRACT

Accumulating evidences indicate that the functional *FAS*−1377G > A, −670A > G and *FASL*−844T > C polymorphisms affect the risk of several kinds of cancers. However, their roles in the development of larynx and hypopharynx squamous cell carcinoma (SCC) were still unknown in the Chinese. In the current study, we examined whether these functional genetic variants were associated with the risk of larynx and hypopharynx squamous SCC in a Han Chinese population. The *FAS* and *FASL* polymorphisms were genotyped in 300 patients with laryngeal and hypopharyngeal SCC and 300 control subjects by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Logistic regression analysis revealed that subjects carrying the *FASL*–844CT or TT genotype had a significantly decreased risk of developing laryngeal and hypopharyngeal SCC [odds ratio (OR) = 0.69; 95% confidence interval (CI) = 0.51–0.93; $P = 0.016$; or, OR = 0.41; 95% CI = 0.20–0.86; $P = 0.009$] compared with those carrying the CC genotype. Joint gene-smoking and gene-drinking effects were also observed, with the OR of CC genotype for smokers or drinkers were 5.15 (95%CI = 3.24–8.97) or 12.52 (95%CI = 7.31–22.47), respectively. Therefore, the *FASL*−844T > C polymorphism is associated with genetic susceptibility of developing laryngeal and hypopharyngeal SCC in a Han Chinese population.

1. Introduction

As the fifth most common cancer in human being, squamous cell carcinoma (SCC) of the larynx and hypopharynx is a heterogeneous group of malignant tumors which arise from the epithelium of the upper aerodigestive tract (Sankaranarayanan et al., 1998; Tai et al., 2010). Epidemiology studies have shown that larynx and hypopharynx transformation is associated with high-dose of tobacco smoking (Ho et al., 2007; Hsu et al., 1991). However, not all smokers develop laryngeal and hypopharyngeal SCC during their normal life span, suggesting that individual genetic background may also play an important role in the etiology of this malignancy.

Accumulating evidences have suggested that the aberrant regulation of apoptosis contributes to many types of human diseases including cancer (Evan and Vousden, 2001; Lowe and Lin, 2000). FAS and FAS ligand (FASL) molecules play an important role in immune escape of tumor cells during carcinogenesis (Griffith and Ferguson, 1997). There was decreased FAS expression but elevated FASL expression in many malignancies including laryngeal and hypopharyngeal SCC (Reimer et al., 2000; Riedel et al., 2001). It has been found that cancer cells may counterattack FAS- mediated T cell killing using heightened expressed FASL and this mechanism may lead to tumor cell immune privilege (Ji et al., 2011; O'Connell et al., 1996). Based on these findings, it was also supposed that FASL gene therapy may provide a new efficient therapeutic modality for laryngeal and hypopharyngeal SCC which is worthy of a clinical trial (ElOjeimy et al., 2006).

Three functional promoter single nucleotide polymorphisms (SNPs) of *FASL* and *FAS* genes have been associated to the differential expression of these two genes. The *FAS*−1377G > A and *FAS*−670A > G polymorphisms locate in the binding sites for transcription factors Sp1 and STAT1, respectively. Since the *FAS*−1377G > A and *FAS*−670A > G polymorphisms could destroy binding elements for transcription factors Sp1 and STAT1, these SNPs have been associated

Abbreviations: SCC, squamous cell carcinoma; OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

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with decreased FAS expression (Huang et al., 1997; Sibley et al., 2003). For *FASL* gene, there is a −844T > C SNP in a binding motif for transcription factor CAAT/enhancer-binding protein β, and a considerably higher basal expression of FASL is associated with the *FASL*–844C allele compared with the–844T allele (Wu et al., 2003). The *FAS* and *FASL* SNPs associated with gene expression changes may lead to alterations in FAS and FASL-mediated apoptosis and, thus, individual susceptibility to cancers, such as laryngeal and hypopharyngeal SCC (Zhang et al., 2007).

It has shown that the *FAS*−1377G > A, −670A > G and *FASL*−844T > C SNPs are associated with increased risk to develop several cancers including breast cancer (Zhang et al., 2007), esophageal cancer (Sun et al., 2004), lung cancer (Zhang et al., 2005), pancreatic cancer (Yang et al., 2008) and cervical cancer (Sun et al., 2005; Ueda et al., 2005) in different populations, suggesting that genetic variations in the death pathway may confer susceptibility to common cancers. Although Zhang et al. has examined the impact of *FAS*−1377G > A, −670A > G and *FASL*−844T > C polymorphisms on squamous cell carcinoma of the head and neck (SCCHN) (including those of the oral cavity, pharynx, and larynx) in US populations (Zhang et al., 2006), there is no report on the role of these genetic variations in laryngeal and hypopharyngeal SCC in Chinese populations. In the current study, we investigated the association between the functional *FAS* and *FASL* SNPs and the risk of developing laryngeal and hypopharyngeal SCC using a case-control study in a Chinese population. It was found that *FASL*−844T > C polymorphism is significantly associated with risk of laryngeal and hypopharyngeal SCC, alone and in combination with environmental risk-factors.

2. Materials and methods

2.1. Study subjects

The case-control study population consisted of 300 patients with laryngeal and hypopharyngeal SCC and 300 cancer-free control subjects. All subjects were unrelated ethnic Han Chinese. Patients were consecutively recruited between October 2009 and February 2011 at the Shandong Cancer Hospital, Shandong Academy of Medical Sciences (Jinan, Shandong Province), and between March 2010 and May 2011 at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing), with response rates of 96 and 97%, respectively. There were no restrictions of age, sex, or cancer stage. All cases were newly diagnosed and previously untreated. Tumors were staged according to the American Joint Committee on Cancer staging criteria and histologically classified according to the World Health Organization classification (Tai et al., 2010). Control subjects were randomly selected from a pool of 3000 individuals based on a physical examination; the response rate was 93%. The selection criteria included no individual history of cancer and frequency matching to cases by sex and age (±5 years). Smokers were identified if they smoked up to one year before cancer diagnosis or if they smoked up to one year before the interview for control subjects. Subjects who never smoked or smoked less than one year before cancer diagnosis for case patients or interview for control subjects were defined as nonsmokers (Yang et al., 2007, 2008, 2009). Participants were classified as drinkers if they consumed alcohol at least twice a week (Tai et al., 2010; Yang et al., 2008). At recruitment, each participant was personally interviewed to obtain detailed information on demographic characteristics and lifetime history of tobacco and alcohol use. The study was approved by the Institutional Review Boards of Shandong Cancer Hospital (Shandong Academy of Medical Sciences) and Cancer Hospital (Chinese Academy of Medical Sciences).

2.2. Polymorphism genotyping

Genomic DNA was isolated from peripheral blood lymphocytes of control subjects and laryngeal and hypopharyngeal SCC patients using standard procedures, as described previously (Sun et al., 2004, 2005; Yang et al., 2007, 2008, 2009, 2011). Genotypes of *FAS*–1377G/A, *FAS*–670A/G, and *FASL*–844T/C polymorphisms were determined by PCR-based restriction fragment length polymorphism (RFLP) assays as described previously (Sun et al., 2004; Yang et al., 2008). Briefly, PCR was performed with a 25-μL reaction mixture containing 100 ng of DNA, 0.1 mmol/L of each primer, 0.2 mmol/L of deoxynucleoside triphosphate, 1.0 U of Taq DNA polymerase (TaKaRa, Dalian, China), 1 × reaction buffer, and 1.5 mmol/L $MgCl_2$. The PCR profile consisted of an initial melting step of 2 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C (for *FAS* –1377G/A SNP), 61 °C (for the *FAS* –670A/G SNP), or 57 °C (for the *FASL*–844 T/C SNP), 45 s at 72 °C, and a final elongation step of 7 min at 72 °C. The restriction endonucleases *Bst*UI, *Scr*FI and *Bsr*DI (New England Biolabs, Beverly, MA) were used to distinguish *FAS*–1377G > A, *FAS*–670A > G, and *FASL*–844 T > C polymorphisms, respectively. The results of RFLP analyses were: *FAS*–1377G allele, fragments of 104 bp and 18 bp; *FAS*–1377A allele, a single fragment of 122 bp; *FAS* –670A allele, a single fragment of 193 bp; *FAS*–670G allele, fragments of 136 bp and 57 bp; *FASL* –844C allele, fragments of 233 bp and 168 bp, and *FASL*–844 T allele, a single fragment of 401 bp. A 10% masked, random sample of subjects was tested twice by different persons and the results were concordant for all of the masked duplicate sets.

2.3. Statistics

Chi-square test was used to examine differences in the distributions of genotypes studied between cases and controls. The association between *FAS* and *FASL* genotypes and the risk of laryngeal and hypopharyngeal SCC was estimated using odds ratios (ORs) and their 95% confidence intervals (CIs), which were computed by unconditional logistic regression. ORs were adjusted for age, sex, and drinking and smoking status, where it was appropriate. A *P* value of less than 0.05 was used as the criterion of statistical significance, and all statistical tests were two-sided. We tested the null hypotheses of multiplicative gene-environment interaction and evaluated departures from multiplicative interaction models (Yang et al., 2008, 2009) by including main effect variables and their product terms in the logistic regression model. All analyses were performed with Statistical Analysis System (version 9.0; SAS Institute, Cary, NC) and SPSS software package (version 16.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Subject characteristics

The baseline clinical characteristics of patients with laryngeal and hypopharyngeal SCC and control subjects are summarized in Table 1. No statistically significant differences were observed between cases and controls in terms of age and sex distributions ($P = 0.861$ and $P = 0.276$, respectively). There were more smokers among patients with laryngeal and hypopharyngeal SCC than healthy controls (80.5% vs. 52.4%; $P < 0.001$). Similarly, more drinkers were found among cases, compared with control subjects (70.3% vs. 21.9%; $P < 0.001$). Among these 300 patients, 224 (74.6%) had laryngeal cancer and 76 (25.4%) had pharyngeal cancer.

3.2. Allele and genotype distribution

As shown in Table 2, the respective allele frequencies for the *FAS*–1377A, –670G and *FASL*–844C were 33.3, 36.5 and 30.3%, respectively, in controls, and 30.5, 35.5 and 22.3% in laryngeal and hypopharyngeal SCC patients. All observed genotype frequencies were consistent with Hardy–Weinberg equilibrium (HWE). Distributions of these genotypes were then compared among cases and controls. The frequencies of the *FASL* –844CC, CT, and TT genotypes in cases differed significantly from those in controls ($\chi^2 = 9.76$, $P < 0.05$,