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Short Communication

Association between 14bp Insertion/Deletion Polymorphism in Exon 8 of *HLA-G* gene and Oral Squamous Cell Carcinoma in Korean Population

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Abnormal *HLA-G* expression occurs in various diseases such as melanoma, renal cell carcinoma, asthma, and classic Hodgkin's lymphoma. The purpose of this study was to determine whether *HLA-G* gene is linked with oral squamous cell carcinoma (OSCC). To investigate the possible link with susceptibility to OSCC, 54 OSCC patients and 120 healthy controls were enrolled in this study. *HLA-G* 14bp insertion/deletion polymorphism is in 3'-untranslated region of *HLA-G* gene. *HLA-G* 14bp insertion/deletion polymorphism was analyzed using the polymerase chain reaction (PCR) method. For the analysis of genetic data, SPSS18.0 program was used. Logistic regression models were performed for odds ratio (OR), 95 percent confidence interval (CI), and *P* value. There was a significant difference in distribution allele between OSCC patients and control subjects (OR=0.018, 95% CI=0.002-0.131, $p<0.001$). Our results suggest that *HLA-G* 14bp insertion/deletion polymorphism may be linked with susceptibility to OSCC in the Korean population.

Key words: association study, oral squamous cell carcinoma, OSCC, *HLA-G* 14bp polymorphism

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Introduction

The 1.4% of total cancer developed in Korea is oral cancer and oral squamous cell carcinoma (OSCC) consists of more than 90% of malignancy in oral cavity (National Cancer Information Center, Republic of Korea, http://www.cancer.go.kr).

Human leukocyte antigen-G (*HLA-G*) is a non-classical HLA class I molecule [1]. Many studies showed that HLA-G involved in the immuno suppressive response and long-term immune escape or tolerance [2]. Level of HLA-G is also significantly higher in patients with various tumors such as malignant melanoma, glioma, breast cancer, ovarian cancer, hepatocellular carcinoma and so on [3-6]. One of the characteristics of the HLA-G gene is that there is a limited genetic polymorphism. The most common polymorphism of *HLA-G* gene is a 14 bp (5'-ATTTGTTCATGCCT-3') insertion /deletion. It is located at the 3' UTR region (exon 8) of the *HLA-G* gene. Several studies suggested that it was associated with a risk factor for various disease including cancer, diabetics, and autoimmune diseases [7]. *HLA-G* polymorphism is evaluated with nasopharyngeal carcinoma risk [8], transitional cell carcinoma of the bladder in a Brazilian population [9], and it also could be a marker for genetic susceptibility to hepatocellular carcinoma in Chinese populations [10]. Although many studies have been conducted, the relationship between 14 bp (5'-ATTTGTTCATGCCT-3')

insertion /deletion of *HLA-G* gene and oral cancer has not been studied yet.

In this study, we evaluated whether the 14 bp (5'-ATTT GTTCATGCCT-3') insertion /deletion of *HLA-G* gene was contributed to susceptibility of OSCC in Korean population.

Materials and Methods

Participants.

This study was approved by the Institutional Review Board of School of Dentistry, Kyung Hee University, Seoul, Republic of Korea (20040915). The OSCC subjects included 54 patients and 120 healthy controls were enrolled in this study. We reviewed the biopsy chart of department of oral pathology, Kyung Hee University and selected the OSCC cases. We found total 92 OSCC cases but some tissues were too small or some samples were not available to extract DNA. Clinical features of experimental subjects were shown in Table 1. Control subjects were selected through general health check-up program that they had no clinical evidence of OSCC or any other severe disorders.

DNA extraction and genotyping assays.

Genomic DNA from OSCC samples was prepared from paraffin embedded block using a Qiagen® DNA Micro kit (Qiagen) and stored at -20℃ before use. Genomic DNA from control subjects was prepared from peripheral blood using a genomic DNA isolation reagent kit (High Pure PCR template preparation kit, Roche, USA). Genotyping for 14bp insertion/deletion polymorphism in exon 8 (3'UTR) of the *HLA-G* gene was performed by polymerase chain reaction (PCR) method using the primers antisense 5′-GGAAGGAA TGCAGTTCAGCATGA-3' and sense 5′-GTGATGGGCTGTTT AAAGTGTCACC-3' (fluorescent labeled). PCR method was carried out basically as described by Tripathi et al. using the following cycling profile: 94°C for 5 minutes, and 39 cycles at 94°C for 30 seconds, 58°C for 40 seconds, and 72°C for 1 minutes[11]. The PCR products were analyzed by gene scan (3730xl DNA Analyzer, Applied Biosystems, USA).

Table 1. Clinical features of experimental subjects diagnosed as OSCC.

Number		54
Sex	Male/female	43/11
Age	Average±SD	61.3±11.6
Location		
	anterior region of the maxilla	1
	posterior region of the maxilla	10
	anterior region of the mandible	3
	posterior region of the mandible	16
	lower lip	2
	mouth floor	5
	salivary gland	1
	hard palate	5
	soft palate	5
	sublingual	1
	tongue	5

SD, standard deviation

Statistical analysis.

We used SPSS 18.0 for analyzing genetic data between OSCC and control subjects. Logistic regression model was used for odd ratio (OR), 95% confidence interval (CI), and *p* value. The level of significance was set at 0.05.

Results

In the present study, we examined whether 14bp insertion/deletion polymorphism in exon 8 (3'UTR) of the *HLA-G* gene has a relation with susceptibility to OSCC. No deviation from Hardy-Weinberg equilibrium was found in the control group ($p>0.05$). Table 2 shows genotypic and allelic frequencies of *HLA-G* 14bp insertion/deletion polymorphism among OSCC and control subjects. We observed a significant difference in genotype and allelic frequencies between OSCC and control subjects (OR=0.017, 95% CI= 0.002-0.129, $p<0.001$, OR=0.019, 95% CI=0.003-0.139, $p<0.001$, respectively). Genotypic frequencies 210bp/210bp, 210bp/224bp, and 224bp/224bp were 98.1%, 1.9% and 0.0% versus (*vs*) 43.3%, 47.5%, and 9.2% (OSCC subjects *vs* control subjects). The allelic frequencies of 210bp and 224bp were 99.1% and 0.9% *vs* 67.1% and 32.9%, respectively. Frequency of 224bp allele in the OSCC group was significantly decreased compared with the control group. There were significant differences between OSCC and control subjects ($p<0.05$).

Table 2. Genotypic and allelic frequencies of *HLA-G* 14bp insertion/deletion polymorphism between oral squamous cell carcinoma (OSCC) and control subjects.

Genotype/ Allele	Control n, (%)	OSCC n, (%)		OR	95 % CI	p
Genotype						
210bp/210bp	52 (43.3%)	53 (98.1%)	Codominant 1	0.017	0.002-0.129	**0.000077**
210bp/224bp	57 (47.5%)	1 (1.9%)	Codominant 2	NA	NA	NA
224bp/224bp	11 (9.2%)	0 (0.0%)	Dominant	0.014	0.002-0.108	**0.000036**
			Recessive	NA	NA	NA
Allele						
210bp	161 (67.1%)	107 (99.1%)				
224bp	79 (32.9%)	1 (0.9%)		0.019	0.003-0.139	**0.000094**

OR and 95% CI were from logistic regression analyses.
OR, odds ratio; CI,confidence interval;n,number of subjects;NA, not applicable.

Discussion

HLA-G is a non classical tolerogenic molecule and many previous reports showed its relationship with various immune reactions. *HLA-G* degrades the function of all immune cells, thus defects in HLA class I expression allow tumor cells to resist cytotoxic immune functions [12]. Immune cells that may be affected by HLA-G antigens include natural killer cells, $CD8^+$ T cells, $CD4^+$ T lymphocyte function, and dendritic cells, which may affect their cytotoxicity [13]. *HLA-G* is selectively expressed in the cytotrophoblast of the feto-maternal interface and plays an important role in protecting the fetus from maternal natural killer cells [14]. Therefore, there were reports that its polymorphism is related to abortion and failure of in vitro fertilization failure [15,16]. *HLA-G* also has a relationship with the infection. *HLA-G* polymorphism is associated with human papilloma virus infection and squamous intraepithelial lesions, which represents a profile of predisposition to cervical cancer in woman [17]. *HLA-G* polymorphism is known to be an estimated susceptibility factor for human cytomegalovirus infection in children [18] and human immunodeficiency virus vertical transmission [19,20]. As previously mentioned, *HLA-G* inhibits natural killer cell cytotoxicity in some tumors. Human melanoma cell secrets *HLA-G* [21] and this enable tumor cells to evade from immune surveillance of the host [12]. *HLA-G* interacts with *HLA-G*-recognizing killer-cell inhibitory receptors (KIRs) and inhibits host immune response in human breast cancer [22].

The 14 bp insertion/deletion polymorphism of *HLA-G* is a large, polymorphic promoter region, which appears to play an important role in the regulation of *HLA-G* expression [23]. The presence of the 14 bp insertion allele (+ 14 bp) destabilizes the mRNA and reduces *HLA-G* protein production. This suggests that differences in genotypes may cause differences in immune responses [13,24]. The 14 bp deletion polymorphism of the HLA-G gene also influences mRNA stability and plays an important role in the expression of soluble HLA-G in plasma [25].

In this study, we evaluated whether 14bp insertion/deletion polymorphism was associated with OSCC in Korean population. We identified genotypic and allelic frequencies of *HLA-G* in OSCC and control subjects. In control subjects, genotypic frequencies 210bp/210bp, 210bp/224bp, and 224bp/224bp were 43.3%, 47.5% and 9.2% and allelic frequencies of 210bp and 224bp were 67.1% and 32.9%, respectively. This result was similar to previous study. In Chinese, genotypic frequencies 210bp/210bp, 210bp/224bp, and 224bp/224bp were 37.3%, 46.6%, and 16.1% and allelic frequencies of 210bp and 224bp were 60.6% and 39.4%, respectively [26]. There were few differences in genotypic and allelic frequencies between normal Chinese and Korean population. However, genotypic frequencies 210bp/210bp, 210bp/224bp, and 224bp/224bp and allelic frequencies of 210bp and 224bp were completely different between OSCC and control subjects. In OSCC subjects, only one 224bp was found, and even there was no 224bp/224bp in OSCC subjects. This result suggests that 14bp insertion/deletion polymorphism of *HLA-G* might be associated with the

development of OSCC.

In conclusion, we found a significant association between OSCC patients and control subjects. Our results suggest that 14bp insertion/deletion polymorphism of *HLA-G* may be contributed to the susceptibility to OSCC in Korean population.

Conflict of Interest

The authors indicate that no potential conflict of interest exist.

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