The Joint Effect of *hOGG1* Single Nucleotide Polymorphism and Betel Quid Chewing on Oral Cancer in Taiwan

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Abstract. *Aim: To evaluate the association and interaction among hOGG1 genotypic polymorphism, betel quid chewing status and oral cancer risk in Taiwan. Materials and Methods: The well-known polymorphic variants of hOGG1, codon 326, were analyzed in association with oral cancer susceptibility, and discussed regarding its joint effect with individual habits on oral cancer susceptibility. In total, 620 patients with oral cancer and 620 healthy controls recruited from the China Medical Hospital were analyzed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Results: The hOGG1 codon 326 genotypes were differently distributed between the oral cancer and control groups (p=0.0266) and the C allele of hOGG1 codon 326 was significantly (p=0.0046) more frequently found in cancer patients than in controls. We further analyzed the joint effects of gene variants and habits on oral cancer risk and found an interaction between hOGG1 codon 326 genotype and betel quid chewing status. The association of the C allele for hOGG1 codon 326 with oral cancer risk was found to be significant only in the betel quid chewer group (p=0.0149), not in the non-chewer group (p=0.8028). Conclusion: Our results provide evidence that the C allele of hOGG1 codon 326 may have a joint effect with betel quid chewing on the development of oral cancer.*

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In Taiwan and South-Eastern Asia, betel quid (BQ) chewing has been associated with the development of oral squamous cell carcinoma (OSCC) through epidemiological studies. BQ, which comprises areca nut, lime and *Piper betel* inflorescence or leaf, has been classified as a human carcinogen by the IARC (2004). According to the *in vitro* results of a chemiluminescence assay, areca nut extract (ANE) reacts with the lime and this generates reactive oxygen species (ROS), including superoxide anion radicals and hydrogen peroxide, which may cause DNA single- and double-strand breaks. Continuous painting of such prepared ANE combined with lime on hamster cheek pouches for 5 days significantly increased the frequency of micronucleate cell formation compared to the controls (1).

Sustained oxidative stress, such as smoking and BQ exposure, induce oxidative DNA adducts in the human genome, and 8-hydroxy-2-deoxyguanine (8-OH-dG) seems to be the major form of these (2, 3). 8-OH-dG is mutagenic and if not repaired on time, the adducts can cause severe transversions of GC to TA in several oncogenes and tumor suppressor genes and in turn lead to carcinogenesis (2, 3). Among the DNA repair pathways, 8-OH-dG and other oxidative DNA adducts are repaired by the base excision repair pathway (4). The human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) gene encodes a DNA glycosylase which catalyzes the cleavage of the glycosylic bond between the oxidized base and the sugar moiety, leaving an abasic apurinic/apyrumidinic site in DNA. The resulting apurinic/apyrumidinic site is then incised, and the repair is completed by successive actions of a phosphodiesterase, a DNA polymerase, and a DNA ligase (5).

Among the common single nucleotide polymorphisms (SNPs) of *hOGG1* gene, that located in exon 7, resulting in an amino acid substitution of serine (Ser) with cysteine (Cys) at codon 326 (Ser326Cys, rs1052133), has been demonstrated to affect hOGG1 function (6). Cells with the Cys-encoding

Table I. *The primer sequences and PCR-RFLP conditions for hOGG1 gene polymorphisms.*

Polymorphism (location)	Primer sequences (5'->3')	Restriction enzyme	SNP sequence	DNA fragment size (bp)
Codon 326	F: ACTGTCACTAGTCTCACCAG	*Fnu4HI*	C (Ser)	200
(rs1052133)	R: GGAAGGTGGGAAGGTG	37˚C for 2 h	G (Cys)	100 + 100

*F and R indicate forward and reverse primers, respectively.

Table II. *Characteristics of oral cancer patients and controls.*

Characteristic	Controls (n=620)			Patients (n=620)			*P*-value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			51.3 (7.4)			52.4 (7.2)	0.78
Gender							1.00
Male	586	94.5%		586	94.5%		
Female	34	5.5%		34	5.5%		
Indulgence							
Cigarette smokers	443	71.5%		458	73.9%		0.37
Betel quid chewers	382	61.6%		399	64.4%		0.35
Alcohol drinkers	413	66.6%		441	71.1%		0.10

[a]Based on chi-square test.

allele exhibited a reduced DNA repair activity (6), which has been reported to be associated with the risk of many types of cancer (7). In the present work, we aimed at analyzing the genetic polymorphisms of the *hOGG1* Ser326Cys in a Taiwanese oral cancer population (control/case=620/620), and investigated the interaction of *hOGG1* Ser326Cys genotypes and BQ chewing habits in a Taiwanese population.

Materials and Methods

Study population and sample collection. Six hundred and twenty cancer patients diagnosed with oral cancer were recruited at the outpatient clinics of general surgery between 1998-2010 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients, including histological details, were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. As many non-oral cancer healthy volunteers as controls were selected by matching for age, gender and habits after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included habits. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping assays. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (8-14). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s, and a final extension at 72˚C for 10 min. Pairs of PCR primer sequences and restriction enzyme for each DNA product are all listed in Table I.

Statistical analyses. Only those individuals with both genotypic and clinical data (control/case=620/620) were selected for final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *hOGG1* codon 326 in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test was used to compare the distribution of the genotypes between cases and controls. Data were recognized as significant when the statistical *p*-value was less than 0.05.

Results

The frequency distributions of selected characteristics of 620 oral cancer patients and 620 controls are shown in Table II. These characteristics of patients and controls are all well matched. None of the differences between the groups were statistically significant ($p>0.05$) (Table II).

The frequencies of the genotypes for *hOGG1* codon 326 in controls and oral cancer patients are shown in Table III. The genotype distributions of *hOGG1* codon 326 was significantly different between oral cancer and control groups ($p=0.0266$) (Table III). The frequencies of the alleles for *hOGG1* codon 326 in controls and oral cancer patients

Table III. *Distribution of hOGG1 codon 326 genetic and allelic frequencies among oral cancer patient and control groups.*

Codon 326 (rs1052133)	Controls		Patients		*P*-value[a]
	n	%	n	%	
Genetic frequency					0.0266
CC	104	16.8%	138	22.3%	
CG	251	40.5%	252	40.6%	
GG	265	42.7%	230	37.1%	
Allele frequency					0.0046
Allele C	459	37.0%	528	42.6%	
Allele G	781	63.0%	712	57.4%	

[a]Based on Chi-square test.

Table IV. *Distribution of hOGG1 codon 326 genotypes in oral cancer patients after stratification by betel quid chewing habit.*

Variable	*hOGG1* codon 326 genotype			*P*-value[a]
	CC (%)	CG (%)	GG (%)	
Betel quid chewers				0.0149
Controls	60 (15.7%)	155 (40.6%)	167 (43.7%)	
Patients	93 (23.3%)	161 (40.4%)	145 (36.3%)	
Non-betel quid chewers				0.8028
Controls	44 (18.5%)	96 (40.3%)	98 (41.2%)	
Patients	45 (20.4%)	91 (41.2%)	85 (38.4%)	

[a]Based on Chi-square test.

are also shown in Table III, and the trend is more significant. The C allele of the *hOGG1* codon 326 polymorphism was significantly associated with oral cancer (p=0.0046). The conclusion deduced from the data in Tables III and IV is that *hOGG1* codon 326 C allele seems to be associated with a higher risk for oral cancer in Taiwanese.

The interaction of genotype of *hOGG1* codon 326 and the BQ chewing habits was of great interest. The genotype distribution of *hOGG1* codon 326 was significantly different between individuals of the oral cancer and control groups who have BQ chewing habit (p=0.0149), while that in these who do not use BQ (p>0.05) (Table IV). Consistent with the findings in Table III, the C allele frequency was still significantly higher in cancer patients with a BQ chewing habit than in BQ-chewing controls. There was no such difference in the non-BQ chewing groups.

Discussion

In order to reveal the role of *hOGG1* in oral cancer, in this study, we selected a common SNP of the *hOGG1* gene, that for codon 326, and investigated its association with the susceptibility for oral cancer in a population of central Taiwan. We found that the C variant genotypes of *hOGG1* codon 326 were significantly associated with a higher susceptibility for oral cancer (Tables III and IV).

Previous studies have implicated the *hOGG1* codon 326 polymorphism in risk for smoking- and/or alcohol-related cancer. Significant increases in risk were found for the homozygous G/G genotype and lung cancer in a Japanese study (15). In addition, non-significant increases in the prevalence of the *hOGG1* G/G genotype were observed in lung cancer cases as compared to controls in two small studies (6, 16). A significant positive association between *hOGG1* genotype and cancer risk was also observed for esophageal cancer (17). However, we found no other study of the joint effect of genotypes of *hOGG1* and BQ chewing habit on oral cancer susceptibility. For this purpose, we further analyzed the association between *hOGG1* codon 326 genotypes and oral cancer risk in patients and controls who have a BQ chewing habit. Interestingly, the interaction between hOGG1 codon 326 and BQ chewing habit is clear (Table IV). We propose that the different genotypes of codon 326 may affect hOGG1 activity, slightly influencing its normal function. Generally speaking, oxidative insults to the genome are continuously conducted, resulting from endogenous oxidative stress and exposure to chemical carcinogens. If hOGG1 is dysfunctional, DNA adducts could be left unrepaired, leading to mutations or carcinogenesis. As individuals with the C allele(s) become older, the alteration towards carcinogenesis may accumulate via the decreasing function of hOGG1. There are several studies suggesting that amino acid changes in hOGG1 may affect the catalytic properties of the enzyme (18, 19). One explanation for the functional relevance of the polymorphism is that the variant allele may be tightly linked to other functional polymorphisms in *hOGG1* and/or other DNA repair genes involved in the removal of oxidative DNA damage. Another possible explanation is that the variant genotype may be deficient in repair of oxidative DNA damage only under conditions of excessive cellular oxidative stress (18). However, both of the hypotheses need to be tested in future studies.

To sum up, this is the first study which focuses on the codon 326 of *hOGG1* and joint effects with BQ chewing habit on oral cancer risk in Taiwanese. The C allele of *hOGG1* codon 326 may be a useful marker in oral oncology for cancer prevention, and early cancer detection.

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