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

Yung-An Tsou[1,2], Chun-Hung Hua[1,3], Hsien-Chang Tseng[1,2], Chia-Fang Hsu[2,5], Chia-Wen Tsai[2,4], Shung-Shung Sun[2,4], Ru-Yin Tsai[2], Ming-Hsui Tsai[1,2,3], and Da-Tian Bau[2,3,4]

[1]Department of Otolaryngology and [2]Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung; Graduate Institutes of [3]Clinical Medical Science and [4]Basic Medical Science, China Medical University, Taichung, Taiwan; [5]Department of Otolaryngology, Chang-Hua Hospital, Chang-Hua, Taiwan, R.O.C.

Correspondence to: Da-Tian Bau and Ming-Hsui Tsai, Terry Fox Cancer Research Lab, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, P.R.C. Tel: +886 422053366 Ext 3312, Fax: +886 422053366 Ext 1511, e-mail: datian@mail.cmuh.org.tw; artbau1@yahoo.com.tw

Running title: Tsou *et al*: *hOGG1* genotypes in oral cancer

Abstract

To evaluate the association and interaction among *hOGG1* genotypic polymorphism, betel quid chewing status and oral cancer risk in Taiwan. The well-known polymorphic variants of *hOGG1,* codon 326, was analyzed in association with oral cancer susceptibility, and discussed of 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 PCR-RFLP. The *hOGG1* codon 326 genotypes were differently distributed between the oral cancer and control groups (*P*=0.0809) and the C allele of *hOGG1* codon 326 was significantly (*P*=0.0198) more frequently found in controls than in cancer patients. We have further analyzed the genetic-environmental joint effects on oral cancer risk and found an interaction between *hOGG1* codon 326 genotypes and betel quid chewing status. The *hOGG1* codon 326 genotypes were in association with oral cancer risk only in the betel quid chewer groups (*P*=0.0149), but not in the non-chewer group (*P*=0.8028). Our results provide the evidence that the C allele of *hOGG1* codon 326 may have a joint effect with betel quid chewing on the development of oral cancer.

***Key Words*: *hOGG1*, single nucleotide polymorphism, oral cancer, betel quid**

Introduction

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 oxy-gen species (ROS) including superoxide anion radicals and hydrogen peroxide, which may further 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 cells formation compared to the controls (Nair, U.J., Obe, G., Friesen, M., Goldberg, M.T., Bartsch, H., 1992. Role of lime in the generation of reactive oxygen species from betel-quid ingredients. Environ. Health Perspect. 98, 203-205).

Sustained oxidative stress, such as smoking and BQ exposure, induced oxidative DNA adducts to the human genome, and 8-hydroxy-2-deoxyguanine (8-OH-dG) seems to be the major form (Chen L, Elahi A, Pow-Sang J, Lazarus P and Park J: Association between polymorphism of human oxoguanine glycosylase 1 and risk of prostate cancer. J Urol *170*: 2471-2474, 2003; Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang BL, Bleecker ER, Walsh PC, Meyers DA and Isaacs

WB: Associations between *hOGG1* sequence variants and prostate cancer susceptibility. Cancer Res *62*: 2253-2257, 2002). The 8-OH-dG is mutagenic which if not repaired on time, can cause severe transversions of GC to TA in several oncogenes and tumor suppressor genes and in turn lead to carcinogenesis (Chen L, Elahi A, Pow-Sang J, Lazarus P and Park J: Association between polymorphism of human oxoguanine glycosylase 1 and risk of prostate cancer. J Urol *170*: 2471-2474, 2003; Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang BL, Bleecker ER, Walsh PC, Meyers DA and Isaacs WB: Associations between *hOGG1* sequence variants and prostate cancer susceptibility. Cancer Res *62*: 2253-2257, 2002). Among the DNA repair pathways, 8-OH-dG and other oxidative DNA adducts are repaired by the base excision repair pathway (Goode EL, Ulrich CM and Potter JD: Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev *11*: 1513-1530, 2002). The human OGG1 (*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 (Dianov GL, Souza-Pinto N, Nyaga SG, Thybo T, Stevnsner T and Bohr VA: Base excision repair in nuclear and mitochondrial DNA.

Prog Nucleic Acid Res Mol Biol *68*: 285-297, 2001).

Among the common single nucleotide polymorphisms (SNPs) of *hOGG1* gene, the one located in the exon 7, resulting in an amino acid substitution of serine (Ser) with cysteine (Cys) at codon 326 (Ser326Cys, rs1052133), has been demonstrated to affect the hOGG1 function (Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, Nohmi T, Kasai H and Yokota J: Genetic polymorphisms and alternative splicing of the *hOGG1* gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene *16*: 3219-3225, 1998). Those cells with CYS allele exhibited a reduced DNA repair activity (Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, Sugimura H, Nohmi T, Kasai H and Yokota J: Genetic polymorphisms and alternative splicing of the *hOGG1* gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene *16*: 3219-3225, 1998), which has been reported to be associated with the risk of many types of cancers (Weiss JM, Goode EL, Ladiges WC and Ulrich CM: Polymorphic variation in *hOGG1* and risk of cancer: a review of the functional and epidemiologic literature. Mol Carcinog *42*: 127-141, 2005). In the present work, we aimed at analyzing the genetic polymorphisms of the *hOGG1* Ser326Cys genotypes in a Taiwan 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 (Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF, Liu CS, Lin CC and Bau DT: Significant association of an *XRCC4* single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1777-1782, 2009; Chang CH, Chiu CF, Liang SY, Wu HC, Chang CL, Tsai CW, Wang HC, Lee HZ and Bau DT: Significant association of *Ku80* single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1275-1279, 2009; Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN, Lin CC and Bau DT: A novel single nucleotide polymorphism in *XRCC4* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol *44*: 898-902, 2008; Chiu CF, Wang CH, Wang CL, Lin CC, Hsu NY, Weng JR and Bau DT: A novel single nucleotide polymorphism in *XRCC4* gene is associated with gastric cancer susceptibility in Taiwan. Ann Surg Oncol *15*: 514-518, 2008; Chiu CF, Wang HC, Wang CH, Wang CL, Lin CC, Shen CY, Chiang SY and Bau DT: A new single nucleotide polymorphism in *XRCC4* gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res *28*: 267-270, 2008; Hsu CF, Tseng HC, Chiu CF, Liang SY, Tsai CW, Tsai MH and Bau DT: Association between DNA double strand break gene *Ku80* polymorphisms and oral cancer susceptibility. Oral Oncol *45*: 789-793, 2009; Hsu NY, Wang HC, Wang CH, Chiu CF, Tseng HC,

Liang SY, Tsai CW, Lin CC and Bau DT: Lung cancer susceptibility and genetic polymorphisms of *Exo1* gene in Taiwan. Anticancer Res *29*: 725-730, 2009). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, 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 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 both 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 a 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 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 higher risk for oral cancer in Taiwan.

The interaction of genotype of *hOGG1* codon 326 and the BQ chewing habits was of great interest. The genotype distribution of various genetic polymorphisms of *hOGG1* codon 326 was significantly different between oral cancer and control groups who have BQ chewing habit (P=0.0132), while that for *hOGG1* codon 326 was not significant (P>0.05) (Table IV). Consistent with the findings in Table III, the C allele frequency was still significantly higher in cancer patients who have 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 common SNP of the *hOGG1* gene, the 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). As we supposed, the effects of the *hOGG1* codon 326 on carcinogenesis are complex, exerting either an adverse effect or an advantageous influence on determining cancer risk. This may be caused by differences among ethnics and larger studies including different ethnic groups with more careful matching between cases and controls should be conducted in future studies. Only by this can make meta-analysis and evaluation the effects of gene-gene and gene-environment interactions clearer and more feasible.

Previous studies have implicated the *hOGG1* codon 326 polymorphism in risk for smoking- and/or alcohol-related cancers. Significant increases in risk were found for the homozygous G/G genotype and lung cancer in a Japanese study (Sugimura-H, et al, 1999). 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 (Kohno,T., Shinmura,K., Tosaka,M., Tani,M., Kim,S-R., Sugimura,H., Nohmi,T., Kasai,H. and Yokota,J. (1998) Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene*, **16**, 3219–3225; Wirkman,H., Risch,A., Klimek,F., *et al.* (2000) *hOOG1* polymorphism and loss of heterozygosity (LOH): significance for lung cancer susceptibility in a Caucasian population. *Int. J. Cancer*, **88**, 932–937). A significant positive association between hOGG1 genotype and cancer risk was also observed for esophageal cancer (Xing,D-Y., Tan,W., Song,N. and Lin,D-X. (2001) Ser326Cys polymorphism in *hOGG1* gene and risk of esophageal cancer in a Chinese population. *Int. J. Cancer*, **95**, 140–143). However, there was no other paper studied the joint effect of genotypes of *hOGG1* and betel quid chewing habit on oral cancer susceptibility. For this purpose, we have 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 obvious (Table V). We propose that the different genotypes of codon 326 may affect hOGG1 activity, slightly influencing its normal function. Generally speaking, oxidative insults to genome DNA are continuously conducted, which are resulted from of endogenous oxidative stress and exposure to chemical carcinogens. If the hOGG1 is dysfunctional, the DNA adducts could be left unrepaired, leading to

mutations or carcinogenesis. As these with the C allele(s) get older, the alteration towards carcinogenesis may accumulate *via* the decreasing functions of hOGG1. There are several studies suggested our idea that the amino acid change in hOGG1 may affect the catalytic properties of the enzyme (Lee AJ, Hodges NJ and Chipman JK: Interindividual variability in response to sodium dichromate-induced oxidative DNA damage: role of the Ser326Cys polymorphism in the DNA-repair protein of 8-oxo-7,8-dihydro-2'-deoxyguanosine DNA glycosylase 1. Cancer Epidemiol Biomarkers Prev *14*: 497-505, 2005; Yamane A, Kohno T, Ito K, Sunaga N, Aoki K, Yoshimura K, Murakami H, Nojima Y and Yokota J: Differential ability of polymorphic *OGG1* proteins to suppress mutagenesis induced by 8-hydroxyguanine in human cell in vivo. Carcinogenesis *25*: 1689-1694, 2004). 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 (Lee AJ, Hodges NJ and Chipman JK: Interindividual variability in response to sodium dichromate-induced oxidative DNA damage: role of the Ser326Cys polymorphism in the DNA-repair protein of 8-oxo-7,8-dihydro-2'-deoxyguanosine DNA glycosylase 1. Cancer

Epidemiol Biomarkers Prev *14*: 497-505, 2005). However, both of the hypotheses need to be confirmed in future studies.

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

Acknowledgements

We thank Wen-Shin Chang and the Tissue Bank at the China Medical University for their technical assistance. This study was supported by research grants from the Terry Fox Cancer Research Foundation, National Science Council (NSC98-2320-B-039-010-MY3 and NSC98-2218-E-039-001), Taiwan Department of Health, China Medical University Hospital Cancer Research of Excellence (DOH99-TD-C-111-005) and China Medical University and Hospital (DMR-99-045).

Table I. The primer sequences, polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) conditions for *hOGG1* gene polymorphisms.

Polymorphism (location)	**Primers sequences (5'->3')**	**Restriction enzyme**	**SNP sequence**	**DNA fragment size (bp)**
Codon 326	F: ACTGTCACTAGTCTCACCAG	*Fnu4HI*	C (Ser)	200
(rs1052133)	R: GGAAGGTGGGAAGGTG	37 ^{o}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

Characteristics	Controls (n = 620)			Patients (n = 620)			P^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (y)			51.3 (7.4)			52.4 (7.2)	0.78
Gender							1.00
Male	586	94.5%		586	94.5%		
Female	34	6.1%		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] *P* based on chi-square test.

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]
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			
	CC (%)	CG (%)	GG (%)	*P-value*[a]
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. ORs were estimated with multivariate logistic regression analysis.

[b] Statistically identified as significant.