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Enclosures

The Joint Effect of *hOGG1* Single Nucleotide Polymorphism and Smoking Habit on Lung Cancer in Taiwan

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Abstract. *Aim:* To evaluate the association and interaction among human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) genotypic polymorphism, smoking status and lung cancer risk in Taiwan. *Materials and Methods:* In total, the gene for *hOGG1* was analyzed via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 358 patients with lung cancer and 716 healthy controls recruited from the China Medical Hospital. *Results:* The *hOGG1* codon 326 genotypes were not differently distributed between the lung cancer and control groups ($p=0.0809$). However, the C allele of *hOGG1* codon 326 was significantly ($p=0.0198$) more frequently found in controls than in cancer patients. We further analyzed the joint effect of genetics and smoking on lung cancer risk and found an interaction between *hOGG1* codon 326 genotypes and smoking status. The *hOGG1* codon 326 C allele-bearing genotypes were significantly associated with lung cancer risk only in the smoker group ($p=0.0132$), but not in the non-smoker group ($p=0.06588$). *Conclusion:* Our results provide evidence that the C allele of *hOGG1* codon 326 may have a joint effect with smoking on the development of lung cancer.

Lung cancer has become one of the most common malignancies all over the world (1, 2). In Taiwan, lung cancer is very prevalent, has a high mortality, and low 5-year survival rate, especially in female adenocarcinoma cases (3).

*These authors contributed equally to this work.

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Key Words: *hOGG1*, single nucleotide polymorphism, lung cancer, smoking.

Among the several well-known factors for lung cancer susceptibility, smoking seems to be the most important (4-6).

Sustained oxidative stress, such as caused by smoking, induces oxidative DNA adducts to form in the human genome, and 8-hydroxy-2-deoxyguanine (8-OH-dG) seems to be the major form produced (7, 8). 8-OH-dG is mutagenic, and, if not repaired in time, can cause severe transversions of GC to TA in several oncogenes and tumor suppressor genes and in turn lead to carcinogenesis (7, 8). Among the DNA repair pathways, 8-OH-dG and other oxidative DNA adducts are repaired by the base excision repair pathway (9). 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/apyrimidinic site in DNA. The resulting apurinic/apyrimidinic site is then incised, and the repair is completed by successive actions of a phosphodiesterase, a DNA polymerase, and a DNA ligase (10).

Benzo[a]pyrene, an abundant tobacco smoke carcinogen, was shown to induce 8-OH-dG production in animal tissues (11). Increased 8-OH-dG levels were observed in lung DNA of smokers (compared with non-smokers), with a correlation between the levels of 8-OH-dG and the number of cigarettes smoked (12). Smokers also have higher levels of 8-OH-dG both in their peripheral leukocyte DNA (13, 14), the nuclei of oral mucosa (15, 16), and urine (17) than do non-smokers. Together, these data suggest that the formation and removal of 8-OH-dG is strongly linked to tobacco smoke carcinogenesis.

Among the common single nucleotide polymorphisms (SNPs) of the *hOGG1* gene, one 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 (18). The protein resulting from this substitution exhibits reduced DNA repair activity (18), and this SNP has been reported to be associated with the risk of many types of cancer (19). In the present

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

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

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

Table II. Characteristics of lung cancer patients and controls.

Characteristic	Controls (n=716)			Patients (n=358)			P-value ^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.58
Gender							0.36
Male	488	68.2%		254	70.9%		
Female	228	31.8%		104	29.1%		
Habit							
Cigarette smokers	563	78.6%		293	81.8%		0.23
Non-smokers	153	21.4%		65	18.2%		

^aBased on Chi-square test.

work, we aimed at analyzing the *hOGG1* Ser326Cys genotypes in a Taiwanese lung cancer population (control/case=716/358), and investigated the interaction of *hOGG1* Ser326Cys genotypes and smoking habits.

Materials and Methods

Study population and sample collection. Three hundred and fifty-eight patients diagnosed with lung cancer were recruited at the Outpatient Clinics of General Surgery between 2005-2008 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. Twice as many non-lung 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 (20-28). 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, with 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=716/358) 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 358 lung cancer patients and 716 controls are shown in Table II. These characteristics of patients and controls are all well matched. None of the differences between groups were statistically significant (*p*>0.05) (Table II).

The frequencies of the genotypes for *hOGG1* codon 326 in controls and lung cancer patients are shown in Table III. The genotype distribution of *hOGG1* codon 326 was not significantly different between lung cancer and control groups (*p*=0.0809) (Table III). The frequencies of the alleles for *hOGG1* codon 326 in controls and lung cancer patients are shown in Table IV. The C allele of the *hOGG1* codon 326 polymorphism was significantly associated with lung cancer (*p*=0.0198). The conclusion deduced from the data in Tables

Table III. Distribution of *hOGG1* codon 326 genotypes in lung cancer patient and control groups.

Genotype	Controls		Patients		<i>P</i> -value ^a
	n	%	n	%	
Codon 326 rs1052133					0.0809
CC	110	15.4%	68	19.0%	
CG	294	41.0%	158	44.1%	
GG	312	43.6%	132	36.9%	

^aBased on Chi-square test.

Table IV. *hOGG1* codon 326 allelic frequencies in the lung cancer patient and control groups.

Allele	Controls		Patients		<i>P</i> -value ^a
	n	%	n	%	
Codon 326 rs1052133					0.0198
Allele C	514	35.9%	294	41.1%	
Allele G	918	64.1%	422	58.9%	

^aBased on Chi-square test.

III and IV is that *hOGG1* codon 326 C allele seems to be associated with higher risk for lung cancer in Taiwan.

The interaction of genotype of *hOGG1* codon 326 and the smoking habit was of great interest. Consistent with the findings in Table III and IV, the C allele frequency was still significantly higher in cancer patients who smoked than in smoking controls ($p=0.0132$; Table V). There was no such difference in the nonsmoker groups.

Discussion

In order to reveal the role of *hOGG1* in lung cancer, in this study, we selected a common SNP of the *hOGG1* gene, that in codon 326, and investigated its association with the susceptibility for lung 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 lung cancer (Tables III and IV). There are several studies investigation the association of *hOGG1* with lung cancer but with controversial results or no association being found (29-35). Thus, the effects of the *hOGG1* codon 326 polymorphism on carcinogenesis are complex, exerting either an adverse effect or an advantageous influence on determining cancer risk. This may be caused by differences in ethnicity and larger studies including different ethnic groups with more careful matching between cases and controls should be conducted in future studies. Only can this

Table V. Distribution of *hOGG1* codon 326 genotypes in lung cancer patients after stratification by smoking habit.

Variable	<i>hOGG1</i> codon 326 genotype			<i>P</i> -value ^a
	CC (%)	CG (%)	GG (%)	
Smokers				0.0132
Controls	80 (14.2%)	227 (40.3%)	256 (45.5%)	
Patients	56 (19.1%)	133 (45.4%)	104 (35.5%)	
Non-smokers				0.6588
Controls	30 (19.6%)	70 (43.8%)	53 (36.6%)	
Patients	12 (18.5%)	25 (38.5%)	28 (43.0%)	

^aBased on Chi-square test.

make meta-analysis and evaluation of the effects of gene – gene and gene – lifestyle interactions clearer.

We have further analyzed the association between *hOGG1* codon 326 genotype and lung cancer risk in patients and controls who have a cigarette smoking habit. Interestingly, the interaction between *hOGG1* codon 326 and cigarette smoking 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 genomic DNA are continuously occurring, 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) get older, the alteration towards carcinogenesis may accumulate *via* the decreasing function of *hOGG1*. There are several studies suggesting that the amino acid change in *hOGG1* may affect the catalytic properties of the enzyme (36, 37). 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 (36). However, both of these hypotheses need to be confirmed in future studies.

This is the first study which focuses on codon 326 of *hOGG1* and its joint effects with a smoking habit on lung cancer risk in Taiwan. The C allele of *hOGG1* codon 326 may be a useful marker in lung oncology for anticancer application, and early cancer detection.

Acknowledgements

We thank Wen-Shin Chang, Hui-Ni Cheng 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 (NSC 98-2320-B-039-010-MY3) and China Medical University and Hospital (DMR-99-014).

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Received June 8, 2010

Revised July 29, 2010

Accepted August 12, 2010

ANTICANCER RESEARCH

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