

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

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To evaluate the association and interaction of *hOGG1* genotypic polymorphism and smoking status with lung cancer in Taiwan. The polymorphic variants of *hOGG1* codon 326 was analyzed of its association with lung cancer susceptibility, and its joint effect with smoking habits on lung cancer susceptibility. In total, 358/716 lung cancer patients/controls were analyzed via PCR-RFLP. The *hOGG1* codon 326 genotypes were not differently distributed between case and control groups ($P=0.0809$). However, the G allele of *hOGG1* codon 326 was significantly ($P=0.0198$) more frequently found in controls than in cases. We have also found an interaction between *hOGG1* codon 326 genotypes and smoking status. The *hOGG1* codon 326 genotypes were in association with lung cancer risk only in the smoker group ($P=0.0198$), but not in the non-smoker group ($P=0.0198$). Our results suggested that C allele of *hOGG1* codon 326 may have a joint effect with smoking on the lung carcinogenesis.

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

Introduction

Lung cancer has become one of the most common malignancies all over the world [1, 2]. In Taiwan, lung cancer is very prevalent with its high incidence, high mortality, and low 5-year survival rate, especially in female adenocarcinoma cases [3]. Among the several well-known environmental factors for lung cancer susceptibility, smoking seems to be the most important [4-6].

Sustained oxidative stress, such as smoking, induced oxidative DNA adducts to the human genome, and 8-hydroxy-2-deoxyguanine (8-OH-dG) seems to be the major form [7, 8]. 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 [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 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/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].

The abundant and carcinogenic tobacco smoke carcinogen, benzo[a]pyrene, was shown to induce 8-OH-dG 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]. The 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 the urine [17] than the non-smokers. Together, these data suggested the idea that the 8-OH-dG formation and removal strongly linked to the tobacco smoke carcinogenesis.

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 [18]. The CYS allele exhibits reduced DNA repair activity [18], and has been reported to be associated with the risk of many types of cancers [19]. In the present work, we aimed at analyzing the genetic polymorphisms of the *hOGG1* Ser326Cys genotypes in a Taiwan lung cancer population (control/case=716/358), and investigated the interaction of *hOGG1* Ser326Cys genotypes and smoking habits in a Taiwanese population.

Materials and Methods

Study population and sample collection. Three hundred and fifty-eight cancer 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 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=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 *hOGGI* 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 both groups were statistically significant ($P>0.05$) (Table II).

The frequencies of the genotypes for *hOGGI* codon 326 in controls and lung cancer patients are shown in Table III. The genotype distributions of *hOGGI* codon 326 was not significantly different between lung cancer and control groups ($P=0.0809$) (Table III). The frequencies of the alleles for *hOGGI* codon 326 in controls and lung cancer patients are shown in Table IV. The C allele of the *hOGGI* codon 326 polymorphism was significantly associated with lung cancer ($P=0.0198$). The conclusion deduced from the data in Tables III and IV is that *hOGGI* 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 habits was of great interest. The genotype distribution of various genetic polymorphisms of *hOGG1* codon 326 was significantly different between lung cancer and control groups who have smoking habit ($P=0.0132$), while that for *hOGG1* codon 326 was not significant ($P>0.05$) (Table V). 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. 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 common SNP of the *hOGG1* gene, the 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 was 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 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.

We have further analyzed the association between *hOGG1* codon 326 genotypes 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 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 [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 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 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.

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Table I. The primer sequences, polymerase chain reaction and restriction fragmentlength 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	<i>Fnu4HI</i>	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 lung cancer patients and controls.

Characteristic	Controls (n = 716)			Patients (n = 358)			<i>P-value</i> ^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.58
Gender							0.36
Male	488	68.1%		254	70.9%		
Female	228	31.9%		104	29.1%		
Habit							
Cigarette smokers	563	78.6%		293	81.8%		0.23
Non-smokers	153	21.4%		65	18.2%		

^a Based on Chi-square test.

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

Genotype	Controls	%	Patients	%	<i>P-value</i> ^a
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%	

^a Based on Chi-square test.

Table IV. *hOGGI* codon 326 allelic frequencies among the lung cancer patient and control groups.

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

^a Based on Chi-square test.

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

Variable	<i>hOGGI</i> codon 326 genotype			<i>P-value</i> ^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%)	

^a Based on Chi-square test. ORs were estimated with multivariate logistic regression analysis.

^b Statistically identified as significant.