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ORIGINAL ARTICLE

Association of *hOGG1*-Cys variants with occurrence of p53 and *EGFR* deletion mutations in non-small cell lung cancer

Ming-Jenn Chen[1,2], Ching-Ju Shen[3], Lee Wang[4], Po-Ming Chen[5], Chih-Yi Chen[6] & Huei Lee[5]

1 Department of Surgery, Chi Mei Medical Center, Tainan, Taiwan
2 Department of Sports Management, College of Leisure and Recreation Management, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
3 Department of Gynecology and Obstetrics, Kaohsiung Medical University Hospital, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
4 Department of Public Heath, Chung Shan Medical University, Taichung, Taiwan
5 Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan
6 Department of Surgery, Chung Shan Medical University Hospital, Taichung, Taiwan

Keywords
Deletion mutation; *hOGG1* Ser326Cys polymorphism; non-small cell lung cancer (NSCLC).

Correspondence
Huei Lee, Graduate Institute of Cancer Biology and Drug Discovery, Taipei Medical University, Taipei, Taiwan.
Email: hl@tmu.edu.tw; Tel: +86 2 2736 1661 ext 7616; Fax: +86 2 2655 8562.

Ming-Jenn Chen, Department of Surgery, Chi Mei Medical Center, Tainan, Taiwan.
minchen117@gmail.com; Tel: +86 9328 66520

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Abstract

Background: The human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) gene encodes a DNA glycosylase that removes 8-hydroxy-2-deoxyguanine (8-OH-dG) DNA damage to protect against gene mutations. The association of *hOGG1* Ser326Cys polymorphism with lung cancer risk has predicted that *hOGG1*-Cys variants are less effective at removing 8-OH-dG damage from DNA; therefore, these variants might show an increased occurrence of tumor suppressor gene and oncogene mutations. However, no evidence has yet supported this hypothesis.
Methods: Direct sequencing was performed to examine the mutations of *p53* and EGFR genes in lung tumors from patients with non-small cell lung cancer (NSCLC). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to examine *hOGG1* Ser326Cys polymorphism in this study population.
Results: A total of 99 *p53*-mutated and 99 *EGFR*-mutated patients with NSCLC were selected to explore the possible associations of these mutations with *hOGG1* Ser326Cys polymorphism. The *p53*-mutated and *EGFR*-mutated patients were divided into nondeletion and deletion subgroups. *P53* deletion mutations were more commonly observed in male than in female patients ($P = 0.030$). However, *EGFR* exon 19 deletion mutations were more prevalent in female and adenocarcinoma patients than in male and squamous cell carcinoma patients ($P = 0.028$ for genders, $P = 0.017$ for tumor histology). Interestingly, *p53* and *EGFR* exon 19 deletion mutations were more frequent in patients with *hOGG1* Ser/Cys + Cys/Cys *hOGG1*-Cys variants than with the *hOGG1* Ser/Ser genotype ($P = 0.010$ for *p53*, $P = 0.032$ for *EGFR*).
Conclusions: We suggest that the association of *hOGG1* Ser326Cys polymorphism with lung cancer risk could be partially explained by increases in *p53* and *EGFR* deletion mutations.

Key points

Significant findings of the study:

- NSCLC patients with *hOGG1*-Cys variants may have a higher risk of *p53* and *EGFR* deletion mutations than with *hOGG1* Ser/Ser genotype.

What this study adds:

- NSCLC patients with *hOGG1*-Cys variants might be helpful to predict patients having higher risk of *EGFR* exon 19 deletion mutations and these patients who were treated with gefitinib or erlotinib could be a higher risk to occur *EGFR* T790M mutation.

Introduction

The human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) gene encodes a DNA glycosylase that catalyzes the cleavage of the glycosylic bond between the oxidized base and the sugar moiety, leaving an abasic apurinic/apyrumidinic site in the DNA.[1] Therefore, the enzyme plays an important role in removing oxidative stress-induced DNA damage, such as 8-hydroxy-2-deoxyguanine (8-OH-dG), which can induce oncogene and tumor suppressor gene mutations and cause tumor formation.[1]

An in vitro cell study has indicated that the *hOGG1*-Cys variant (Cys/Cys + Ser/Cys) may have less repair capability for removing 8-OH-dG in damaged DNA when compared with the *hOGG1*-Ser/Ser genotype.[2–4] This finding supports several epidemiological studies revealing that individuals with a *hOGG1*-Cys variant, and particularly smokers, had a higher risk of non-small cell lung cancer (NSCLC) than did those with the *hOGG1*-Ser/Ser genotype.[5–9] This difference arose because the *hOGG1* Ser/326Cys polymorphism was associated with removal of 8-OH-dG and was particularly induced by cigarette smoking.[3,4] However, the *hOGG1* Ser326Cys polymorphism was not associated with tobacco-related G:C to T:A mutations of the *p53* gene in patients with NSCLC.[10] No association was observed for *hOGG1* Ser326Cys polymorphism with *p53* mutation in patients with NSCLC, as we previously reported.[11]

Our previous report indicated a high frequency of deletion mutations in the *p53* gene in squamous cell lung cancer patients in Taiwan.[12] Conversely, an *EGFR* exon 19 deletion mutation frequently occurred in Asian nonsmoking female NSCLC patients, including nonsmoking female NSCLC Taiwanese patients.[13–15] The association of 8-OH-dG accumulation with the occurrence of deletion mutations has been shown in human lung tissues and in cardiac mitochondrial DNA during aging.[16,17] The accumulation of 8-OH-dG has also been correlated with mitochondrial DNA deletion in the kidneys of diabetic rats.[18] We therefore hypothesized that the *hOGG1* Ser326Cys polymorphism could be associated with deletion mutations of the *p53* and *EGFR* genes in NSCLC patients. In the present study, we discovered that deletion mutations in *p53* exon 5–8 and *EGFR* exon 19 were more frequent in Taiwanese NSCLC patients with *hOGG1*-Cys variants than with NSCLC the hOGG1 Ser/Ser genotype.

Methods

Study population

A total of 99 p53-mutated and 99 *EGFR*-mutated NSCLC patients were selected from our database to explore whether hOGG1 Ser326Cys polymorphism might be associated with the occurrence of p53 and *EGFR* deletion mutations. The selection procedure is outlined in Fig. 1. The hOGG1 Ser326Cys polymorphisms in adjacent normal lung tissues of this study population were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The *EGFR*-mutated patients in this study were divided into deletion and nondeletion mutation subgroups based on the presence of an exon 19 deletion mutation and exon 21 L858R point mutation of the *EGFR* gene for this study.

Direct sequencing of the p53 and EGFR genes

The DNA extraction procedures and DNA sequencing methods used in this study have been previously described.[11,19] Genomic DNA was isolated from primary tumor samples by overnight digestion with SDS and proteinase K at 37°C, followed by standard phenol-chloroform extraction and ethanol precipitation. Exons 5–8 *p53* gene mutations and exons 19 and 21 *EGFR* gene mutations were determined by direct sequencing. The primers used for

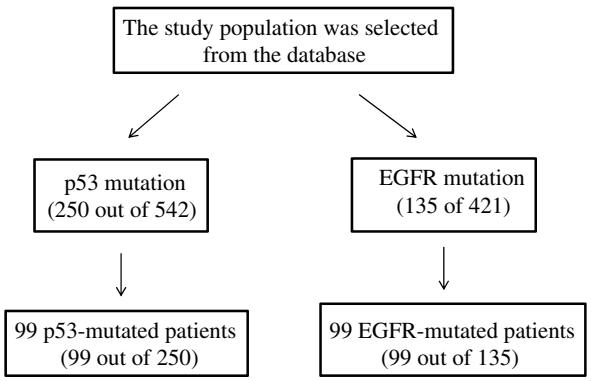

Figure 1 The flow chart of selection of study population for this study.

detection of both gene mutations were according our previous reports.[11,19] All polymerase chain reaction products were incubated with exonuclease 1 and shrimp alkaline phosphatase and then sequenced directly using an automated sequencing system (3100 Avant Genetic Analyzer; Applied Biosystems, Hitachi, Japan).

hOGG1 Ser326Cys polymorphism assays

Genotyping analysis of the *hOGG1* Ser326Cys polymorphism from genomic DNA of adjacent normal lung tissue from NSCLC patients was conducted by PCR-RFLP, as previously described.[11] Briefly, a 156 bp fragment was amplified by PCR in a 50 μL reaction volume that contained 50 ng of buccal cell genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 mM $MgCl_2$, 0.2 mM of each deoxynucleotide trisphosphate, 10 pmol of the hOGG1 sense (59-ACTGTCACTAGTCTCACCAG-39) and antisense (59-CCTTCCGGCCCTTTGGAAC-39) primers, and 2.5 units of Taq DNA polymerase (Eppendorf). The reaction mixtures were incubated at 95°C for two minutes, then for 40 amplification cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension step at 72°C for 10 minutes. 10 μL of each PCR sample was digested with five units of ItaI (New England Biolabs) at 37°C overnight and resolved on 8% native polyacrylamide gels to detect differences in RFLP patterns. The gels were stained with ethidium bromide, then examined and photographed over UV light. Selected PCR-amplified DNA samples from subjects possessing each of the three potential *hOGG1* genotypes were examined by dideoxy DNA sequencing (performed at the Molecular Biology Core Facility in the H. Lee Moffitt Cancer Center) for further confirmation of the *hOGG1* genotyping results.

Statistical analysis

Statistical analysis was performed using SPSS statistical software (Version 13.0 SPSS Inc., Chicago, IL, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Correlation of deletion mutation in *p53* and *EGFR* gene with patients' clinical parameters

A total of 99 *p53*-mutated and 99 *EGFR*-mutated lung cancer patients were randomly selected from our database and *hOGG1* Ser326Cys polymorphism was then examined in their adjacent normal lung tissues by PCR-RFLP. We divided the patients into nondeletion and deletion mutations of the *p53* and *EGFR* genes to explore whether deletion mutations of both genes could be associated with patients' clinical parameters, including age, gender, tumor histology, and stage. As shown in Table 1, *p53* deletion mutation was more common in male than in female patients ($P = 0.030$). No association of *p53* deletion mutation was observed with any other clinical parameters (Table 1). *EGFR* deletion in exon 19 was more frequently observed in female than in male patients ($P = 0.028$). In addition, *EGFR* deletion mutations were more likely to occur in exon 19 patients with adenocarcinomas than in patients with squamous cell carcinomas ($P = 0.017$, Table 1). However, the occurrence of an *EGFR* deletion mutation in exon 19 was not associated with age and tumor stage in this study population.

Association of *hOGG1* Ser326Cys polymorphism with occurrence of *p53* and *EGFR* deletion mutation in NSCLC patients

We next examined the possibility that deletion mutations of the *p53* and *EGFR* genes were more prevalent in patients with *hOGG1*-Cys variants than with the *hOGG1* Ser/Ser genotype. As shown in Table 2, 17 of 99 patients with *p53* deletion mutations had *hOGG1*-Cys variants, but no *p53* deletion mutations occurred in patients with the *hOGG1* Ser/Ser genotype ($P = 0.001$, Table 1). Consistently, *EGFR* exon 19 deletion mutations occurred more frequently in patients with *hOGG1*-Cys variants than with the *hOGG1*

Table 1 Association of p53 and *EGFR* deletion mutation with clinical characteristic of patients with NSCLC

	p53		*EGFR*	
Characteristic	Nondeletion	Deletion	Nondeletion	Deletion
Age				
<66	31	5	24	12
≥66	51	12	42	21
P-value	0.589		1.000	
Gender				
Female	39	3	49	31
Male	43	14	17	2
P-value	0.030		0.028	
Tumor histology				
AD	38	6	52	32
SQ	44	11	14	1
P-value	0.436		0.017	
Tumor stage				
I	12	1	29	17
II	7	0	7	1
III	63	16	30	15
P-value	0.247		0.400	

AD, adenocarcinoma; SQ, squamous cell carcinoma.

Table 2 Association of hOGG1 Ser326Cys polymorphism with occurrence of p53 and **EGFR** deletion mutation in patients with NSCLC

hOGG1 Ser326Cys polymorphism	Nondeletion	Deletion	*P*-value
p53 gene (*n* = 99)			
Ser/Ser	24 (28)	0	
Ser/Cys + Cys/Cys	58 (72)	17 (100)	0.010
EGFR gene (*n* = 99)			
Ser/Ser	29 (44)	6 (18)	
Ser/Cys + Cys/Cys	37 (56)	27 (82)	0.032

The deletion mutation of *EGFR* gene occurred on exon 19 and the nondeletion mutation of *EGFR* gene occurred on exon 21.

Ser/Ser genotype (82% vs. 18%, $P = 0.032$; Table 2). These results appear to support our hypothesis that *hOGG1*-Cys variants may be associated with the occurrence of *p53* and *EGFR* deletion mutations in Taiwanese NSCLC patients.

Discussion

The contribution of *hOGG1* Ser326Cys polymorphism to lung cancer risk has been reported to be increased by cigarette smoking habits.[7,8] A previous study indicated no association between *hOOG1* Ser326Cys polymorphism and the smoking-related *p53* G:C to T:*A* transversion mutation.[10] In the present study population, 34 of the 57 male patients (59.6%) were smokers, but no female patients were smokers (0 of 42, 0%). In addition, the *p53* deletion mutation was more commonly observed in male than in female patients ($P = 0.03$, Table 1). We therefore expected that the increase in lung cancer risk due to the interaction between *hOGG1* Ser326Cys polymorphism and cigarette smoking could be partially explained by the higher level of *p53* deletion mutations in patients with NSCLC. This observation was consistent with our previous report indicating that *p53* deletion mutations were more commonly observed in male than in female patients.[12]

The observation of *EGFR* mutations in this study seemed to be consistent with previous reports from our group and others indicating that the occurrence of an *EGFR* deletion mutation in exon 19 was found more frequently in females and adenocarcinoma patients.[13–15] Our previous report indicated that the association between the increase in 8-OH-dG levels due to human papillomavirus (HPV) 16/18 infection and the occurrence of *EGFR* mutations in patients with NSCLC might be due to a reduction in DNA repair capabilities.[19] Other groups have reported an association between lower ERCC1 expression, the SNP rs744154c in *ERCC4*, and *EGFR* exon 19 deletion mutations, thereby revealing the possibility that *EGFR* exon 19 deletion mutations could be associated with decreasing DNA repair capabilities.[20,21] In the present study, we observed that *EGFR* deletion mutations in exon 19 were more common in patients with *hOGG1*-Cys variants with less ability to remove 8-OH-dG than with *hOGG1* Ser/Ser genotype. The response to treatment with the tyrosine kinase inhibitors (TKIs) gefitinib or erlotinib was also more favorable for patients with *EGFR* exon 19 deletion mutations than with *EGFR* exon 21 L858R mutation.[14,15,22] Surprisingly, patients with *EGFR* exon 19 deletion mutations who were treated with gefitinib or erlotinib were more likely to have an *EGFR* T790M mutation and therefore resistance to gefitinib or erlotinib treatment.[23,24] The recurrence-free survival was also shorter and extrathoracic recurrence more frequent in patients with *EGFR* exon19 deletion mutations than with *EGFR* exon 21 L858R mutations.[25] Therefore, we suggest that detection of *hOGG1* Ser326Cys polymorphism could be used to predict *EGFR* exon 19 deletion mutations for early decisions on clinical treatment.

In summary, the association of *hOGG1* Ser326Cys polymorphism with lung cancer risk could be partially explained by an increasing occurrence of *p53* and *EGFR* deletion mutations. This possibility would seem to support previous reports indicating that *hOGG1*-Cys variants have a poorer ability to remove 8-OH-dG.

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Disclosure

The authors declare that there are no conflict of interests.

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