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

Ming-Jenn Chen1,2, Ching-Ju Shen3, Lee Wang4, Po-Ming Chen5, Chih-Yi Chen6 & Huei Lee5

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 Health, 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

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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 glycosyl bond between the oxidized base and the sugar moiety, leaving an abasic apurinic/apyrimidinic site in the DNA. 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.

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. 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. This difference arose because the hOGG1 Ser326Cys polymorphism was associated with removal of 8-OH-dG and was particularly induced by cigarette smoking. 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. No association was observed for hOGG1 Ser326Cys polymorphism with p53 mutation in patients with NSCLC, as we previously reported.

Our previous report indicated a high frequency of deletion mutations in the p53 gene in squamous cell lung cancer patients in Taiwan. Conversely, an EGFR exon 19 deletion mutation frequently occurred in Asian nonsmoking female NSCLC patients, including nonsmoking female NSCLC Taiwanese patients. 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. The accumulation of 8-OH-dG has also been correlated with mitochondrial DNA deletion in the kidneys of diabetic rats. 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. 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

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detection of both gene mutations were according our previous reports.\textsuperscript{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.\textsuperscript{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 MgCl₂, 0.2 mM of each deoxynucleotide triphosphate, 10 pmol of the hOGG1 sense (59-ACTGTCACATGCTCTACAG-39) and anti-sense (59-CCTTCCCGGCCTTTGAAC-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 Ital (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). \textit{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 (\textit{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 (\textit{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 (\textit{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 (\textit{P} = 0.001, Table 1). Consistently, EGFR exon 19 deletion mutations occurred more frequently in patients with hOGG1-Cys variants than with the hOGG1 Ser/Ser genotype.

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

AD, adenocarcinoma; SQ, squamous cell carcinoma.
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 hOGG1 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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