Although colorectal cancer is a highly malignant disease, little is known about the genetic factors that could alter the physiology of the colorectum and increase the risk of colorectal cancer development. Inherited deficiencies in the DNA repair system are associated with increased cancer susceptibility,1 and DNA repair gene polymorphisms may increase the risk of colorectal cancer. Oxidative DNA damage induced by reactive oxygen species (ROS) can cause mutations that are substrates for DNA repair systems in prokaryotes and eukaryotes.2 Base excision repair (BER) pathway genes are involved primarily in repair of ROS-associated DNA damage.3 The 8-oxoguanine DNA glycosylase (OGG1) gene, a BER gene family member, encodes a DNA repair enzyme that can directly remove 8-hydroxyguanine (8-oxoG), a major base lesion produced by ROS causing G:C to T:A transversions.4 The hOGG1 gene is located on human chromosome 3p25, a region frequently missing in various cancers, particularly lung and kidney tumors, which show loss of heterozygosity of markers.5

The 1245C>G polymorphism (Ser326Cys) is a well-known hOGG1 gene polymorphism that results in an amino acid substitution from serine to cystein in codon 326. Compared to the OGG1-Ser326 protein, the OGG1-Cys326 protein was shown to be less able to suppress spontaneous mutations in an Escherichia coli strain defective in 8-oxoG repair.6 Recently, the hOGG1 Ser326Cys variant has been consistently associated with an increased risk for a range of cancers.7

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polymorphism is associated with an increased risk of lung, esophageal and prostate cancer, whereas no association has been observed with breast cancer or basal cell carcinoma.\(^\text{1-13}\) The issue of whether the *hOGG1* Ser326Cys polymorphism is associated with colorectal cancer remains controversial.\(^\text{14,15}\)

In the present study, we investigated whether the *hOGG1* Ser326Cys polymorphism was associated with colorectal cancer risk by genotyping over 1,000 colorectal cancer patients and normal controls from the Korean population. In addition, we investigated whether the Ser326Cys polymorphism and colorectal cancer risk might be modulated by clinico-pathological characteristics such as tumor location, tumor-node-metastasis (TNM) stage or microsatellite instability (MSI) status.

### Samples and DNA Extraction

We collected 373 colorectal cancer patients from Seoul National University Hospital and the remaining 66 cases from National Cancer Center. All 676 healthy controls were collected from National Cancer Center (Korea). Eight out of 439 colorectal cancer patients were HNPCC cases and the others are all sporadic colorectal cancers. All colorectal cancer patients were pathologically diagnosed as colorectal cancers and surgically operated. Normal controls were selected from cancer-free samples enrolled from the Cancer Cohort Study Branch of the National Cancer Center. The mean ages of the cases and controls were 59.3 ± 12.5 and 50.3 ± 11.7 years, respectively. The case group comprised 283 males and 156 females, while the control group consisted of 400 males and 276 females. Of the 439 colorectal cancer subjects, 423 were able to be classified according to TNM. DNA was extracted from normal colorectal tissue of cancer patients, and from peripheral blood lymphocytes of normal controls. Total genomic DNA was extracted using Trizol\textsuperscript{®} reagent (Invitrogen, CA, USA) according to the manufacturer's instructions.

### Genotyping

The *hOGG1* Ser326Cys polymorphism (rs1052134) was genotyped using the TaqMan\textsuperscript{®} assay (7900HT, Applied Biosystems, CA, USA).\(^\text{16}\) Briefly, 5 μL reactions contained 2X Universal PCR Master Mix, 900 \text{nM} primers, 200 \text{nM} probe and 10 ng genomic DNA. Cycling conditions were as follows; 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 sec, and 60 °C for 1 min performed in 384-well plates. For quality control purposes, each assay run included all three genotype controls plus non-template controls. The *hOGG1* Ser326Cys genotyping was analyzed using allele discrimination plots using the Sequence Detection Software (SDS) program (version 5.0, Applied Biosystems, Foster City, CA). A part of samples were randomly confirmed using direct sequencing (ABI 3100 DNA sequencer, Perkin-Elmer, CA, USA).

### Statistical Analysis

Chi-square or Fisher's exact tests were used to assess differences in genotype distribution. Genotype-specific risks were estimated as odds ratios (OR) with 95% confidence intervals (CI) using logistic regression models. All statistical tests were performed using SPSS\textsuperscript{®} software (version 12.0, SPSS Inc, Chicago, IL). A p value of less than 0.05 was considered to indicate a significant difference.

We found that for colorectal cancer patients, 91/439 (20.7%) were homozygous for the C allele, 220/439 (50.1%) were heterozygous

### Table 1. *hOGG1* Ser326Cys polymorphisms in colorectal cancer patients and control subjects in this study and previous reports.

| Genotype       | Number (%)     | odds ratio (95% CI) | P      | Kim et al.\(^\text{*}\) | Hansen et al.\(^\text{*}\) |
|----------------|----------------|--------------------|--------|-------------------------|--------------------------|
|                | controls (n=676) | cases (n=439)      |        |                          |                          |
| Ser/Ser        | 120 (17.7)      | 91 (20.7)          | 1.00 (reference) | 0.454 | 21.1/19.2 | 52.5/61.2 |
| Ser/Cys        | 333 (49.3)      | 220 (50.1)         | 0.89 (0.64-1.25) | 0.502 | 53.0/52.8 | 41.4/33.3 |
| Cys/Cys        | 223 (33.0)      | 128 (29.2)         | 0.79 (0.55-1.14) | 0.215 | 25.9/28   | 6.1/5.5   |
| Combined genotype |               |                    |        |                          |                          |
| Ser/Ser + Ser/Cys | 453 (67.0) | 311 (70.8) | 1.00 (reference) |        |            |            |
| Cys/Cys        | 223 (33.0)      | 128 (29.2)         | 0.86 (0.66-1.13) | 0.289 |            |            |

Odds ratios adjusted by logistic regression for age and sex.

\*: genotype distribution of colorectal adenocarcinomas

Kim et al. from reference 14; Hansen et al. from reference 15.

CI: confidence interval
Table 2. Association between Ser326Cys genotypes and clinicopathological colorectal cancer features.

| Tumor location | n=439 | Ser/Ser (%) | Ser/Cys (%) | Cys/Cys (%) | P for trend |
|----------------|-------|-------------|-------------|-------------|-------------|
| Distal         | 273   | 57 (20.9)   | 144 (52.7)  | 72 (26.4)   |             |
| Proximal       | 166   | 34 (20.5)   | 76 (45.8)   | 56 (33.7)   | 0.232       |
| MSI status     |       |             |             |             |             |
| MSS\*          | 369   | 77 (20.9)   | 190 (51.5)  | 102 (27.6)  |             |
| MSI\'          | 58    | 13 (22.4)   | 26 (44.8)   | 19 (32.8)   | 0.619       |
| TNM stages     |       |             |             |             |             |
| I              | 17    | 2 (11.8)    | 10 (58.8)   | 5 (29.4)    |             |
| II             | 163   | 36 (22.1)   | 89 (54.6)   | 38 (23.3)   |             |
| III            | 151   | 34 (22.5)   | 75 (49.7)   | 42 (27.8)   |             |
| IV             | 92    | 15 (16.3)   | 43 (46.7)   | 34 (37.0)   | 0.381       |

* MSS, microsatellite stability; MSI, microsatellite instability

(C/G), and 128/439 (29.2%) were homozygous for the G allele. For normal controls, 120/676 (17.7%) were homozygous for the C allele, 333/676 (49.3%) were heterozygous (C/G), and 223/676 (33.0%) were homozygous for the G allele. The normal control genotype distributions were in Hardy-Weinberg equilibrium. Consistent with a previous report, we found that the G allele for the Ser326Cys polymorphism was more prevalent in Asian populations than in Caucasian populations. Analysis showed there was no association between the Ser326Cys polymorphism and colorectal cancer risk (Table 1). Analysis of Cys/Cys carriers versus combined Ser/Ser and Ser/Cys carriers also showed there was no association between Cys/Cys carriers and colorectal cancer risk (p=0.289) (Table 1). We stratified cases into HNPCC and non-HNPCC and re-analyzed to see any relationship between hOGG1 Ser326Cys polymorphism and stratified cases. There was no significant relationship between Ser326Cys and stratified cases (data not shown).

We investigated whether there was any association between the Ser326Cys polymorphism and clinico-pathological features such as tumor location, TNM stage and MSI status. This analysis found no association between the polymorphism and MSI status (p=0.619) (Table 2), tumor location (p=0.232) or TNM stage (p=0.381) (Table 2). The present study investigated hOGG1 polymorphism in colorectal cancers, and found no association between the Ser326Cys polymorphism and colorectal cancer risk. Of the two similar previous studies, Kim et al\(^{14}\) also found no association between the Ser326Cys polymorphism and colorectal cancer risk. In contrast to these findings, a study of over 1,000 Norwegians by Hansen et al\(^{15}\) concluded the hOGG1 Ser326Cys variant was indeed associated with colon cancer. They found that carriers of the rare Cys allele had a lower risk of colorectal cancer, particularly of adenocarcinoma (166 patients), but not a lower risk of adenoma. They concluded that the hOGG1 S326C polymorphism may be protective against colorectal cancer development but not carcinogenesis. These findings are not consistent with those of the present study involving over 400 carcinoma cases in the Korean population.

Previous reports suggest that the frequency of the hOGG1 Ser326Cys polymorphism depends on race and ethnicity.\(^{7,8}\) Studies involving a number of cancer types have shown that the Asian population tends to have a greater proportion of GG(Cys/Cys) genotypes than the Western population.\(^{7,11,18}\) Consistent with those reports, the present study found a higher proportion of Cys/Cys genotype frequencies in this Korean population (33.0% in controls, 29.2% in cases) compared to that reported for Western populations, in particular Norwegians (12.1% in controls, 7.9% in cases).\(^{15}\) Thus, it appears the hOGG1 S326C polymorphism may be differently distributed among different ethnic groups. These findings indicate the need for studies examining whether the Ser326Cys polymorphism is associated with colorectal cancer in larger Asian populations such as the Chinese or Japanese. Moreover, hOGG1 Ser326Cys polymorphism may not be a risk factor of colorectal cancers in the Korean population.

No study on the relationship between MSI and hOGG1 Ser326Cys polymorphism was reported in human cancers. Interestingly, it was reported that monoallelic MYH, one of BER genes, germline mutations showed a negative correlation with MSI in colorectal cancer patients.\(^{22}\) It was also reported that BER deficiency was rarely accompanied by CIN (Chromosomal insta-
Another study showed that MLH1-93G>A polymorphism was associated with an increased risk of MSI-H colorectal cancer. Thus, we investigated whether MSI might be associated with hOGG1 Ser326Cys polymorphism in colorectal cancers and found no significant result. This result suggests that there is no impact of hOGG1 Ser326Cys polymorphism to the MSI.

It is important to consider environmental factors in a case-control genotyping analysis. However, we could not get information on the alcohol consumption, physical exercise, smoking status, and folate consumption in our samples. This is a limitation on this study.

In summary, the present large-scale study of Koreans found that the Ser326Cys polymorphism was not associated with colorectal cancer development. Thus, although the hOGG1 Ser326Cys polymorphism is reported to be a risk factor in some cancer types, it does not appear to play a major role in colorectal cancer development, at least in the Korean population.

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