

Infrequent Mutations of the *hOGG1* Gene, That Is Involved in the Excision of 8-Hydroxyguanine in Damaged DNA, in Human Gastric Cancer

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DNA glycosylase, encoded by the *hOGG1* gene, repairs 8-hydroxyguanine (oh⁸Gua), which is an oxidatively damaged mutagenic base. To clarify whether the DNA repair activity of hOGG1 protein is involved in gastric carcinogenesis, we examined 9 gastric cancer cell lines and 35 primary gastric cancers for mutations and genetic polymorphisms of the *hOGG1* gene by polymerase chain reaction-single strand conformation polymorphism analysis. A G-to-A transition was detected in a gastric cancer cell line, MKN1. This nucleotide change caused the conversion of the amino acid from Arg to His at codon 154, which is located in a domain highly conserved among human, mouse, and yeast OGG1 proteins. No mutation was detected in primary gastric cancers. We compared the distribution of the polymorphic alleles associated with enzymatic activity (hOGG1-Ser³²⁶ vs. hOGG1-Cys³²⁶) between 35 gastric cancer patients and 42 healthy individuals. Although the frequency of the Cys³²⁶ allele, associated with low enzymatic activity, in gastric cancer patients was a little higher than that in healthy individuals, the difference did not reach statistical significance. These results suggest that low hOGG1 activity due to mutations and genetic polymorphisms is involved in the development of only a small subset of gastric cancers.

Key words: 8-Hydroxyguanine — hOGG1 — Oxidative DNA damage — DNA repair — Gastric cancer

8-Hydroxyguanine (oh⁸Gua) is a modified base found in oxidatively damaged DNA.¹⁾ The presence of oh⁸Gua in DNA causes G:C-to-T:A transversion, since oh⁸Gua pairs with cytosine and adenine nucleotides opposite the lesion.²⁻⁴⁾ Genes coding for a repair enzyme to excise oh⁸Gua from DNA have been cloned in bacteria (*mutM*) and yeast (*OGG1*).⁵⁻⁹⁾ Recently we and others isolated a structural human homologue (*hOGG1*) of the yeast OGG1 (*yOGG1*).¹⁰⁻¹⁶⁾ Since hOGG1 protein excises oh⁸Gua from DNA in the same manner as yOGG1, the *hOGG1* gene is considered to be a functional human homologue of the *yOGG1* gene.^{10, 12-17)}

oh⁸Gua is generated by oxidative metabolism as well as by chemical carcinogens, and the *hOGG1* gene is ubiquitously expressed in a variety of organs.¹¹⁾ Therefore, it is possible that DNA repair activity of hOGG1 protein is inversely associated with mutagenesis leading to the carcinogenic conversion of cells in diverse organs. It has already been reported that the level of oh⁸Gua was elevated in cancerous tissues compared with cancer-free surrounding tissues in the stomach.¹⁸⁾ Thus, it would be worth investigating whether the *hOGG1* gene is inactivated by genetic alterations in gastric cancers. Recently

we demonstrated that due to a genetic polymorphism at codon 326 of the *hOGG1* gene, hOGG1-Ser³²⁶ and hOGG1-Cys³²⁶ proteins were produced in human cells.¹⁹⁾ By a complementation assay using an *E. coli mutM mutY* strain, it was shown that the activity to suppress spontaneous mutagenesis of hOGG1-Cys³²⁶ protein was weaker than that of hOGG1-Ser³²⁶ protein.¹⁹⁾ Thus, it should also be elucidated whether or not the weaker repair ability of the hOGG1-Cys³²⁶ allele is associated with susceptibility to gastric cancer. Therefore, we searched for mutations as well as genetic polymorphisms in the *hOGG1* gene in 9 gastric cancer cell lines and 35 primary gastric cancers.

MATERIALS AND METHODS

Samples The nine gastric cancer cell lines used in this study were MKN1, MKN7, MKN28, MKN45, MKN74, OKAJIMA, TMK1, KATOIII, and HSC39.²⁰⁾ Gastric cancers and corresponding normal mucosae from a total of 35 sporadic cases were obtained at the time of surgery. Histopathologically, the 35 cases consisted of 24 intestinal types and 11 diffuse types according to Lauren's criteria, and were composed of 9 early and 26 advanced gastric cancers.^{21, 22)} Freshly frozen samples were obtained from Hamamatsu University Hospital, Fujieda Municipal Hos-

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pital, Fujinomiya City General Hospital, Seirei Mikatahara General Hospital, and Hamamatsu Rosai Hospital, in Shizuoka Prefecture in Japan. High-molecular-weight DNA was prepared as described previously.²³⁾

Mutation analysis of the *hOGG1* gene Exons 1 to 7 of the *hOGG1* gene were amplified by polymerase chain reaction (PCR) for single strand conformation polymorphism (SSCP) analysis using 6 sets of primers as described previously.^{19, 24)} Briefly, PCR was carried out by using 50 ng of DNA as a template, in a 20 μ l reaction mixture containing 0.25 μ l of [α -³²P]dCTP (3000 Ci/mmol, 10 Ci/ml). After 35 cycles of 95°C (60 s) for denaturation, 55°C (60 s) for annealing, and 72°C (90 s) for extension, PCR products were denatured and electrophoresed on 5 % polyacrylamide gel with/without 5% (vol/vol) glycerol under cooling with a fan in a cold room. The gels were dried and exposed to X-ray film at -80°C.

DNA sequencing DNA fragments corresponding to the shifted band were reamplified by PCR. PCR products were purified using a QIA quick-spin PCR purification kit (QIAGEN Inc., Chatsworth, CA) and directly sequenced with an *fmol* DNA cycle sequencing kit (Promega, Madison, WI).

Comparison of the genotypes and allele frequencies of the polymorphism at codon 326 The distribution of the three genotypes, two forms of homozygotes (Ser³²⁶/Ser³²⁶ and Cys³²⁶/Cys³²⁶) and a heterozygote (Ser³²⁶/Cys³²⁶), and the frequency of each allele were compared between 35 primary gastric cancer patients and 42 healthy individuals.¹⁹⁾ χ^2 analysis was performed for comparison.

RESULTS

Nine gastric cancer cell lines and 35 sporadic gastric cancer cases were analyzed for *hOGG1* mutations and polymorphisms. Six pairs of intron-based primers were used to amplify exons 1-7 of the *hOGG1* gene for PCR-SSCP analysis. A point mutation was detected in a gastric cancer cell line, MKN1 (Fig. 1, A and B). By a G-to-A transition in exon 3, arginine at codon 154 was replaced by histidine in MKN1. The wild-type allele was retained in this cell line. No mutation was detected in 35 primary gastric cancers. Five different types of genetic polymorphisms were detected in this study, and all the types of polymorphisms were the same as those we had previously identified.¹⁹⁾ A polymorphism leading to an amino acid exchange (Ser/Cys) and a silent polymorphism were found in exon 7 and exon 2, respectively, while three others were present in the noncoding regions or introns. We compared the distribution of genotypes and the allele frequencies of the polymorphism at codon 326 in exon 7 between 42 healthy individuals and 35 gastric cancer patients (Table I). The frequency of the Cys³²⁶ allele in

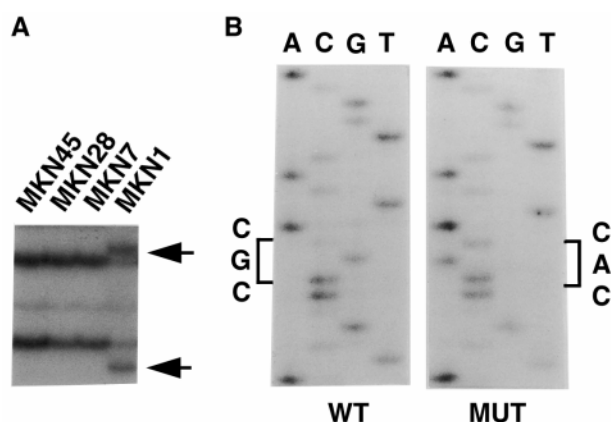


Fig. 1. A) PCR-SSCP analysis of exon 3 of the *hOGG1* gene in gastric cancer cell lines. Arrows (\blackleftarrow) indicate the mobility shifts detected in MKN1. B) Sequence analysis of the PCR product corresponding to the shifted band of MKN1. A G-to-A transition at codon 154 was detected in MKN1 (MUT) in comparison with the sequence of the wild-type (WT) allele.

Table I. Genotypes and Allele Frequencies of the Polymorphism at Codon 326 of the *hOGG1* Gene in Healthy Individuals and Gastric Cancer Patients

	Healthy individuals ^{a)}		Gastric cancer patients ^{b)}	
	<i>n</i>	%	<i>n</i>	%
Genotype ^{c)} :				
Ser ³²⁶ /Ser ³²⁶	15	35.7	9	25.7
Ser ³²⁶ /Cys ³²⁶	20	47.6	16	45.7
Cys ³²⁶ /Cys ³²⁶	7	16.7	10	28.6
Allele ^{d)} :				
Ser ³²⁶	50	59.5	34	48.6
Cys ³²⁶	34	40.5	36	51.4

a) *N*=42; genotypes and allele frequencies in healthy individuals were previously reported.¹⁹⁾

b) *N*=35.

a, b) All the subjects are Japanese.

c) *P*=0.396.

d) *P*=0.174.

gastric cancer patients was a little higher than that in healthy individuals. However, the distribution of the genotypes as well as the frequency of each allele were not significantly different between the two groups by χ^2 analysis (*P*=0.396 and *P*=0.174, respectively).

DISCUSSION

A nucleotide change at codon 154 detected in MKN1 gastric cancer cell line causes the conversion of the corresponding amino acid from arginine to histidine in a highly conserved domain among human, mouse, and yeast

OGG1 proteins.^{8, 11, 25} Since the corresponding normal tissue DNA for this cell line was not available, we could not determine whether it was a rare genetic polymorphism or a somatic mutation. However, since this nucleotide change was not detected in over 100 individual DNAs, it is likely that this variant is a somatic mutation rather than a genetic polymorphism. On the other hand, mutations of the *hOGG1* gene were not detected in primary gastric cancers. These results indicate that the *hOGG1* gene is infrequently mutated in human gastric cancers. Thus, although elevated levels of oh⁸Gua in gastric cancer tissues were previously reported,¹⁸ it is unlikely that such an elevation is due to mutational inactivation of the *hOGG1* gene in cancer cells.

Genotypes and allele frequencies of the polymorphisms at codon 326 were slightly different between healthy individuals and gastric cancer patients. The frequency of the Cys³²⁶ allele, which encodes a protein with low enzyme activity, was a little higher in gastric cancer patients. This result suggests that low hOGG1 activity in cells expressing the hOGG1-Cys³²⁶ protein may in part influence gastric carcinogenesis. However, differences in the distribution of the genotypes as well as the frequency of each allele at codon 326 between healthy individuals and gastric cancer patients did not reach statistical significance. Since relatively few cases were compared in this study and they were not matched strictly, a larger survey for polymorphism at codon 326 among gastric cancer patients and matched healthy individuals is in progress.

To elucidate the effect of the *hOGG1* mutation and polymorphisms on the oh⁸Gua levels in the cells, the amounts of oh⁸Gua in 9 gastric cancer cell lines were examined by high-performance liquid chromatography with electrochemical detection. The oh⁸Gua levels were not significantly different among these cell lines, irrespec-

tive of the presence of mutation and the types of genetic polymorphism. This result is consistent with our previous finding that the oh⁸Gua levels were similar among several lung cancer cell lines, irrespective of the types of hOGG1 protein expressed.¹⁹ Furthermore, the oh⁸Gua levels in gastric and lung cancer cell lines were not significantly higher than those in normal leukocytes. Thus, it was suggested that the enzymatic activities of any mutated and polymorphic hOGG1 proteins are high enough to maintain the oh⁸Gua contents at a steady level in the absence of severe oxidative stress. However, since there are several methodological problems involved in measuring the oh⁸Gua levels in DNA,^{26, 27} further studies will be necessary to assess critically the differences in the oh⁸Gua contents between cancer cells and non-cancerous cells, and to evaluate the differences in the ability of mutated and polymorphic hOGG1 proteins to repair oh⁸Gua *in vivo*.

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