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GENE-SPECIFIC OXIDATIVE DNA DAMAGE IN *HELICOBACTER PYLORI*–INFECTED HUMAN GASTRIC MUCOSA

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To study the status of oxidative DNA damage in *Helicobacter pylori* infection in more detail, we examined oxidative DNA damage to individual genes by determining the loss of PCR product of a targeted gene before and after gastric mucosal DNA was treated with 8-hydroxyguanine glycosylase, which cleaves DNA at the 8-hydroxyguanine residues. The results showed that, of the 5 genes tested, *p53, insulin-like growth factor II receptor* and *transforming growth factor-β receptor type II* showed significant oxidative DNA damage in *H. pylori*-positive tissues and that the *BAX* and *β-ACTIN* genes were relatively undamaged. These results suggest that in *H. pylori* infection, oxidative DNA damage does not occur homogeneously throughout the genomic DNA but, rather, in a gene-specific manner. We conclude that the progressive accumulation of preferential oxidative DNA damage in certain genes, such as *p53,* likely contributes to gastric carcinogenesis.

Key words: Helicobacter pylori; *8-hydroxydeoxyguanosine; gene-specific DNA damage; p53; gastric carcinogenesis*

Helicobacter pylori causes type B gastritis and is strongly associated with the development of gastric carcinoma.[1–4] Previous studies[5–8] have shown that oxidative DNA damage is elevated in *H. pylori*–infected human gastric mucosa by demonstrating increased amounts of 8-hydroxyguanine (oh^{8}G) in the DNA compared to noninfected subjects. oh^{8}G, a guanine residue that is hydroxylated at position 8 by reactive oxygen species (ROS), mismatches with A instead of C and causes GC to TA transversion; thus, this modified base is expected to mediate mutations in *H. pylori*–infected gastric tissue. The mutagenic action of oh^{8}G may cause substantial impairment of genetic integrity because the longstanding and lifelong active inflammation of gastric mucosa by *H. pylori* might override the ability to repair DNA damage. Thus, we suggest that an increased level of oxidative DNA damage mechanistically links *H. pylori* infection and gastric carcinoma.

Mutations in cancers tend to show gene preference, and mutations of this type have been frequently studied for diagnostic and prognostic purposes. Moreover, such mutations are also useful to elucidate the mechanistic basis of the carcinogenic processes involved.[9–13] In the case of gastrointestinal cancers, particularly in gastric carcinoma, *p53,*[14,15] *insulin-like growth factor II receptor (IGFIIR),*[16] *transforming growth factor-β receptor type II (TGFβRII)*[17] and *Bax*[18] are examples of genes that are preferentially mutated, which suggests that genes have individual susceptibilities to attack by various damaging agents. Based on this gene-selective mutation in cancer, we hypothesized that the oxidative DNA damage observed in *H. pylori* infection is not homogeneous throughout the genome but heterogeneous and gene-dependent.

To test this hypothesis, we used quantitative PCR and the principle that the formation of a PCR product is disrupted by cleavage of a DNA strand. Cleavage was induced at oh^{8}G sites by treating genomic DNA with 8-hydroxyguanine glycosylase (OGG1), which cleaves DNA at oh^{8}G residues after removing the oh^{8}G residue as a free base from the DNA strand.[19–21] Oxidative gene damage was evaluated by comparing quantitative differences between the PCR products of the targeted gene before and after treatment of gastric mucosal DNA with OGG1.

MATERIAL AND METHODS

Selection of volunteers

Our study was performed in accordance with the Declaration of Helsinki and approved by the Ethnics Committee at Gyeongsang National University Hospital. All volunteers gave written informed consent and were recruited from young people (aged 21–23) who had undergone endoscopy between October 1999 and March 2000 at the Department of Internal Medicine for various abdominal complaints. Patients either were normal or had ulcers, and none had cancer.

Endoscopic biopsy and diagnostic tests for H. pylori

Endoscopic biopsy and the laboratory tests used for *H. pylori* were performed as described in our previous study.[5] Briefly, the same endoscopist took 7 biopsy specimens of the gastric antrum and a serum sample from each volunteer. Biopsy samples were normal, erosive or ulcerous. Two biopsy specimens were used for microbial tests (culture and urease tests) and 2 for routine histologic and Warthin-Starry silver staining. The remaining 3 samples were immediately frozen and stored at –70°C until required.

OGG1 preparation

OGG1 was prepared using OGG1 cDNA synthesized from the mRNA of mouse (R1 strain of senescence-accelerated mice)[22] liver by RT-PCR, as described in our previous study.[23] Briefly, fragments of the entire coding sequence were amplified from cDNAs synthesized using the Expand Long Template PCR system (Boehringer-Mannheim, Mannheim, Germany), using 5′-CAT ATG TTA TTC AGT TCC TG-3′ and 5′-GGA TTC CTA GCC CTC TGG CCT CTT-3′ as primers, which contained sequences with NdeI and BamHI sites at the 5′ and 3′ ends of the resulting cDNA fragments, respectively. Amplified cDNA fragments were inserted into TA cloning vector 2.1 (Invitrogen, Groningen, the Netherlands), and the sequences of the inserts were confirmed using an ABI PRISM 377 DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Fragments containing the OGG1 coding region were isolated by NdeI and BamHI digestion and ligated to pET-15b vector into the 6-histidine tag. The vector, carrying the coding regions, was transfected in *Escherichia coli* BL21(DE3)pLysS, and the bacteria were cultured at 25°C for 7–8 hr. To induce the T7 RNA polymerase gene of *E. coli,* bacteria were cultured for 3–4 hr in the presence of isopropyl-thio-β-D-galactoside. Bacteria were then harvested and sonicated. To purify

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the OGG1 protein, supernatants so obtained were chromatographed twice at 4°C, using His-Bind resins (Novagen, Madison, WI) and an S-Sepharose column (Pharmacia, Uppsala, Sweden). The homogeneity of the purified protein was confirmed by a single band on SDS-polyacrylamide gel electrophoresis. The protein was kept at –20°C until use.

Endonuclease activity assay of OGG1 and treatment of genomic DNA with OGG1

The endonuclease activity of purified OGG1, *i.e.,* its ability to nick DNA at oh^{8}G residues, was assayed as follows. Two 21 mer oligonucleotides, 1 containing oh^{8}G (5′-CAGCCAATCAGTG*CACCATTC-3′, G* = oh^{8}G) and the other containing G at the oh^{8}G site, were chemically synthesized and labeled with ^{32}P at the 3′ terminus, according to the method of Tu and Cohen[24] with slight modification, as described previously.[25] They were then annealed with the unlabeled complementary oligonucleotide. Duplex DNAs (0.2 pmol) were incubated with or without the purified OGG1 in 20 μl of 50 mM TRIS-HCl, 50 mM KCl and 1 mM EDTA (pH 7.5) at 37°C for 15 min. Substrate DNA fragments, cleaved at the oh^{8}G residue, were detected autoradiographically, as described previously.[19,25] One unit was defined as the amount of enzyme that cleaved 1 pmol of substrate DNA at the oh^{8}G residues in 15 min.

For quantitative PCR, the genomic DNAs (50 ng) isolated from gastric samples were treated with 1 unit of OGG1 in 10 μl of reaction mixture for 1 hr, as described above. This reaction condition resulted in cleavage at all sites of oh^{8}G in 50 ng DNA (see Results).

Measurement of oxidative DNA damage at each gene level

This measurement was based on the assumptions that certain DNA regions are more likely to form oh^{8}G residues when subjected to oxidative stress and that PCR of such regions after OGG1 treatment produces a smaller amount of product due to nicks by OGG1 at the oh^{8}G residue sites. Thus, the difference between the amounts of PCR products of a targeted region before and after OGG1 treatment indicates the extent of oxidative damage to that DNA region.

An outline of the experimental procedure is shown in Figure 1. Briefly, DNA was isolated from each human gastric mucosa specimen using a QIAamp DNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Two DNA samples (50 ng) were prepared from each specimen, and 1 sample was treated with 1 unit of OGG1 at 37°C for 1 hr, as described above. The 2 DNA samples were then subjected to PCR amplification directed toward the same genomic region.

Quantitative PCR conditions

Each PCR solution (20 μl) contained DNA (50 ng) treated or untreated with OGG1, primers (20 pmol), Taq polymerase (1 unit; Stratagene, La Jolla, CA), dNTP (250 μM), KCl (40 mM), $MgCl_2$ (1.5 mM) and 50 mM TRIS-HCl (pH 7.5). PCR was carried out in a Perkin-Elmer GeneAmp PCR system 2400. The oligonucleotides used as primers for the 5 genes examined were as follows (numbers in parentheses indicate lengths of the resulting PCR products): 5′- GAA GAC CCA GGT CCA GAT GA -3′, 5′- GAA GAC GGC AGC AAA GAA AC -3′ for *p53* (901 bp); 5′- GAG GTT CTG ACC ATC CCA GA -3′, 5′- ATC TTT GGG CAG GTT GTT TG -3′ for *IGFIIR* (1,051 bp); 5′- TTT TCC ACC TGT GAC AAC CA -3′, 5′- CCC ACT GTT AGC CAG GTC AT -3′ for *TGFRII* (1,080 bp); 5′- GCA GAT CAT GAA GAC AGG GG -3′, 5′- CAG ACA CGT AAG GAA AAC GC -3′ for *Bax* (607 bp); and 5′- AAA TCT GGC ACC ACA CCT TC -3′, 5′- CAC CTT CAC CGT TCC AGT TT -3′ for *β-actin* (1,041 bp).

DNA untreated or treated with OGG1 was used for PCR, which was programmed to operate sequentially with a denaturation step of 94°C for 5 min, optimized reaction steps for each gene and a final extension step at 72°C for 10 min. The optimized reaction steps for each gene were as follows: 30 cycles of 94°C for 30 sec, 62°C for 1 min and 72°C for 1 min for *p53;* 30 cycles of 94°C for 30 sec, 64°C for 1 min and 72°C for 1 min for *IGFIIR;* 30 cycles of 94°C for 30 sec, 60°C for 1 min and 72°C for 1 min for *TGFβRII;* 26 cycles of 94°C for 30 sec, 59°C for 1 min and 72°C for 1 min for *Bax*; and 26 cycles of 94°C for 30 sec, 62°C for 1 min and 72°C for 1 min for *β-actin.* The optimal condition for each gene was determined from the experimental results shown below.

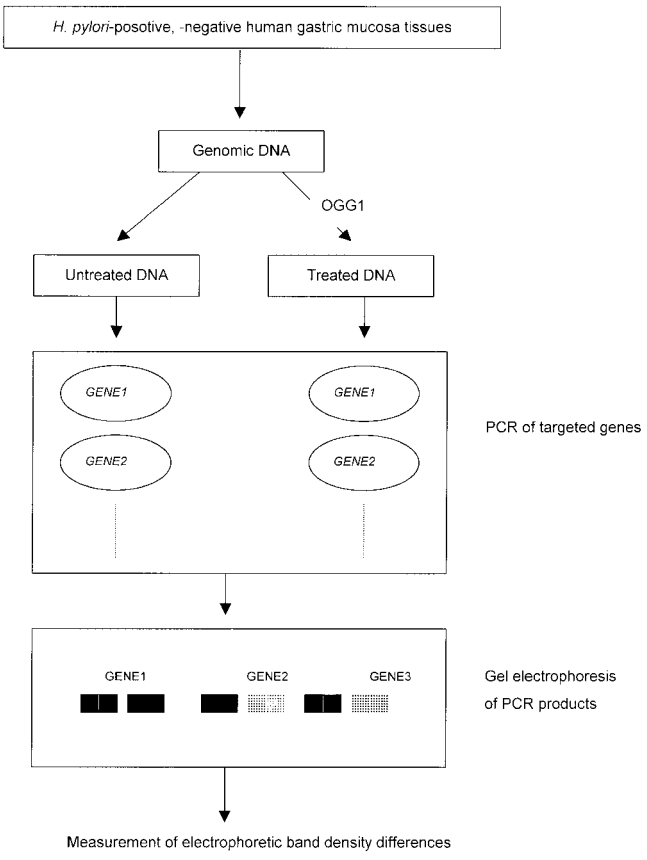

FIGURE 1 – Experimental scheme for the measurement of gene-dependant oxidative DNA damage. Details are described in Material and Methods.

After the PCR, mixtures were stored at 4°C until required. Each PCR was mixed with 5 μl of 6 × loading buffer (Promega, Madison, WI), loaded on a 1.2% agarose gel and electrophoresed at 100 V for 30 min in 0.5 × TBE buffer. PCR products were identified using a UV detector and photographed. Product band densities were determined using a densitometer (MCID; Imaging Research, St. Catharines, Canada) and used to quantify the oh^{8}G in the genomic DNA strand.

Quantification of oxidative DNA damage

Oxidative DNA damage in the region of interest of each gene was quantified in terms of the frequencies of DNA lesions using the difference between the amounts of PCR products obtained before and after OGG1 treatment. Assuming a random distribution of lesions and using a Poisson equation [$f(x) = e^{-\lambda}\lambda^x/x!$, where λ is the average lesion frequency] for the nondamaged molecules (*i.e.,* zero class, $x = 0$), the average lesion frequency per genomic DNA strand was determined using $\lambda = -\ln A_D/A_O$, where A_D is the band density of the PCR product obtained from the OGG1-treated DNA and A_O is the band density of the PCR product obtained from the untreated DNA.[26,27] Statistical analysis was performed using unpaired Student's *t*-test.

RESULTS

Bacteriologic, serologic and histopathologic data of human gastric mucosa biopsy samples

Twenty volunteers were examined for *H. pylori* infection. A volunteer was regarded as *H. pylori*-positive when the samples showed positive results on all of the following tests: bacterial culture, urease, immunoblot analysis for anti-*H. pylori* antibody and histopathology. If a subject showed negative results on all of these tests, he or she was regarded as *H. pylori*-negative. Results are summarized in Table I. Ten volunteers proved to be negative and the others positive. Gastric biopsy samples were described according to the diagnosis and used to investigate the oxidative damage of individual genes.

Determination of the optimal amount of OGG1 for genomic DNA nicking

The method used to measure the oxidative damage of individual genes is summarized in Figure 1. One of the crucial requirements for the accuracy of this method is that the oh^{8}G sites present in the DNA must be completely nicked. Thus, we undertook to determine the amount of OGG1 required. When 0.2 pmol of the substrate DNA was incubated with OGG1, the substrate DNA was completely cleaved in 15 min (Fig. 2). Based on this result, 1 unit of OGG1 was defined as the amount of this enzyme that cleaves 1 pmol of substrate DNA in 15 min. The calculation below indicates that 50 ng of genomic DNA used in the quantitative PCR contains 0.01 pmol of oh^{8}G. We decided to use 1 unit of OGG1 for 1 hr, which has the capacity to cleave 4 pmol of oh^{8}G and, thus, to cleave all oh^{8}G sites present in 50 ng of DNA. According to a previous report,[5] the amount of oh^{8}G in *H. pylori*-positive mucosa is $12.7 \pm 1.9/10^5$ G (normal guanine). Assuming that the level of oh^{8}G in *H. pylori*-positive mucosa was $30/10^5$ G, which doubles the mean value, 50 ng of the DNA from *H. pylori*-positive mucosa would contain about 3.75 pg of oh^{8}G [50 ng $\times$ 1/4 (assuming that 1 of the 4 bases is G) $\times$ $30/10^5$ (assuming that the m.w. of G and oh^{8}G is equal)], which gives about 0.01 pmol of oh^{8}G (assuming that the m.w. of oh^{8}G is about 400). Therefore, the capacity of 1 unit of OGG1 for 1 hr incubation is 400 times the maximal amount (0.01 pmol) of oh^{8}G present in 50 ng of genomic DNA.

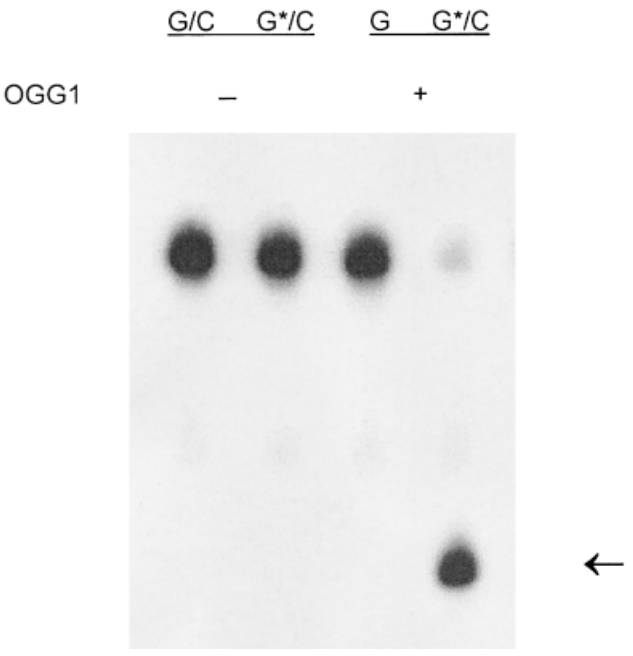

FIGURE 2 – Endonuclease activity of OGG1. Oligonucleotides (21 mer), 1 containing oh^{8}G (5′-CAGCCAATCAGTG*CACCATTC-3′, G* = oh^{8}G) and the other containing G at the oh^{8}G sit, were synthesized, labeled with ^{32}P at the 3′ terminus and annealed with the unlabeled complementary oligonucleotide. Each of the duplex DNAs (0.2 pmol) was incubated with or without purified OGG1 in 20 μl of 50 mM Tris-HCl, 50 mM KCl and 1 mM EDTA (pH 7.5) at 37°C for 15 min. Substrate DNAs and cleaved fragments created by oh^{8}G site cleavage were detected autoradiographically. G*/C, duplex DNA containing oh^{8}G; G/C, duplex DNA containing G at the oh^{8}G position. Arrow indicates the fragment cleaved at the oh^{8}G position.

TABLE I – BACTERIOLOGIC, SEROLOGIC AND HISTOPATHOLOGIC DATA OF HUMAN GASTRIC MUCOSA BIOPSY SAMPLES[1]

Diagnosis	Number of volunteers	Age (years)	Sex	Microbiologic examination			Histopathologic examination		
				Culture	Urease	Immunoblot	Lymphocytic infiltration[2]	Neutrophilic infiltration[2]	*H. pylori* density[3]
H. pylori-positive	1	20.1	M	+	+	+	2	2	3
	2	21.0	F	+	+	+	3	2	2
	3	23.4	F	+	+	+	3	2	3
	4	21.5	M	+	+	+	3	1	3
	5	22.4	M	+	+	+	3	3	3
	6	23.1	F	+	+	+	3	1	2
	7	20.9	F	+	+	+	3	1	2
	8	20.8	M	+	+	+	3	3	2
	9	21.6	F	+	+	+	3	2	1
	10	22.3	M	+	+	+	3	3	2
H. pylori-negative	1	22.5	F	–	–	–	1	0	0
	2	20.1	M	–	–	–	1	0	0
	3	22.5	F	–	–	–	1	0	0
	4	23.1	M	–	–	–	1	0	0
	5	22.5	M	–	–	–	1	0	0
	6	21.9	M	–	–	–	1	0	0
	7	22.6	M	–	–	–	1	0	0
	8	21.6	F	–	–	–	1	0	0
	9	20.9	F	–	–	–	1	0	0
	10	22.1	F	–	–	–	1	0	0

[1]As described in Material and Methods, 7 biopsy samples were taken from 1 volunteer. Two were used for culture and urease test within 1 hr of collection and 2 for routine histologic and Warthin-Starry stainings. The remaining 3 samples were used for the oxidative DNA damage study. The remaining samples were diagnosed as *H. pylori*-positive if taken from a positive volunteer or as *H. pylori*-negative if taken from a negative volunteer. Ten volunteers were *H. pylori*-positive and 10 were *H. pylori*-negative.–[2]Based on routine histologic staining: grade 0, absent; grade 1, mild; grade 2, moderate; grade 3, severe (graded according to the Sydney System).–[3]Based on Warthin-Starry silver staining; grades determined by arbitrary criteria.

Quantitative PCR

The cycle number and template DNA quantity are crucial requirements for the accuracy of the method, described in Figure 1, and can give a constant and reproducible amount of product in each PCR. To determine the optimal values for these 2 parameters, the kinetics of PCR product formation were studied for the candidate genes *vs.* cycle number (Fig. 3*a*) and the amount of template DNA (Fig. 3*b*). As can be seen in Figure 3, *p53, IGFIIR* and *TGFβRII* showed exponential kinetics when the cycle number ranged between 25 and 30. *Bax* and *β-actin* also showed exponential phases but in a slightly lower cycle range of 20–30 (data not shown). A linear relationship between the PCR yield and the quantity of template DNA used (20–200 ng) was observed for all 5 genes. Thus, the conditions for the oxidative damage assay of each gene in Figure 1 were fixed as follows: the amount of DNA used for PCR was 50 ng, 1 unit of OGG1 was used for 1 hr and 30 PCR cycles were used for *p53, IGFIIR* and *TGFβRII* and 26 cycles for *Bax* and *β-actin.*

Oxidative DNA damage at individual gene levels in H. pylori *infection*

To determine the level of oxidative DNA damage in individual genes in *H. pylori* infection, the DNA isolated from *H. pylori*-negative and -positive samples was treated as described in Figure 1 under conditions determined as above. Figure 4 shows electrophoretic comparisons of the PCR products of each gene obtained before and after OGG1 treatment. Of the 5 genes, *p53, IGFIIR* and *TGFβRII* showed definite differences in many of the *H. pylori*-positive samples (samples 1–4 and 6–10 for *p53,* samples 2–4 and 6–10 for *IGFIIR* and samples 1, 3–5 and 7–10 for *TGFβRII*). *Bax* also showed differences (samples 1–4, 7 and 8), which, however, were not as marked as those of the other 3. In contrast, *β-actin* showed no difference in any of the *H. pylori*-positive samples. However, none of the 5 genes in the *H. pylori*-negative samples showed such marked differences; only 3 (samples 1, 4 and 8) showed differences in *Bax*, 1 (sample 1) in *IGFIIR* and 2 (samples 3 and 10) in *TGFβRII.* No differences were found in *p53* or *β-actin.*

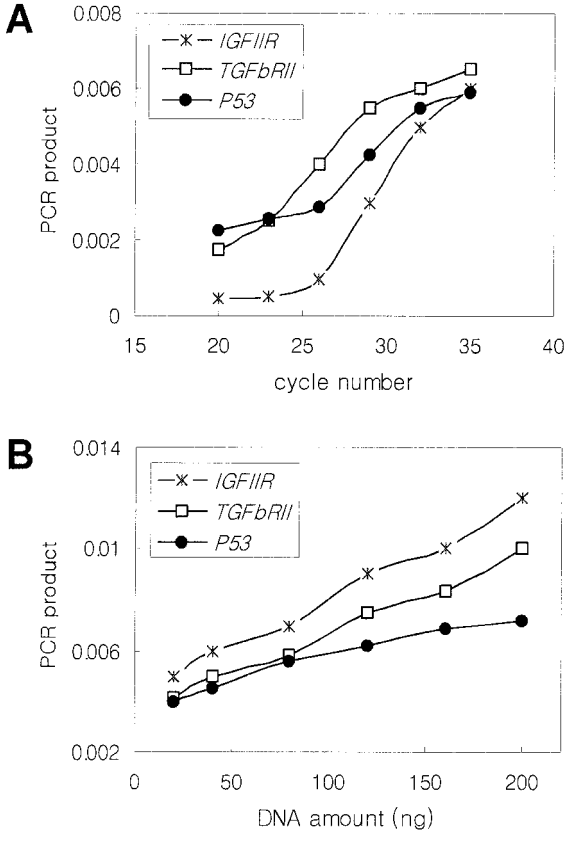

FIGURE 3 – Kinetics of PCR product formation for *IGFIIR, TGFβRII* and *p53.* PCR was performed with different cycle numbers *(a)* or different amounts of template DNA *(b).* PCR product quantities are expressed as electrophoretic band densities, as determined by densitometry ($n = 3$).

Differences in the amounts of PCR product of each gene were quantified as OGG1-sensitive sites/kb using the Poisson equation.[26,27] Results are summarized in Table II. Of the 5 genes, *p53, IGFIIR* and *TGFβRII* showed significant *H. pylori*-negative and -positive differences in OGG1-sensitive sites. *BAX* also showed differences, but these were not statistically significant. No difference was observed in the *β-ACTIN* gene.

DISCUSSION

H. pylori infection induces active inflammation with neutrophilic infiltration and chronic inflammation with infiltration of lymphocytes and macrophages/monocytes into the lamina propria of the mucosa of the human gastric antrum.[28–31] These neutrophils and macrophages/monocytes produce ROS or reactive nitrogen species,[9,32] which have the potential to cause DNA damage to adjacent gastric cells, such as gastric glandular epithelial cell and deep foveolar cells.[8] Moreover, the life-long effect of *H. pylori*–induced inflammation might cause accumulation of DNA damage. This DNA damage, particularly in some important genes in the stomach tissue, could have harmful consequences, including gastric carcinoma. Our observations support this presumptive scenario by showing that oxidative DNA damage to gastric cells occurs preferentially in some genes, *e.g., p53, IGFIIR* and *TGFβRII* (Fig. 4, Table II).

The reasons for this preferential oxidative attack on *p53, IGFIIR* and *TGFβRII* in *H. pylori* infection are unclear. The extent of damage and repair in certain DNA regions appears to be influenced by several factors, *e.g.,* the nucleotide sequence, the transcriptional or replicative state, the nature of the damaging agents and the type of lesion.[33–38] The mutations observed in these 3 genes and *Bax* were found to be due to microsatellite (oligonucleotide repeat sequence) instability, which is considered a mutator phenotype that results from insufficient mismatch repair.[15–18,39] However, in the present study, preferential attack of *p53, IGFIIR* and *TGFβRII* was observed in regions not containing repeat sequences because the PCR was designed to avoid these repeat sequences. Therefore, in addition to microsatellite instability, preferential oxidative damage may contribute to preferential mutation in these genes. *p53, IGFIIR, TGFβRII* and *Bax* function mainly as cell growth regulators.[40–42] Under such stressful conditions as *H. pylori* infection, these genes are believed to be in an active state of cellular defense preparedness and are thus rendered susceptible to ROS attack. The ROS damage causes functional impairment or loss of each of these genes, and the resulting imbalance in their functional equilibrium would be expected to increase the likelihood of uncontrolled cell growth.

There are a number of shortcomings in the present study. First, the stomach lesions were multifocal in nature, and each biopsy specimen was taken from a different site. For this reason, the biopsy specimens used for the oh^{8}G examinations and the diagnostic laboratory tests were unavoidably different, which suggests that *H. pylori*-negative samples might exist in the *H. pylori*-positive group and *vice versa.* In Figure 4, *H. pylori*-negative samples showing different band densities and *H. pylori*-positive samples showing negligible differences may provide examples of this limitation. Another problem concerns the size determination of the PCR products. Naturally, the longer the product, the more correct the estimation of oxidative damage. However, the longer strands tend to be associated with

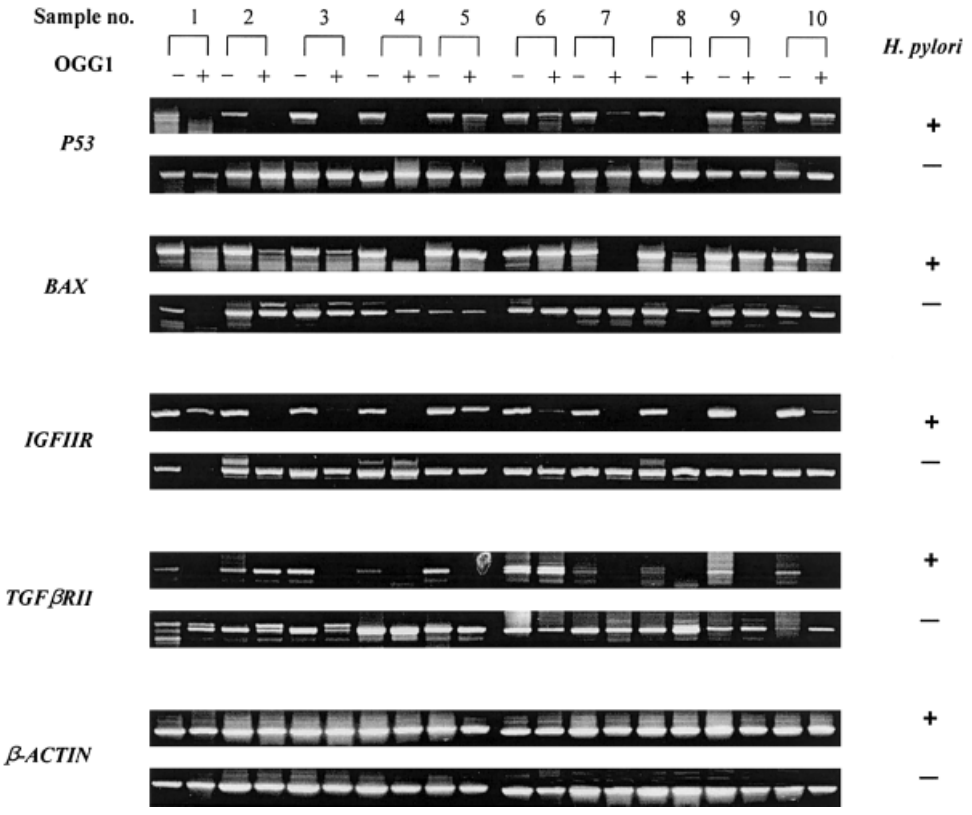

FIGURE 4 – Electrophoretic detection of PCR products of a targeted gene before and after OGG1 treatment of DNA isolated from individual biopsy samples. DNA was isolated from each biopsy sample. Two DNA samples (50 ng) were prepared for each gene, and 1 DNA sample was treated with OGG1. The 2 DNA samples were subjected to PCR using primers targeting the gene region of interest. PCR products obtained from each of the DNAs were loaded as a pair into a 1.2% agarose gel and visualized as bands; their band densities were compared. Paired band densities were used to determine the amount of oxidative DNA damage to the gene, which was expressed as OGG1-sensitive sites/kb, as summarized in Table II.

TABLE II – EVALUATION OF *H. PYLORI*-INDUCED OXIDATIVE DNA DAMAGE ON AN INDIVIDUAL GENE[1]

Genes	OGG1-sensitive sites/kb		p (n = 10) (Student's t-test)
	H. pylori		
	Positive	Negative	
p53	0.343 ± 0.088	0.004 ± 0.010	0.001
Bax	0.335 ± 0.107	0.114 ± 0.067	0.083
IGFIIR	0.437 ± 0.087	0.048 ± 0.041	0.001
TGFRII	0.441 ± 0.091	0.032 ± 0.018	<0.001
β-*actin*	0.009 ± 0.003	0.006 ± 0.004	0.600

[1]Using the electrophoretic results detailed in Figure 4, oxidative DNA damage to each gene was estimated using the relative differences of band densities of PCR products obtained before and after OGG1 treatment of DNA isolated from each biopsy sample.

fluctuations in the amounts of PCR products. We examined various PCR product sizes up to several thousand base pairs and found that a size of about 1,000 bp was satisfactory in terms of producing reproducible amounts of PCR products and oxidative damage detection sensitivity. Lastly, DNA damage might be overestimated by additional DNA damage other than that caused by oh^{8}G or by apoptotic degradation of DNA in infected tissues,[6] which could block the progression of thermostable polymerase during PCR. However, even with such limitations, this method was capable of detecting susceptible genes since significant differences were found in oxidative DNA damage of genes, whereas no significant differences were found in others.

In conclusion, we present a method of assaying gene-specific oxidative damage. Using this method, we found that *p53, TGFβRII* and *IGFIIR,* which function as cell growth regulators, are preferentially damaged in *H. pylori* infection. In the present study, the gene-dependant assay of oxidative damage was applied only to the 5 genes described. However, it is hoped that this assay method will aid the identification of further susceptible genes. Particularly, to improve our understanding of the role of *H. pylori* in gastric carcinogenesis, this method should be applied to genes that are active but not linked to increased mutation in gastric cancer.

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