RESEARCH NOTE

OGG1, MYH and *MTH1* gene variants identified in gastric cancer patients exhibiting both 8-hydroxy-2′-deoxyguanosine accumulation and low inflammatory cell infiltration in their gastric mucosa

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Introduction

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the main DNA modifications produced by reactive oxygen species (ROS). Because 8-OHdG can pair with cytosine and adenine bases during DNA synthesis, when 8-hydroxyguanine (8-OHG) is present in the DNA template, it causes G:C to T:A transversions (Shibutani *et al*. 1991), and it induces A:T to C:G transversions when 8-hydroxy-dGTP in the nucleotide pool is incorporated into DNA (Maki and Sekiguchi 1992). Because of these transversions, 8-OHdG accumulation is thought to cause carcinogenesis. 8-OHdG accumulation in mammalian cells is prevented by the base excision repair enzymes OGG1, MYH and NEIL1, and by MTH1, an enzyme that removes 8-hydroxy-dGTP from the intracellular nucleotide pool (Nakabeppu 2001). A variety of factors, including sodium chloride, *Helicobacter pylori* infection and smoking (Tredaniel *et al*. 1997; Farinati *et al*. 1998), induce inflammation in the stomach tissue. A considerable inflammatory cell infiltrate in the gastric mucosa causes the production of ROS (Ernst 1999), and ROS are thought to lead to 8-OHdG accumulation in the gastric mucosa (Farinati *et al*. 1998), suggesting that the level of inflammatory cell infiltration in the stomach may be one of the factors that determine the 8-OHdG level in the stomach. We, therefore, hypothesized that gastric cancer patients with both 8-OHdG accumulation and low level of inflammatory cell infiltration in their stomach have genetic factors that cause them to have a low ability to repair 8-OHdG. We selected 23 patients exhibiting mild or no neutrophil and mononuclear infiltration, and intense immunoreactivity for 8-OHdG in noncancerous gastric antral tissue, by hematoxylin-eosin (HE) staining and 8-OHdG immunostaining, respectively, of specimens from 231 gastric cancer patients. The 23 patients selected were then searched for variants of the *OGG1, MYH, NEIL1* and *MTH1* genes by direct sequencing. Three, three, and four single nucleotide polymorphisms (SNPs) were detected in *OGG1, MYH*, and *MTH1*, respectively. They included *OGG1*-c.977C>G (p.Ser326Cys), which is associated with a difference in 8-OHdG repair activity, and *MTH1*-c.247G>A (p.Val83Met), which has been reported to be associated with increased risk of gastric cancer. These results suggest that the SNPs of *OGG1*-c.977C>G (p.Ser326Cys) and of *MTH1*-c.247*G*>A (p.Val83Met) may be a cause of 8-OHdG accumulation in the gastric mucosa.

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Materials and methods

Case selection by histopathological analysis

In this study, the stomach tissue from 231 gastric cancer patients treated at Hamamatsu University Hospital were used. Their mean age was 64.7 years (standard deviation (SD: 11.9)), and the sample included 160 men and 71 women. The tumour histology was classified as the intestinal-type in 116 cases and the diffuse-type in 115 cases. Paraffin blocks of noncancerous gastric antral tissue from each patient were sectioned and stained with HE for histopathological analysis. The Sydney System was used to grade infiltration by neutrophils and mononuclear cells (0, absent; +, mild; ++, moderate; +++, severe) (Dixon *et al*. 1996). Sections graded 0 or +, determined by the diagnosis, were selected for

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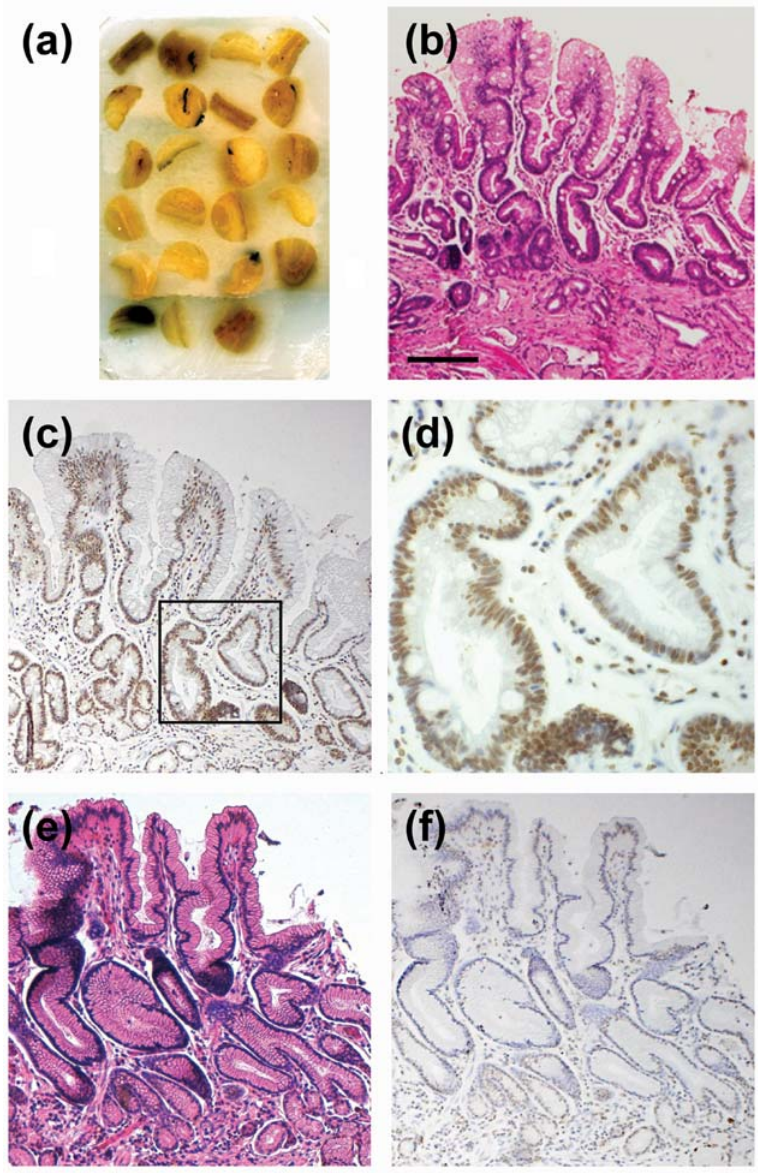

Figure 1. Evaluation of inflammatory cell infiltration and 8-OHdG accumulation in unaffected gastric mucosa. (*a*) Tissue microarray block used for 8-OHdG immunostaining. The diameter of cylinder is 5 mm. (*b*) HE-stained gastric mucosa section from a case selected for mutational analysis. Scale bar represents 0.1 mm. Mild neutrophil and mononuclear cell infiltration is seen. (*c*) Immunohistochemically stained section of gastric mucosa from the same case as in (b). Intense immunoreactivity for 8-OHdG is seen. (*d*) A higher magnification of the area enclosed by the square in (c). (*e*) HE-stained gastric mucosa section from a case not selected for mutational analysis. Mild neutrophil and mononuclear cell infiltration is seen. (*f*) Immunohistochemically stained section from the case shown in (*e*). Mild 8-OHdG immunoreactivity is seen.

immunohistochemical analysis. This study was approved by the Institutional Review Board of Hamamatsu University School of Medicine (12–14).

Case selection by immunohistochemical analysis

A tissue microarray block was prepared by transferring a cylinder with a diameter of 5 mm from each paraffin-embedded tissue selected by histopathological analysis (figure 1a), and sectioned. After deparaffinization and rehydration of the sections, they were microwaved for 5 min in 0.01 mol/l citric acid buffer for antigen retrieval. Endogenous peroxidase activity was blocked by incubation for 10 min in a 0.3% hydrogen peroxide/methanol buffer, and the sections were incubated with an anti-8-OHdG monoclonal antibody

(Nikken SEIL, Fukuroi, Japan) at a dilution of 1:50 for 1 h at room temperature. After the primary antibody reaction, the sections were incubated with the Envison$^+$ polymer reagent, which is a secondary antibody conjugated with peroxidase-labelled dextran (DAKO, Kyoto, Japan) for 1 h at room temperature. The antigen–antibody complex was visualized with 3, 3′-diaminobenzidine tetrahydrochloride (DAB) hydrogen peroxide solution and counterstained with hematoxylin. Immunohistochemical staining was graded according to the following system: +, very mild; ++, mild; +++, moderate; ++++, intense; +++++, very intense. Based on the results of the evaluation by HE staining and 8-OHdG immunostaining, 23 gastric cancer cases were selected from 231 gastric cancer patients. The clinicopathological data of these 23 cases were: mean age, 69.1 years (SD: 11.6); 13 men and 10 women; histological classification, intestinal-type in 16 and diffuse-type in 7; early-stage gastric cancer in 14 and advanced-stage gastric cancer in 9.

Mutation analysis by direct sequencing

DNA was extracted from noncancerous gastric mucosa with a DNeasy Tissue Kit (QIAGEN, Valencia, CA). All the coding exons of the *OGG1, MYH, NEIL1*, and *MTH1* genes and their boundary regions were amplified by PCR with HotStarTaq DNA polymerase (QIAGEN, Valencia, CA). Information on the *OGG1, MYH, NEIL1* and *MTH1* PCR primers is summarized in table 1. The PCR primers were designed so that the PCR products would be no longer than 320 bp. The PCR products were directly sequenced with a BigDye Terminator Cycle Sequencing Kit and the ABI 3100 Genetic Analyzer (Applied Biosystems, Tokyo, Japan).

Statistical Analysis

Linkage disequilibrium was measured by Lewontin's D′, which is calculated by using SNPAlyze version 3.2 software (Dynacom, Yokohama, Japan).

Table 1. Primers used for PCR amplification of *OGG1, MYH, NEIL1* and *MTH1* exons.

Gene	Exon	Forward sequence (5′ → 3′)[a]	Reverse sequence (5′ → 3′)[a]	Size (bp)
OGG1	1	(c.1-99) cttaagggtcgtggtccttg	(c.137+33) gaggggacaggcttctcag	268
OGG1	2	(c.138-26) agggttgtcatgtgccttg	(c.385+44) cttatgtccaagaaccctaaccc	317
OGG1	3	(c.386-41) cagcaggtacctctcctaccc	(c.565+55) gaacagatcttgaaagctgatgg	275
OGG1	4	(c.566-43) cttgaagatgcctgatgcttg	(c.747+35) gtagagagggcagctcctacc	259
OGG1	5	(c.748-69) gggctataagcaagatgctgg	(c.898+55) gagaagtttctaccatcccagc	274
OGG1	6	(c.899-79) cacctctccctcagaccctac	(c.948+35) gaggaaacctagggaggacac	163
OGG1	7	(c.948+197) ctgaccccagtgtaccctc	(c.948+364) tggggaatttctttgtccag	167
OGG1	8-1	(c.949-51) cttgtgcaggacagcaatctc	(c.1275-148) ggtccagcagtggtcacacg	230
OGG1	8-2	(c.949+123) agcttccctccagcctctc	(c.1275+41) ctccaggcttacatcctaccag	243
MYH	1a	(c.1-45) gaagctgcgggagctgaaac	(c.36+79) cggcgacccgacggcgagac	159
MYH	1b	(c.36+143) aattgcctattggcctgtgc	(c.36+362) ttctctggaaagcccaaacc	219
MYH	2	(c.37-47) ttggcctcattgtgactgac	(c.157+25) cttgggccacaacctagttc	192
MYH	3	(c.158-11) agcctgtgcagggatgattg	(c.339+29) cccactgtccctgctcctc	221
MYH	4	(c.340-30) ctaactcctcatctggggttgc	(c.379+93) gaggacactgctgacctgcc	162
MYH	5	(c.380-34) cagcagtgtcctcatgcca	(c.453+55) gaggctctcatctggggtctg	162
MYH	6	(c.454-44) cttcaccttgaccttgtctc	(c.495+70) cagaggtcaaagagatcaccc	155
MYH	7	(c.496-34) gggtgatctctttgacctctg	(c.567+78) cacctgattggagtgcaagac	183
MYH	8	(c.568-57) ggtaggaacccaggagtcttg	(c.681+70) caaagagttagcctgggctg	240
MYH	9	(c.682-36) ccagcccaggctaactctttg	(c.779+40) cagacacccctgaagcacc	169
MYH	10	(c.780-43) ctgcaaaggagctctgcttc	(c.924+41) ctccttaggacttctcactgcc	228
MYH	11	(c.925-29) cctatgacactcaaccctgtgc	(c.988+51) cttctgactgggccaggaag	143
MYH	12	(c.989-33) gcttgagtagggttcgggg	(c.1177+41) gttactcatgccactgccctc	262
MYH	13	(c.1178-52) gagggaatcggcagctgag	(c.1314+32) caacatccttggctattccgc	220
MYH	14	(c.1315-29) ctatttgaacccccttgaccc	(c.1467+106) ctccgtctcaaaaaaaaatgc	287
MYH	15	(c.1468-39) aaaaagtgccagccctcacc	(c.1509+54) acctatggactcaggcctgg	134
MYH	16	(c.1510-41) tcccccaactacaaggcctc	(c.1641+33) aggattctcagggaatgggg	205
NEIL1	1-1	(c.1-79) agccgctacctcacaaagtc	(c.1+148) gaagctgagatgcggtagg	228
NEIL1	1-2	(c.1+101) caaccctgaggtgccctttg	(c.434-106) gtccacgaaacatagggcgag	247
NEIL1	1-3	(c.434-143) ccacctgcgcttttacacg	(c.434+78) cttgttggccaagaaggcac	222
NEIL1	2	(c.435-25) acgcacccagaccgtgttc	(c.554+78) ggcaagccatgctaggcag	223
NEIL1	3	(c.555-45) cacattccccactgcctagc	(c.618+30) cacgtgtgctccatccctc	139
NEIL1	4	(c.619-50) ctttacatggccctgctgac	(c.718+40) catttgctgaagcagaggttg	190
NEIL1	5	(c.719-109) tcagtccatcagcttgtgtag	(c.846+32) ggtctctgcctgtgtgaac	269
NEIL1	6	(c.847-72) cctgcctctccaaggaatac	(c.874+52) aggcgtgaagtcctttctg	152
NEIL1	7	(c.875-32) gaccctccaactccaacacc	(c.936+113) gaggttggttagggcccac	207

Table 1 *(contd.)*

Gene	Exon	Forward sequence (5′ → 3′)[a]	Reverse sequence (5′ → 3′)[a]	Size (bp)
NEIL1	8	(c.937-66) tgaactgctttctgagcccc	(c.1102+61) ccaccatcccatcctctcc	270
NEIL1	9	(c.1103-46) cagcccctggagtcttagc	(c.1173+93) accttcagatattgcctgctc	210
MTH1	2	(c.1-47) ggagcaatcagatcacacg	(c.57+74) gaaccatgagtttggcagg	177
MTH1	3	(c.58-39) cacgtcatggctgactctg	(c.221+41) tgggaaagccggttctatg	243
MTH1	4	(c.222-72) tcctccctgccatcgtgtg	(c.367+43) gacccgcatagtggggagg	260
MTH1	5	(c.368-29) cagtgcctcctcttccccc	(c.540+75) aatgcccccaggtgaagatg	277

[a]The positions of 5′ end of primers are indicated by the number of nucleotides upstream (−) or downstream (+) from the nearest coding region. The reference sequences of the coding regions for the *OGG1, MYH, NEIL1*, and *MTH1* genes are CCDS ID CCDS2576.1, CCDS520.1, CCDS10278.1 and CCDS5329.1, respectively.

Table 2. The *OGG1, MYH*, and *MTH1* SNPs detected in 23 gastric cancer patients exhibiting both 8-OHdG accumulation and mild or no inflammatory cell infiltrate in unaffected gastric mucosa.

Gene	Nucleotide change[a]	Predicted effect	Cases with alleles heterozygous for the variant type (%)	Cases with alleles homozygous for the variant type (%)
OGG1	c.-24A>G	Unknown	2/23 (8.7%)	0/23 (0%)
OGG1	c.751-15C>G	Unknown	10/23 (43.5%)	7/23 (30.4%)
OGG1	c.977C>G	p.Ser326Cys	13/23 (56.5%)	4/23 (17.4%)
MYH	c.36+267C>T	Unknown	1/23 (4.3%)	0/23 (0%)
MYH	c.462+35G>A	Unknown	7/23 (30.4%)	0/23 (0%)
MYH	c.972G>C	p.Gln324His	12/23 (52.2%)	5/23 (21.7%)
MTH1	c.-1598T>C[b]	p.X14ArgextX185[c]	6/23 (26.1%)	0/23 (0%)
MTH1	c.247G>A[b]	p.Val83Met	5/23 (21.7%)	0/23 (0%)
MTH1	c.357C>T	p.Asp119Asp	1/23 (4.3%)	0/23 (0%)
MTH1	c. ∗49C>T	Unknown	1/23 (4.3%)	0/23 (0%)

[a]Nucleotide +1 is the A of the ATG-translation initiation codon. The reference sequences for the *OGG1, MYH*, and *MTH1* genes are accession number NM_002542, NT_32977.8, and NM_002452, respectively.

[b]Linkage disequilibrium parameter, D′ = 1.00.

[c]This SNP leads to the production of isoform-p26, which is not expressed in the wild-type (c.-1598T). However, the SNP does not affect isoform-p18, a major form of MTH1.

Results

To extract gastric cancer cases that might have mutations in 8-OHdG repair genes, we selected cases exhibiting both high 8-OHdG accumulation and low inflammatory cell infiltrate in their stomach. Noncancerous gastric antral tissue from 231 gastric cancer patients was evaluated histopathologically (figure 1,b&e) and the 77 cases with mild or no neutrophil, and mononuclear cell infiltration were selected. Further, the antral tissue from these 77 cases was evaluated immunohistochemically (figure 1,c,d&f), and the 23 cases with intense or very intense 8-OHdG immunoreactivity were selected (figure 1,c&d: a representative case). 8-OHdG was found to have accumulated predominantly in the nuclei of gastric mucosal epithelial cells, as reported previously (Toyokuni *et al*. 1997).

We examined the 23 gastric cancer patients selected for mutations and genetic polymorphisms of the 8-OHdG repair genes *OGG1, MYH, NEIL1*, and *MTH1*. Although no mutations resulting in a premature stop codon were detected in any of these genes, three SNPs were detected in *OGG1* (c.-24A >G, c.751-15C >G, and c.977C >G), three in *MYH* (c.36+267C >T, c.462+35G >A, and c.972*G* >C), and four in *MTH1* (c.-1598T >C, c.247G >A, c.357C >T, and c. ∗49C>T), but none were detected in *NEIL1* (table 2). *OGG1*-c.977C>G, *MYH*-c.972G>C, and *MTH1*-c.247G>A were associated with amino acid exchange of p.Ser326Cys, p.Gln324His, and p.Val83Met, respectively. Both the c.-1598T >C SNP and c.247G >A SNP in the *MTH1* gene were found to be in linkage disequilibrium (D′ = 1.00). One of the SNPs detected, c.36+267C >T in the *MYH* gene, was a novel variant.

Discussion

Three SNPs associated with an amino acid exchange (OGG1-Ser326Cys, MTH1-Val83Met and MYH-

Gln324His) were detected in this study. Functional differences in the Ser326Cys polymorphic proteins of OGG1 have been investigated by several groups. The 8-OHdG repair activity of OGG1-Ser326 has been shown to be greater than that of OGG1-Cys326 in complementation assay of an *E. coli* mutant defective in 8-OHdG repair (Kohno *et al*. 1998), a *supF* forward mutation assay employing an 8-OHG-containing plasmid in human cells (Yamane *et al*. 2004), and a cleavage assay for 8-OHG containing duplex DNA (Hill and Evans 2006). In addition, dynamic relocalization of OGG1-Cys326 during the cell cycle has been found to be disrupted in human cells (Luna *et al*. 2005), and the Cys326 SNP has been shown to be associated with an increased risk of various human cancers, including gastric cancer (Sugimura *et al*. 1999; Chen *et al*. 2003; Tsukino *et al*. 2004). These findings suggest that the *OGG1*-Ser326Cys SNP contributed to the 8-OHdG accumulation in the gastric cancer cases selected for this study. The Met83 of the *MTH1* gene has been shown to be more frequent in gastric cancer patients than in healthy controls (Kimura *et al*. 2004), and recombinant Met83 protein has been reported to be more heat labile than Val83 protein in terms of both structure and catalytic function (Yakushiji *et al*. 1997). However, it is unknown whether the difference in heat lability is associated with any difference in 8-OH-dGTP hydrolysing activity under physiological conditions. In this study *MTH1*-c.247*G*>A (Val83Met) showed linkage disequilibrium with *MTH1*-c.-1598T>C. *MTH1*-c.-1598T>C SNP (GT/GC) is located at the beginning of an alternative exon 2c and it modifies the patterns of alternative splicing (Oda *et al*. 1997). Alternative translation initiation occurs on MTH1 transcripts, in the *MTH1*-c.-1598C allele, resulting in the production of MTH1-isoform p26, a protein that possesses a functional mitochondrial targeting signal (Nakabeppu 2001). Although no explanation for the association between Val83 and gastric cancer has been proposed, the association suggests that the Val83 allele may be related to 8-OHdG accumulation in gastric-mucosal cells. No clear difference between the repair activity of MYH-Gln324His SNP and wild-type was found (Shinmura *et al*. 2000), suggesting that the Gln324His SNP does not contribute to 8-OHdG accumulation.

A novel variant of *MYH*-c.36+267C>T was found in this study, but it is unlikely to affect *MYH* expression, because the SNP site is not located on a putative transcription factor binding sequence based on an analysis with Genomatix software (http://www.genomatix.de/matinspector.html). However, interestingly, c.36+267C>T contains a consensus sequence for a GAGA box in an orientation opposite to that of the *MYH* sequence. The *MYH* gene lies immediately adjacent to the *TOE1* gene in a 5′-to-5′ orientation, and *MYH*-c.36+267C>T is located 301 bp upstream of the initiation codon of the *TOE1* gene. *TOE1* is an *Egr1* target gene and has been characterized as a cell growth inhibitor by altering the cell cycle through induction of p21 (De Belle *et al*. 2003). Thus, this SNP may be associated with differential *TOE1* expression level.

In conclusion, two SNPs, *OGG1*-c.977C>G (p.Ser326 Cys) and *MTH1*-c.247G>A (p.Val83Met), may be the cause of 8-OHdG accumulation in the gastric mucosa.

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