Overexpression of the hOGG1 Gene and High 8-Hydroxy-2'-deoxyguanosine (8-OHdG) Lyase Activity in Human Colorectal Carcinoma: Regulation Mechanism of the 8-OHdG Level in DNA

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ABSTRACT

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most abundant oxidatively modified lesions in DNA. Our previous study (Kondo et al., Free Radic. Biol. Med., 27: 401–410, 1999) revealed that human colorectal carcinoma cells are oxidatively stressed based on 8-OHdG determination. To elucidate 8-OHdG metabolism and its clinical significance in colorectal carcinoma, we studied the 8-OHdG repair system in DNA by measuring specific lyase activity and hOGG1 expression using quantitative-competitive reverse transcription-PCR. In addition, we searched for the presence of mutations and single nucleotide polymorphisms of the hOGG1 gene by single-strand conformational polymorphism and sequencing analyses. It was found that 8-OHdG-specific lyase activity and hOGG1 expression were significantly up-regulated in carcinoma, and a proportional association between 8-OHdG levels and either 8-OHdG lyase activity (r = 0.641, P < 0.05) or hOGG1 expression (r = 0.702, P < 0.05) was present. Whereas no difference was detected in the 8-OHdG level between early- and advanced-stage cancer, lyase activity (1.2-fold) and hOGG1 expression (1.6-fold) were significantly increased in advanced-stage cancer. No mutation was found in the 25 tumors examined. Three kinds of single nucleotide polymorphism were observed, including that of codon 326 (Ser/Cys) in exon 7. However, there was no correlation between any of the three polymorphic patterns and either 8-OHdG level or lyase activity. These results suggest that increased 8-OHdG levels in colorectal carcinoma are attributed to increased formation and are maintained by induced 8-OHdG repair activity at appropriate high levels. Our results may offer a unique approach in the development of preventive and therapeutic interventions as well as new insights into the pathogenesis of colorectal carcinoma.

INTRODUCTION

Oxidative stress caused by ROS plays a role in a variety of tumor-associated cellular events such as carcinogenesis, UV- or radiation-induced damage, chemotherapy-induced cytotoxicity, and reperfusion injury (1, 2). Thus far, oxidative stress in tumor cells has not attracted much attention.

In 1991, Szatrowski and Nathan (3) first reported that some human cancer cell lines can produce large amounts of H2O2. Other studies, including our own, have concluded that several kinds of human cancer tissues such as lung (4), renal (5), and colorectal carcinoma (6, 7) show higher levels of DNA oxidation compared to their nontumorous counterparts, based on 8-OHdG determination. 8-OHdG, one of the major DNA base-modified products, is induced by either hydroxyl radical, singlet oxygen, or photodynamic action (8), and its pairing with adenine as well as cytosine is known to be mutagenic, leading to G:C to T:A transversion on DNA replication (9).

We (6) have previously found that colorectal carcinoma but not adenoma cells are exposed to more oxidative stress than the corresponding nontumorous epithelial cells. Furthermore, immunohistochemical methods revealed that the oxidative stress in carcinoma cells is associated with cellular proliferation. Based on these observations, we hypothesized that cancer cells are under “persistent oxidative stress” (10). However, no unifying concept has emerged thus far to explain the general metabolism of oxidatively modified DNA products in tumor cells. Oxidative stress might be defined as “ROS load minus total antioxidant activities.” Total antioxidant activities are hypothetical concepts including a variety of antioxidants and antioxidant enzymes associated with glutathione, thioredoxin, or direct ROS metabolites.
olism as well as different kinds of repair mechanisms for modified molecules (11).

In the present study, we focused on 8-OHdG metabolism in the DNA of human colorectal carcinoma to determine whether any deviation from nonneoplastic epithelial cells is present. 8-OHdG must be cleaved from the DNA strand to maintain any deviation from nonneoplastic epithelial cells is present. The responsible gene was recently cloned and assigned as human OGG1 or MutM homologue gene (12–16). It removes 8-oxoguanine in double-stranded DNA by glycosylase activity and further excises a phosphodiester bond at 3’ end of the apurinic site by apurinic lyase activity via a β-elimination reaction (12, 13, 16). Thus, we measured 8-OHdG-specific lyase activity and hOGG1 expression in colorectal carcinoma and in its nontumorous counterpart, with a subsequent analysis of their correlation with clinical factors. Furthermore, we searched for the presence of SNPs and mutations in the hOGG1 gene of colorectal carcinoma because mutations in lung and renal carcinoma (17) as well as polymorphisms (18) were reported recently.

MATERIALS AND METHODS

Tissue Samples. Twenty-five sporadic primary colorectal adenocarcinoma specimens that were surgically resected at the First Department of Surgery, Kyoto University Hospital or its affiliated hospitals during the period between January 1997 and February 1999 were evaluated in this study. Histopathological examination was carried out by two independent registered pathologists (S. T. and H. H.) on H&E-stained sections. Both of the histological type and clinical stage of all of the specimens were classified according to the WHO (19) and tumor-node-metastasis (TNM) classification system (20), respectively. None of the patients received chemotherapy or radiation before surgery. Cancerous tissue, together with corresponding normal mucosal tissue, was dissected separately immediately after surgery. Tissue samples were carefully removed by using scissors. The specimens were snap-frozen in liquid nitrogen and stored at −80°C until RNA, DNA, and protein extractions.

8-OHdG Determination by HPLC/ECD. 8-OHdG was assayed by HPLC/ECD according to the method of Helbock et al. (21), with minor modifications. To minimize artifactual 8-OHdG generation during sample preparation, DNA extraction was achieved by using the chaotropic NaI method, and an iron chelate, deferoxamine mesylate (Sigma Chemical Co., St. Louis, MO), was added to suppress iron-catalyzed Fenton reaction. Nuclear DNA was extracted from colorectal tissue (approximately 200 mg) using a DNA Extractor WB kit (Wako, Osaka, Japan), denatured at 95°C for 5 min, and digested with 10 μl of nuclease P1 (20 units/μl; Sigma) in 10 mm sodium acetate buffer (pH 4.5) at 37°C for 1 h, followed by incubation with 4 μl of alkaline phosphatase (100 mill units/μl; Sigma) in 40 mm Tris buffer (pH 8.5) at 37°C for 1 h. The solution was then treated with an ion exchange resin (Muromac; Muroumach Ka-gaku, Tokyo, Japan) to remove NaI and centrifuged at 5,000 × g for 5 min. The supernatant was further filtered through a 0.22-μm filter membrane (Millipore, Bedford, MA) and centrifuged again at 15,000 × g for 5 min. The filtrate was then injected onto a HPLC column (ODS-80Ts; Tosoh, Tokyo, Japan) as described previously (22). 2’-Deoxyguanosine (Sigma) and 8-OHdG (Wako) solutions were used as standards. The molar ratio of 8-OHdG per 105 deoxyguanosine (8-OHdG/105 dG) was calculated.

8-OHdG-specific DNA Lyase Activity. The 8-OHdG-specific DNA lyase activity assay was performed according to the method of Yamamoto et al. (23), with minor modifications. Colorectal tissue was homogenized with 5 volume/weight of buffer A [50 mm Tris-HCl (pH 7.4), 50 mm KCl, 3 mm EDTA, 5 mm magnesium acetate, and 3 mm β-mercaptoethanol] containing protease inhibitors (5 μg/ml each of antipain, pepstatin A, chymostatin, and leupeptin). The homogenate was centrifuged at 25,000 × g for 1 h at 4°C, and the cytosolic fraction was obtained. Total protein concentration was determined using the BCA protein assay kit (Pierce, Rockford, IL) and adjusted to 5 mg/ml in each sample. Table 1 illustrates the substrate and size markers for the 8-OHdG-specific lyase activity assay. The substrate is a double-stranded 22-bp oligonucleotide containing one 8-OHdG paired with deoxythymidine at the complementary strand, and it was labeled with FITC. The cytosolic extract (50 μg of protein) was incubated with 40 fmol of the DNA substrate at 30°C for the indicated period of time. After two ethanol precipitations, the pellet was dried and dissolved in 10 μl of loading buffer (80% formamide, 10 mm NaOH, and 1 mm EDTA). The solution was then denatured by heating at 95°C for 5 min. Sample solution (4 μl) was applied to a 20% denaturing polyacrylamide gel containing 8 μl urea in 1× Tris-borate EDTA buffer and run at 10 W for 30 min at room temperature. After electrophoresis, the fluorescence intensity of each band was measured using FMBio (Takara, Shiga, Japan). The nicked substrate size was identified by coelectrophoresis of the 11-mer and 12-mer oligonucleotides used as markers. The lyase activity was calculated from the ratio of the excised fragment/total substrate. Each assay was performed in duplicate.

RNA Preparation and Reverse Transcription Reaction. Total RNA was extracted from frozen tumors as well as nontumorous counterparts by means of a modified acid guanidinium phenol chloroform method (Isogen; Nippon Gene, Tokyo, Japan) according to the manufacturer’s protocol. cDNA was prepared from 5 μg of total RNA by random priming using a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech, Tokyo, Japan).

Table 1  Substrate and markers for 8-OHdG lyase activity assay

<table>
<thead>
<tr>
<th>Substrate/marker</th>
<th>Size</th>
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<tbody>
<tr>
<td>Substrate</td>
<td></td>
</tr>
<tr>
<td>$F^<em>$-GGTGGCCCTGAC$^</em>$CATTCCTCCTAAACCCGGAGCTGGA$^*$TAAAGGGGTTC</td>
<td>22 bp</td>
</tr>
<tr>
<td>Single-strand markers</td>
<td></td>
</tr>
<tr>
<td>F-GGTGGCCCTGAC</td>
<td>11-mer</td>
</tr>
<tr>
<td>F-GGTGGCCCTGACG</td>
<td>12-mer</td>
</tr>
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$^*$F. FITC.  $^*$G. 8-OHdG.
PCR Primers and Competitor Synthesis. PCR primers were synthesized to amplify a 300-bp fragment of \textit{hOGG1} cDNA. The forward primer, SK1 (5'-ACACTGGATGGTG-TACATGCCG-3') was situated in exon 3, and the reverse primer, SK2 (5'-GCCGATGTGTTGTTGAGG-3'), was situated in exon 5. The PCR product spanned three exons to differentiate the amplified products from genomic DNA. A heterologous competitor for competitive RT-PCR was prepared using the competitive DNA construction kit according to the manufacturer’s protocol (Takara). Briefly, each 3'-terminal site of SK1 and SK2 primer was ligated with two additional 20-bp oligonucleotides that had an annealing sequence to the same sequence as SK1 or SK2 primer. The PCR (MP3000; Takara) conditions were as follows: denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 62°C for 40 s, and extension at 72°C for 40 s, with a final extension reaction for 4 min. The competitor was serially diluted to produce 10^3, 10^4, 10^5, 10^6, and 10^7 copies/μl.

Quantitative RT-PCR Analysis. \textit{hOGG1} expression was analyzed using a quantitative competitive PCR method with the purified competitor. In the presence of PCR buffer [1.5 mM MgCl₂, 50 mM KCl, and 10 mM Tris-HCl (pH 8.3)], 0.25 mM deoxynucleotide triphosphate, 5 pmol of SK1 and SK2 primer, and 0.5 unit of Taq DNA polymerase (Takara), the reaction mixture contained 1 μl of cDNA solution derived from 1 μg of total RNA and 1 μl of competitor of indicated dilution in a total volume of 12.5 μl. PCR conditions were as follows: denaturation at 94°C for 4 min followed by 28 cycles of denaturation at 94°C for 40 s, annealing at 60°C for 40 s, and extension at 72°C for 40 s, with a final extension for 4 min. PCR products were separated on 8% polyacrylamide gel, and the bands were visualized by ethidium bromide staining. For quantitation, absorbance of each band at 605 nm was measured using FMBio. Copy number of the target cDNA was calculated by finding the condition of equivalent PCR amplification of the original and competitor products; namely, the intensity of each product was plotted against the known copy number of the competitor, and the equivalent point was determined as an intersection of the two lines.

PCR-SSCP Analysis of the \textit{hOGG1} Gene. Mutation and polymorphism in the \textit{hOGG1} gene were screened by PCR-SSCP. Six pairs of primers for exons 1–7 of the \textit{hOGG1} gene were used for SSCP analysis as described by Kohno et al. (18). PCR amplification was carried out in a total reaction volume of 12.5 μl containing 50 ng of genomic DNA of sample tissue, 5 pmol of each of the primer pairs, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.25 mM deoxynucleotide triphosphate, and 0.5 unit of Taq DNA polymerase (Takara). PCR conditions were as follows: denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, and extension at 72°C for 90 s, with a final 10-min extension. After amplification, 2 μl of the product were electrophoresed on 4% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME) to confirm specific amplification of the targeted fragment. The second PCR amplification was then carried out in a total volume of 10 μl, in which 0.1 μl of the first PCR product was used as a template, and 0.1 μl of [α-32P]dCTP (3000 Ci/mmmol; Amersham, Arlington Heights, IL) in the presence of 0.025 mM dCTP was used instead of 0.25 mM. PCR products were mixed with 100 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, and 0.05% bromphenol blue). Before loading, samples were denatured by incubation at 95°C for 5 min and cooled on ice. Each sample (2 μl) was loaded onto 6% nondenaturing polyacrylamide gel with and without 10% glycerol and electrophoresed at 40 W for 4–6 h, followed by autoradiography.

DNA Sequencing of the \textit{hOGG1} Gene. The detected band of altered mobility from SSCP analyses was further evaluated by sequencing analyses. DNA fragments extracted from excised bands of altered mobility were reamplified by PCR with the same pair of primers and subcloned into the PCR II vector using the TA cloning kit (Invitrogen, San Diego, CA). Sequencing was performed using an ABI Prizm 377 DNA sequencer (Perkin-Elmer, Branchburg, NJ), and the results were compared with the original DNA sequence.

Statistical Analyses. Results of 8-OHdG-specific lyase activity and \textit{hOGG1} expression were analyzed by Wilcoxon’s signed rank test. Comparison between the unpaired two groups was achieved by using the Mann-Whitney U test. Correlation between the two variables was estimated by Pearson’s correlation coefficient. P < 0.05 was considered statistically significant.

RESULTS

8-OHdG Determination by HPLC/ECD. The 8-OHdG levels in the DNA of colorectal carcinoma were significantly higher than those of the nontumorous counterparts (P < 0.001), as described in our previous report (Ref. 6; Table 2).

8-OHdG-specific Lyase Activity. We initially studied the time course of the reaction at 30°C. During the first 120 min, the fluorescence intensity of the excised substrate (12-mer) increased constantly. However, thereafter it gradually plateaued in the reactions of both carcinoma and its nontumorous counterpart. The entire time course up to 24 h was consistent with a semilogarithmic correlation, as shown in Fig. 1. We selected a 60-min incubation period for this assay. A representative electrophoretic pattern is shown in Fig. 2. Colorectal carcinoma revealed higher 8-OHdG-specific lyase activity than its nontumorous counterpart (P < 0.001; Table 2).

Optimization of Quantitative Competitive RT-PCR. We first tested the effect of PCR cycles on the amount of products as follows: coamplification of \textit{hOGG1} cDNA derived...
from 1 μg of total RNA and 10⁴ copies of the prepared competitor fragment was performed for various PCR cycles between cycles 24 and 42 by competitive PCR. The two products were amplified with the same efficiency, based on the result that the ratio of target cDNA-derived product:competitor-derived product was constant for each of the examined PCR cycles (Fig. 3). Twenty-two cycles of PCR amplification were necessary to detect hOGG1 products by fluorescence analyses after ethidium bromide staining. The amplification plateaued after 36 cycles, and the proportional relationship with the substrate level was lost. A semi-logarithmic correlation was observed. See “Materials and Methods” and “Results” for details. Results are presented as the means ± SE.

Quantitative Results. The amount of target cDNA was determined on the basis of selecting an equivalent amount of the competitor PCR signal. Fig. 4 is a representative example of hOGG1 expression detected by quantitative competitive RT-PCR. The amount of hOGG1 transcript in nontumorous counterpart and carcinoma was 4.55 ± 0.54 × 10⁵ and 30.58 ± 3.21 × 10⁵ copies/μg RNA, respectively, and the difference was statistically significant (P < 0.001; Table 2). When 8-OhdG levels of carcinoma, together with those of their nontumorous counterparts, were plotted against either 8-OhdG-specific lyase activity or hOGG1 transcript amount, a proportional association was observed between the 8-OhdG content and clinical staging, 25 cases of colorectal carcinoma were divided into two groups according to the TNM staging classification: (a) the early-stage group (stage 0, stage I, and stage II; n = 10); and (b) the advanced-stage group (stage III and stage IV; n = 15). A significant difference was detected between the two clinical groups for 8-OhdG-specific lyase activity and hOGG1 expression by the Mann-Whitney U test (P = 0.03 and P = 0.02, respectively; Fig. 6). However, 8-OhdG levels of colorectal cancer and nontumorous counterparts, were plotted against either 8-OhdG-specific lyase activity or hOGG1 expression in colorectal carcinoma and nontumorous mucosal tissue from the same patient. An increasing amount of target cDNA was coamplified with serial dilutions of known amounts of the competitor fragment. The 300-bp (target cDNA) and 341-bp (competitor) PCR products were analyzed on an 8% polyacrylamide gel. The signals of target cDNA and competitor were determined by measuring the intensity of ethidium bromide fluorescence. An aliquot of the nontumorous counterpart contains 7.94 × 10⁵ copies/μg RNA, whereas an aliquot of the carcinoma sample contains 2.52 × 10⁶ copies/μg RNA, as determined by calculating the equivalent point (△) of both PCR products of target DNA or the competitor.

Fig. 1 Time course study of 8-OhdG-specific lyase activity. A semi-logarithmic correlation was observed. See “Materials and Methods” and “Results” for details. Results are presented as the means ± SE.

Fig. 2 Representative electrophoretic pattern of 8-OhdG-specific lyase activity. A cytosolic fraction of the tissue was used as the enzyme, and 5′ fluorescence-labeled double-stranded oligonucleotide containing one 8-OhdG (Table 1) was used as the substrate. See “Materials and Methods” for details. 4, 7, 8, and 14, case numbers of four paired cases of colorectal cancer and nontumorous counterparts. M11, 11-mer single-strand marker; M12, 12-mer single-strand marker; E↓−), no cytosolic fraction was added to the substrate; T, colorectal carcinoma tissue; N, nontumorous mucosal tissue.

Fig. 3 Kinetics of coamplification of the hOGG1 gene and a competitor DNA. cDNA reverse-transcribed from 1 μg of total RNA and competitor (10⁴ copies) was coamplified using the same primer pair for 24–42 cycles at increments of 3 cycles to evaluate the amplification efficiency. The densitometric values from target (T) and competitor (C) were plotted against the number of amplification cycles and used for calculation of the T:C ratio for each cycle. The T:C ratio was constant. After 36 cycles, PCR amplification plateaued. See “Results” for details.

Fig. 4 Competitive RT-PCR analysis of hOGG1 expression in colorectal carcinoma and nontumorous mucosal tissue from the same patient. An increasing amount of target cDNA was coamplified with serial dilutions of known amounts of the competitor fragment. The 300-bp (target cDNA) and 341-bp (competitor) PCR products were analyzed on an 8% polyacrylamide gel. The signals of target cDNA and competitor were determined by measuring the intensity of ethidium bromide fluorescence. An aliquot of the nontumorous counterpart contains 7.94 × 10⁵ copies/μg RNA, whereas an aliquot of the carcinoma sample contains 2.52 × 10⁶ copies/μg RNA, as determined by calculating the equivalent point (△) of both PCR products of target DNA or the competitor.
early-stage carcinoma and those of advanced-stage carcinoma were not statistically different (means $\bar{x}$, SE, 1.31 $\pm$ 0.13/10$^5$ dG and 1.28 $\pm$ 0.05/10$^5$ dG, respectively).

SSCP Analysis and Sequencing of the hOGG1 Gene. No mutation was detected in the 25 tumors. However, polymorphisms were observed in the 5’-noncoding region (−18 bp from the first codon; G/T), exon 1 (codon 98; G/A), and exon 7, in agreement with previous reports (18, 24). Among these polymorphisms, only the polymorphism in exon 7 was accompanied by an amino acid substitution. However, there was no difference in the 8-OHdG-specific lyase activity among the three genotypes as shown in Table 3.

DISCUSSION

Major advances in discovering genetic alterations of human colorectal cancer have been made during the last decade (25). There is an increasing interest in genomic instability as an important factor for cancer promotion and progression as well as carcinogenesis (26, 27). In particular, DNA repair systems for replication error have been studied extensively since the elucidation of a gene responsible for hereditary nonpolyposis colorectal cancer (28).

We have been working on the metabolism of cancer cells from the standpoint of ROS to develop novel therapeutic as well as preventive approaches to human cancers. We have reported previously that higher levels of ROS-modified products, 8-OHdG, 4-hydroxy-2-nonenal histidine adducts, and 3-nitrotyrosine were observed in colorectal carcinoma cells (but not in adenoma cells) than in the nontumorous counterparts, leading to the conclusion that carcinoma cells are more oxidatively stressed (6). Several reports have indicated the presence of higher levels of 8-OHdG in the DNA of cancer cells than in that of nontumorous counterparts (5, 7, 29, 30). In the present study, we attempted to determine: (a) whether higher levels of 8-OHdG in cancerous DNA result from increased generation of 8-OHdG or from decreased repair of this oxidative lesion; (b) whether there is a mutation in the hOGG1 gene of colorectal carcinoma; and (c) whether polymorphism of hOGG1 is associated with 8-OHdG-specific lyase activity.

Our results showed clearly that hOGG1 expression and 8-OHdG-specific lyase activity are increased in carcinoma cells. A proportional correlation was obtained between the 8-OHdG level and hOGG1 expression or 8-OHdG-specific lyase activity.

**Table 3** Genotype in exon 7 and 8-OHdG repair enzyme activity

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (%)</th>
<th>Normal mucosa</th>
<th>Carcinoma</th>
</tr>
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<tbody>
<tr>
<td>Ser$^{326}$/Ser$^{326}$</td>
<td>32</td>
<td>19.8 $\pm$ 1.2</td>
<td>32.5 $\pm$ 2.3</td>
</tr>
<tr>
<td>Ser$^{326}$/Cys$^{326}$</td>
<td>44</td>
<td>18.6 $\pm$ 1.4</td>
<td>34.5 $\pm$ 1.6</td>
</tr>
<tr>
<td>Cys$^{326}$/Cys$^{326}$</td>
<td>24</td>
<td>18.9 $\pm$ 2.1</td>
<td>32.1 $\pm$ 1.4</td>
</tr>
</tbody>
</table>

*Not statistically significant.*

Fig. 5 8-OHdG-specific lyase activity (A) and expression of hOGG1 transcript (B) in colorectal tissues are proportionally associated with 8-OHdG level. Correlations were evaluated statistically by Pearson’s correlation coefficient ($r$). $A$, $r = 0.641, P < 0.05$; $B$, $r = 0.702; P < 0.05$.

Fig. 6 Comparison between early-stage and advanced-stage colorectal carcinoma for (A) 8-OHdG-specific lyase activity and (B) hOGG1 expression. Results are presented as the means $\bar{x}$ SE, with $P$ determined by the Mann-Whitney U test. *, $P < 0.05$; ***, $P < 0.001$.
This could represent an appropriate feedback mechanism. An increase in 8-OHdG-specific lyase activity under oxidative stress has been reported under several pathological conditions such as smoking (31), ionizing irradiation (32), and exposure to transition metal (33). Moreover, levels of 8-OHdG were directly proportional to the lyase activity in these studies, whereas oxidative stress of these kinds is usually temporary. In cancer, however, oxidative stress is persistent, associated with its own metabolic activity (10).

Therefore, our data demonstrate that an increased load of ROS is responsible for higher levels of 8-OHdG in colorectal carcinoma. Levels of oxidative stress in colorectal carcinoma appear to be suitable for both cellular proliferation and genomic instability. It is established that low levels of oxidative stress promote cellular proliferation instead of inducing apoptosis and necrosis (11). We also found that hOGG1 expression and 8-OHdG-specific lyase activity were associated with the clinical stage of colorectal carcinoma (Fig. 6). These results, together with the finding that 8-OHdG levels did not differ between early- and advanced-stage cancers, suggest that carcinoma cells adjust the levels of oxidative stress to a suitable level by modulating the repair activity. It was also reported that the human MutT homologue, an enzyme for hydrolyzing 8-hydroxy-dGTP in the nucleotide pool, is overexpressed in human renal cell carcinoma or in lung cancer cell lines (5, 34).

Whereas hOGG1 expression levels were increased by 4–11-fold in colorectal carcinoma, 8-OHdG-specific lyase activity showed an increase of only 1.5–3.0-fold. It seems that 8-OHdG-specific lyase activity does not reflect hOGG1 expression levels. There are three possible explanations for this. First, an inhibitor of hOGG1 protein or an 8-OHdG-binding protein in the cytosol might be present (35, 36). Second, we may have underestimated the enzyme activity because of the different localization of each isoform. hOGG1 is known to possess four major alternative splicing isoforms [type 1a, type 1b, type 1c, and type 2 (16, 37)], with each type distributed to different organelles. The main hOGG1 isoform is type 1a, which contains nuclear transfer signal sequence and is localized in the nucleus after translation (16, 37), and the other hOGG1 types without the nuclear transfer signal (type 1b, type 1c, and type 2) might be localized in intracellular organelles. We thought that using the cytosolic compartment as an enzyme source in the lyase activity assay rather than using crude extract containing nuclear protein was a better choice because the system excludes nuclear DNA fragments. On the other hand, transcripts of all of the hOGG1 mRNA isoforms were amplified in the RT-PCR reaction due to the presence of common primer annealing site in hOGG1 gene. It remains unclear how different isoforms of hOGG1 respond to oxidative stress at the transcriptional level.

Third, the half-life of hOGG1 mRNA or protein might be different. In a recent report, Chevillard et al. (17) showed that there was no significant difference in hOGG1 expression between tumorous and nonneoplastic tissues in human kidney cancer. Although the reason for this discrepancy is not clear, this difference might be due to an organ-specific tumor metabolism.

The hOGG1 gene was found to be localized on chromosome 3p25 (12, 13, 16). Because several kinds of human carcinoma reveal a frequent allelic deletion at chromosome 3p, the hOGG1 gene has been a good candidate for a responsible tumor suppressor gene. However, a small percentage of mutations detected were in carcinomas of the lung (17, 18), kidney (17), and stomach (24). There was no hOGG1 mutation in the 25 cases of colorectal carcinoma in the present study. Three kinds of SNPs were observed, and the patterns and frequencies were in accordance with a recent study (18). Reports indicate the presence of a C/G SNP at codon 326 in exon 7, with serine/cysteine amino acid substitution and higher 8-OHdG-specific lyase activity in purified hOGG1-Ser326 protein than in purified hOGG1-Cys326 (18, 24). In this study, no significant difference in lyase activity was found in any of the hOGG1 genotypes (Ser326/Ser326, Cys326/Ser326, and Cys326/Cys326) of carcinoma cells or in the noncancerous counterparts (Table 3). It is possible that there is another enzyme responsible for 8-OHdG-specific DNA lyase activity, hence additional careful studies are necessary.

We have reached the following conclusions: (a) increased load of ROS is responsible for high 8-OHdG levels in human colorectal carcinoma; (b) there is a general proportional correlation between 8-OHdG levels and 8-OHdG-specific lyase activity or hOGG1 expression; (c) however, 8-OHdG levels are maintained at a constant high level among early- and advanced-stage colorectal carcinoma, and this adjustment appears to be regulated by hOGG1 transcription; and (d) no mutation in hOGG1 was detected in the present study. Furthermore, polymorphism of the gene was independent of 8-OHdG-specific lyase activity. Further understanding of tumor metabolism in “persistent oxidative stress” might facilitate the development of a new therapeutic and preventive strategy for colorectal carcinoma.

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REFERENCES


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