Metallothionein-III Prevents γ-Ray-induced 8-Oxoguanine Accumulation in Normal and hOGG1-depleted Cells*

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Metallothioneins (MT) play an important biological role in preventing oxidative damage to cells. We have previously demonstrated that the efficiency of the protective effect of MT-III against the DNA degradation from oxidative damage was much higher than that of MT-I/II. As an extension of the latter investigation, this study aimed to assess the ability of MT-III to suppress 8-oxoguanine (8-oxoG), which is one of the major base lesions formed after an oxidative attack to DNA and the mutant frequency of the HPRT gene in human fibroblast GM00637 cells upon exposure to γ-rays. We found that human MT-III expression decreased the level of 8-oxoG and mutation frequency in the γ-irradiated cells. Using an 8-oxo guanine DNA glycosylase (OGG1)-specific siRNAs, we also found that MT-III expression resulted in the suppression of the γ-radiation-induced 8-oxoG accumulation and mutation in the OGG1-depleted cells. Moreover, the down-regulation of MT in human neuroblastoma SKNSH cells induced by MT-specific siRNA led to a significant increase in the 8-oxoG level, after exposure to γ-irradiation. These results suggest that under the conditions of γ-ray oxidative stress, MT-III prevents the γ-radiation-induced 8-oxoG accumulation and mutation in normal and hOGG1-depleted cells, and this suppression might, at least in part, contribute to the anti-carcinogenic and neuroprotective role of MT-III.

The metallothioneins (MT)† are a group of intracellular metal-binding proteins of low molecular mass (6–7 kDa) that are widely distributed in a broad range of eukaryotic species from yeast to mammals (1, 2). In both mice and humans, there are four classes of quite similar MT proteins, MT-I to MT-IV. MT-I and MT-II are widely expressed in all tissues, whereas MT-III and MT-IV are expressed mainly in the central nervous system and the squamous epithelia, respectively (3–7). Whereas much is known about the chemical properties and genetic regulation of MT, the actual physiological role of MT is largely unknown. The first recognized function of MT is the detoxification of heavy metals such as cadmium and mercury (8, 9). Subsequently, a number of cellular functions have been proposed for MT, including regulating essential metal homeostasis (10, 11), contributing to the control of cellular proliferation and apoptosis (12, 13), and protecting against radiation and oxidative damage (14, 15). The role of MT in oxidative damage has been aggressively investigated, and the vast majority of studies show that MT is a potent antioxidant that protects against various oxidative damage from reactive oxygen species (ROS) in vitro and in vivo because of their multiple cysteines. In vitro up-regulation of MT has been correlated with resistance to cytotoxicity induced by various hydroxyl radical generators, and the rate constant of MT for a reaction with hydroxyl radicals is more than 100× higher than that of glutathione (16–21). In addition, MT is 50× more effective in protecting DNA from hydroxyl radicals than glutathione on a molar basis (22). In vivo, the induction of MT expression by different stress associated with oxidative injury is consistent with MT functioning as an antioxidant (23–25).

MT-III, the brain-specific member of MT family, was discovered as an inhibitory neuronal growth factor that appeared at lower levels in Alzheimer’s disease brains (4, 26). However, the mechanism of this inhibition and the physiological significance of MT-III are not clear. MT-III regulation has been studied in a number of animal models of brain damage (27, 28); such studies have suggested that this MT isoform is involved in reparative and/or protective processes in the brain. Moreover, it has been recently shown that mice deficient in MT-III are more susceptible to seizures induced by kainic acid and exhibit greater neuronal injury than normal mice, and transgenic mice containing elevated levels of MT-III were more resistant to neuronal injury (29). These results suggest that MT-III could play a neuroprotective role; however, the mechanisms underlying such a protective role have not been fully known. In view of the proposed protective role of MT as a free radical scavenger, MT may also have relevance in brain neurological diseases. Therefore, MT-III, which is a unique protein, requires further study to define its antioxidant nature and its involvement in neuroprotection.

ROS-induced damage to cellular macromolecules has been implicated in the etiology of cancer and aging as well as in other human diseases (30). Among the oxidative lesions, 8-oxoguanine (8-oxoG) is one of the major base lesions formed after an oxidative attack to DNA (31). Relatively large quantities of...
8-oxoG are produced in mammalian cells, either as a byproduct of the normal oxidative metabolism or as a result of the exogenous sources of ROS, such as ionizing radiation (32). 8-oxoG preferentially mistranslates with adenosine during replication and thereby gives rise to G to T/A transversion mutations (33). Because of the relative abundance and potent mutagenicity, 8-oxoG is believed to represent a major source of ROS-induced mutagenesis in all aerobic cells. The cellular damage caused by radiation is mainly oxidative damage as a result of the formation of several types of ROS including hydroxyl radicals and superoxide radicals by the radiolysis of water in cells (34, 35). DNA is the presumed target of attack of either the primary radiolysis products of ionizing radiation such as hydroxyl radicals or secondary radicals, which are derived from the reactions of hydroxyl radicals with species in close proximity to the DNA (36). A number of reports have demonstrated that MT can protect against radiation-induced DNA damage (14). Therefore, MT may be one of the most important defense mechanisms against ROS-induced mutagenesis, particularly those produced by irradiation. Supporting this hypothesis, several groups have found that a correlation exists between the elevated MT levels and the decreased number of mutations after exposure to oxidative stress (37, 38). We have previously demonstrated that the MT-III-expressing cells are highly protected from the ROS-induced DNA damage (39) and that purified MT-III has the most efficient protective effect against hydroxyl radical-induced DNA single-strand breaks compared with that of MT-I/II (40).

Based on these considerations, this study evaluated the protection provided by MT-III in γ-irradiated human fibroblast GM00637 cells. Attempts were made to determine whether or not MT-III expression could modulate the γ-irradiation-induced 8-oxoG accumulation and mutagenesis. The amount of 8-oxoG accumulation was determined using HPLC combined with an electrochemical detector, and the mutant frequency was determined by measuring the mutation frequency of the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene locus. This study demonstrates that MT-III expression can effectively inhibit the accumulation of 8-oxoG as well as suppress the mutant frequency of the HPRT gene after exposure to γ-irradiation in GM00637 cells. Most notably, this study demonstrates that MT-III expression leads to a reduction in the level of γ-ray-induced 8-oxoG formation and mutation in 8-oxoguanosine DNA glycosylase (OGG1)-depleted cells, which is the major repair enzyme of 8-oxoG. Finally, this study shows that a decrease in MT expression levels lead to a significant increase in 8-oxoG accumulation after exposure to γ-irradiation in human neuroblastoma SKNSH cells. These findings suggest that MT-III plays a critical role in the protection of γ-irradiation-induced 8-oxoG accumulation as well as in γ-irradiation-induced mutation in normal and hOGG1-depleted cells.

**EXPERIMENTAL PROCEDURES**

**Maintenance of Cell Lines**—The human fibroblast GM00637 cells (Coriell Institute for Medical Research) and human neuroblastoma SKNSH cells (American Type Culture Collection) were maintained in Earle’s minimum essential medium supplemented with 10% fetal bovine serum, 100 units of penicillin/ml, and 100 μg of streptomycin/ml (Invitrogen). Cells were maintained in 5% CO2, 95% air at 37 °C in a humidified incubator.

**Preparation of Constructs and Clones**—Constructs of the human MT-III, MT-I, and MT-II are described elsewhere (39, 40). Human MT-III, MT-I, and MT-II are described elsewhere (39, 40). Human MT-III, MT-I, and MT-II are described elsewhere (39, 40).

**LDH Activity Measurement**—After cells were irradiated on ice with 50 or 100 Gy of γ-rays, culture medium was collected. Total LDH activity was determined by using the LDH Activity Assay Kit (Sigma). A total of 100 μl of supernatant was used to estimate LDH activity by measuring the oxidation of NADH at 340 nm. Extinction was recorded at 340 nm for 2 min. The results were expressed as international units of enzyme activity per liter of medium (units/liter).

**Production and Purification of the Human Metallothionein III Antibody**—Polyclonal antibodies were produced by immunizing New Zealand White rabbits with conjugated bovine serum albumin and a synthetic peptide (CKGEEGAKIEAEE) corresponding to residues 51–62 of human MT-III. A cysteine residue was attached to the N terminus of the peptide to introduce an SH group for coupling. Approximately 500 μg of the conjugated peptide in Freund’s complete adjuvant (Sigma) was subcutaneously injected into two New Zealand White rabbits, followed by boosting the conjugate in Freund’s incomplete adjuvant (Sigma) four times at 2-week intervals. Two weeks after the final boost, the serum was harvested, the immunoglobulin was purified using protein A-agarose (Oncogene), and serum was collected. Specific antibodies were prepared from the antisera by affinity column chromatography using the peptide antigen (CKGEEGAKIEAEE) coupled to SulfoLink Coupling Gel (Pierce).

**Western Blotting**—Cells were washed with phosphate-buffered saline and lysed at 0 °C for 30 min in a lysis buffer (20 mM Heps, pH 7.4, 2 mM EGTA, 50 mM glycerol phosphate, 1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 5 mM NaF). The protein content was determined using a dye-binding microassay (Bio-Rad), and 20 μg of protein per lane were electrophoresed on 10% SDS-polyacrylamide gels after boiling for 5 min in a Laemmli sample buffer. The proteins were blotted onto Hybon ECL membranes (Amersham Biosciences). The markers (MBI) were used as size standards. After electroblotting, the membranes were blocked with Tris-buffered saline with Tween-20 (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% milk, and were incubated with the anti-hOGG1, α-tubulin (Santa Cruz Biotechnology), and MT-I/II (BD Biosciences, San Diego, CA) and MT-III antibodies diluted in a blocking buffer with the appropriate secondary antibodies (1:4,000) in a blocking buffer for 2 h, and then rinsed after washing. The blotted proteins were detected using an enhanced chemiluminescence detection system (INNORON Biotech, Seoul, Korea).

**Semiquantitative Reverse Transcriptase-Polymerase Chain Reaction**—RNA extraction was conducted using the RNA-STAT-60 according to the manufacturer’s instructions (TEL-TEST, Inc., Friendswood, TX). Briefly, after homogenizing cells in the RNA STAT-60, the homogenate was mixed with chloroform (5:1, v/v), shaken vigorously for 15 s, and then centrifuged at 13,000 rpm for 15 min at 4 °C. The RNA present in the upper colorless aqueous phase was precipitated by adding isopropyl alcohol, which was washed twice with 70% ethanol and then air-dried for 1 h (1.7 mg). The RNA was then resuspended in DEPC water. Two RNA aliquots were prepared and stored at −70 °C until needed. 2 μg of the total RNA was reverse-transcribed using a M-MLV DNA synthesis system (Promega), and the reverse-transcribed DNA was subjected to PCR. The profile of the replication cycles was denaturation at 94 °C for 50 s, annealing at 58 °C for 50 s, and polymerization at 72 °C for 1 min. In the absence of reverse transcriptase, the same PCR product was amplified. A forward primer for the gapdh (GAPDH) was used as the internal control. The primers used for the PCR are as follows: hOGG1 forward, 5'-CTG CCT TCT GGA CAA TCT TT-3'; hOGG1 reverse, 5'-TAG CCC GCC CTG TTC TTC-3' designed to amplify a 551-bp region; hMT-III forward, 5'-TCA GGT CAT CCT GCA TCT GCA-3'; hMT-III reverse, 5'-ATG GAC GAC GAT CCC TGC CCG-3' designed to amplify a 227-bp region; hMT-I forward, 5'-ATG GAT CAT AAC TGC TCC TG-3'; hMT-I reverse, 5'-ACT TCT CCT ATG CCC CCG-3' designed to amplify a 227-bp region; hMT-II forward, 5'-ATG GAT CAT AAC TGC TCC TG-3'; hMT-II reverse, 5'-AGC TGC ACT TGT CCG ACG-3' designed to
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plify a 175-bp region; GAPDH forward, 5'-CCA TGG AGA AGG CTG GG3'-3' and GAPDH reverse 5'-CAA AGT TGT CAT GGA TGA CC3'-3' designed to amplify a 194-bp region (total number of cycles: 26). The PCR products were resolved on 1% agarose gels, stained with ethidium bromide, and then photographed.

siRNA—Sequence information regarding the human OGG1 and MT-III mRNA was extracted from the NCBI Entrez nucleotide data base. Three target sites within the human OGG1 and MT-III genes were chosen from the human OGG1 and MT-III mRNA sequences (GenBank™ accession no. AF003595 and NM_005954, respectively). Following selection, each target site was screened with NCBI BLAST to confirm specificity only to the human OGG1 and MT-III. The sequences of the 21-nucleotide sense and antisense RNA are as follows: hOGG1-sense 5'-CACCATTCGAGGAGGGUCUCAUUCU-3' (antisense) for the hOGG1 gene (nt 185–205); hOGG1-siRNA2, 5'-GUACUCUGGAGUAAUUUUG3' (sense) and 5'-AACUACUCUGCGGAGAUAAUUU-3' (antisense) for the human MT gene (nt 148–168); LacZ siRNA, 5'-GUCGUGGGAUCAAUAGAAGUU-3' (sense) and 5'-AACUACUCUGCGGAGAUAAUUU-3' (antisense) for the LacZ gene. These siRNAs were prepared by a transcription-based method using a Silencer siRNA construction kit (Ambion, Austin, TX) according to the manufacturer’s instructions. LacZ siRNA was used as a negative control. Cells were transfected with the siRNA duplexes by using Oligofectamine (Invitrogen).

The 8-oxoG levels in the genomic DNA of 8-oxoG-containing 21-mer with the sequence 5'-CAGCCCATGATCCACATGC-3' (X = 8-oxoG) and its complementary oligonucleotide were chemically deaminated (The Midland Certified Reagent Co., Midland, TX). The oligonucleotides were 3'-end-labeled using terminal transferase and [α-32P]dCTP (Amersham Biosciences, 3000 Ci/mmole). The end-labeled oligomer was annealed with its complementary oligonucleotide, and the resulting duplex DNA was used as the assay substrate. The duplex substrate DNA (20 pmol) was incubated with the cell extracts (10 μg of protein) at 37 °C for 1 h in 1 ml of the reaction mixture (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 5% glycerol, and 0.05% 2-mercaptoethanol, pH 7.5) and homogenized. The homogenates were mixed with streptomycin (final concentration: 1.5%) to remove nucleic acids.

The supernatants obtained by centrifugation were dialyzed extensively (Millipore, Bedford, MA), and a 20-μl aliquot of the sample was injected into an HPLC column (YMCpack ODS-AM, 5 μm, 4.6 × 300 mm) equipped with an electrochemical detector (ECD) (Coulochem II; ESA, Chelmsford, MA) at a flow rate of 1 ml/min. The mobile phase consisted of 12.5 mM citric acid, 25 mM sodium acetate, 30 mM NaOH, and 10 mM acetic acid (pH 4.5). The nucleotide solution was filtered with an Ultrafilter-Probe filter (Millipore, Bedford, MA), and a 20-μl aliquot of the sample was injected into an HPLC column (YMCpack ODS-AM, 5 μm, 4.6 × 300 mm) equipped with an electrochemical detector (ECD) (Coulochem II; ESA, Chelmsford, MA) at a flow rate of 1 ml/min. The mobile phase consisted of 12.5 mM citric acid, 25 mM sodium acetate, 30 mM NaOH, and 10 mM acetic acid (pH 4.5). The nucleotide solution was filtered with an Ultrafilter-Probe filter (Millipore, Bedford, MA), and a 20-μl aliquot of the sample was injected into an HPLC column (YMCpack ODS-AM, 5 μm, 4.6 × 300 mm) equipped with an electrochemical detector (ECD) (Coulochem II; ESA, Chelmsford, MA) at a flow rate of 1 ml/min.

The consumption rate of 0.15 mM NADPH at 37 °C indicated a consumption of 20 mM H2O2 (Fridovich (43)). Briefly, the reduction rate of 20 μM ferricytochrome c was monitored at a wavelength of 550 nm in the presence of 10 μM

GSH, and 0.57 mM GTP (for 5 min. The cell pellets (106 cells per each assay) were then suspended in 2 volumes of a homogenization buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 5% glycerol, and 0.05% 2-mercaptoethanol, pH 7.5) and homogenized. The homogenates were mixed with streptomycin (final concentration: 1.5%) to remove nucleic acids.

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2.9–19.4 ± 3.3 per 10^7 dG, respectively (Fig. 2B). In contrast to the MT-III-expressing cells, MT-I- and MT-II-expressing cells had only a 20–25% decrease in γ-ray-induced 8-oxoG accumulation compared with empty vector-transfected cells (Fig. 2C). Therefore, MT-III, MT-I, and MT-II expression prevent the γ-ray-mediated increase in the accumulation of 8-oxoG in human fibroblast GM00637 cells, and that the effect of MT-III is greater than that of MT-I and MT-II.

The Effect of MT-III on the Expression of hOGG1—8-oxoG is excised by the DNA glycosylase/lyase activity of hOGG1, which is a representative member of the DNA glycosylase/lyase family of repair enzymes (46–48). Therefore, low amounts of 8-oxoG in genomic DNA from the MT-III-expressing clones might reflect the increased repair of 8-oxoG. In order to test this hypothesis, the hOGG1 mRNA and protein in the empty vector-transfected clone and the MT-III-expressing clones were compared. Using semiquantitative RT-PCR with specific primers for hOGG1 and Western blotting with a specific antibody against hOGG1, to evaluate the level of the hOGG1 mRNA and protein, it was found that the levels of the hOGG1 mRNA and protein in the MT-III-expressing and control cells were similar (Fig. 3, A and B). These suggested that MT-III expression had no effect on the hOGG1 expression level.

The Effect of MT-III on the Antioxidant Enzyme Activity—Because the direct consequence of ROS interaction with DNA is the generation of oxidative DNA damage, such as 8-oxoG, this study next examined whether or not MT expression leads to the modulation of antioxidant enzyme activity. The activities of catalase, superoxide dismutase, and glutathione peroxidase were determined in the control and MT-III-expressing clones. As shown in Table I, the levels of these antioxidant enzyme activities in the MT-III-expressing and control cells were similar, suggesting that MT-III expression had no effect on the antioxidant enzyme activity.

8-oxoG Glycosylase Activities in γ-Irradiated Cells—A previous study revealed that the 8-oxoG repair activity was reduced in the γ-irradiated cells (49). Therefore, we investigated whether γ-radiation can affect hOGG1 expression and its activity in MT-III-expressing cells. The MT-III-expressing clone-7 cells were treated with 20, 40, and 60 Gy of γ-radiation, and the hOGG1 protein levels were determined in the attached cells. Western blotting with a specific antibody against hOGG1 showed that the hOGG1 was reduced by 21 and 52% in the 40 and 60 Gy of γ-irradiated cells compared with those of control cells, respectively (Fig. 4A). In order to confirm the γ-radiation-mediated decrease in hOGG1 expression, nuclear extracts from γ-irradiated cells were prepared and examined for their ability to cleave 8-oxoG using a 21-mer oligonucleotide containing a single 8-oxoG at nucleotide 13. As shown in Fig. 4B, the γ-irradiated cells resulted in a concentration-dependent decrease in the ability of nuclear extracts to cleave the 8-oxoG:C substrate. These results suggest that the γ-radiation-mediated decrease in hOGG1 expression has a lower 8-oxoG repair activity.

siRNA-mediated Down-regulation of hOGG1—The above results suggest that γ-irradiation decreased the 8-oxoG repair activity. Therefore, the potential role of MT-III in the γ-ray-induced 8-oxoG formation in hOGG1-depleted cells was next analyzed using the hOGG1-specific siRNAs. Two target regions of the human hOGG1 mRNA (i.e. 241–261 and 348–368 sequences) were selected by scanning the length of the hOGG1 gene for the AA-dinucleotide sequences and downstream 19 nucleotides without a significant homology to the other genes using an appropriate genome data base. The antisense strands of the synthesized hOGG1 siRNAs are the reverse complement of the target sequences. The sense strands of the hOGG1 siRNAs have the same sequences as the target mRNA sequences with the exception that they lack the 5’-AA sequence. A uridine dimer was incorporated at the 3’-end of the sense strand siRNAs. Therefore, the end products are three double-stranded 21-mer siRNAs (i.e. hOGG1-siRNA1 and hOGG1-siRNA2), which should theoretically reduce the hOGG1 mRNA and protein expression levels, and the control siRNA (lacZ-
siRNA), which should not be effective in hOGG1 gene silencing. Two different 21-base pair siRNA constructs, hOGG1-siRNA1, hOGG1-siRNA2, the mock, and the control siRNA, were transfected into the GM00637 cells to test their ability to suppress hOGG1 expression. In order to obtain the quantitative hOGG1 mRNA expression values, semiquantitative RT-PCR experiments were performed 24 h after treating cells with either hOGG1 siRNAs, mock, or control siRNA. Treatment with the hOGG1 siRNAs resulted in a decrease in the hOGG1 mRNA level to 85 ± 6–90 ± 5%, compared with the mock- and control siRNA-transfected cells (Fig. 5A). The protein extracts were obtained 24–96 h after transfection, and Western blot analyses were performed for the hOGG1 protein and normalized to the α-tubulin expression level. It was found that both siRNAs for the different sequences within the hOGG1 gene effectively inhibited hOGG1 protein expression 48 h after transfection (Fig. 5B). By 96-h post-transfection, the hOGG1 protein levels had increased to levels comparable to the control siRNA-transfected cells (data not shown). The level of 8-oxoG repair activity was also tested, and it was confirmed that the hOGG1 siRNAs-transfected cells had almost no 8-oxoG repair activity (Fig. 5C). These results demonstrate that all hOGG1 siRNAs were specific to hOGG1. Because there is a strong correlation between hOGG1 mRNA and protein suppression by siRNA, these results suggest that hOGG1 silencing in the GM00637 cells result from a reduction in the amount of hOGG1 mRNA available.
for translation. This suggests that the hOGG1-siRNAs are highly specific and efficient in hOGG1 gene silencing in the GM00637 cells.

**Down-regulation of hOGG1 via siRNA Leads to an Increase in the Level of γ-Radiation-induced 8-oxoG—**Because hOGG1 is involved in the BER pathway and is known to play a crucial role in the repair of 8-oxoG (46–48), 8-oxoG levels were measured in genomic DNA from hOGG1-depleted GM00637 cells. The GM00637 cells were transfected with either the mock, control-siRNA, or hOGG1-siRNAs. Forty-eight hours after transfection, cells were then exposed to 10, 20, 30, 40, and 50 Gy of γ-rays, and the amount of 8-oxoG in the genomic DNA was then measured by HPLC with an electrochemical detector. As shown in Fig. 6, the mock- and control-siRNA-transfected cells demonstrated that 8-oxoG levels for cells exposed to 10, 20, 30, 40, and 50 Gy of γ-radiation were 6.2 ± 2.5–9.1 ± 3.4, 10.6 ± 3.7–14.8 ± 4.1, 13.8 ± 3.9–17.2 ± 4.1, 19.3 ± 6.2–21.3 ± 7.5, and 26.5 ± 7.1–29.6 ± 7.4 per 10^7 dG, respectively. However, cells transfected with the hOGG1-siRNA were significantly higher in the γ-ray-induced 8-oxoG levels compared with those of the mock- and control-siRNA-transfected cells. The hOGG1 siRNA-transfected cells demonstrated an 8-oxoG level for cells exposed to 10, 20, 30, 40, and 50 Gy of γ-radiation as 29.5 ± 3.9–35.3 ± 4.5, 42.4 ± 4.1–48.3 ± 4.9, 60.1 ± 6.7–64.3 ± 7.2, 83.4 ± 11.4–84.1 ± 12.3, and 101.5 ± 12.3–105.6 ± 14.5 per 10^7 dG, respectively.

**Expression of MT-III Suppresses the Level of γ-Ray-induced 8-oxoG Accumulation in the hOGG1-depleted Cells—**In order to investigate the effect of MT-III on the accumulation of the 8-oxoG in hOGG1-depleted cells, the empty vector pcDNA3-transfected cells and the MT-III clone-7 cells were treated with hOGG1-siRNAs. Forty-eight hours after treatment, cells were then exposed to 50 Gy of γ-irradiation, and the 8-oxoG level in the genomic DNA was measured. We found that the amount of 8-oxoG in genomic DNA from the hOGG1-siRNA-treated pcDNA3 cells exposed to 50 Gy of γ-radiation were 95.3 ± 6.4–106.1 ± 12.5 per 10^7 dG. However, the genomic DNA of the hOGG1-siRNA-treated MT-III clone-7 contained a 57–61% decrease in γ-ray-induced 8-oxoG accumulation compared with hOGG1-siRNA-treated empty vector-transfected cells (Fig. 7). The amount of 8-oxoG in the genomic DNA from the hOGG1-siRNA treated MT-III clone-7 cells exposed to 50 Gy of γ-radiation was 39.7 ± 5.6–43.4 ± 9.5 per 10^7 dG. In contrast to the effect of MT-III, the hOGG1-siRNA-treated MT-I and MT-II clones had only a 25–29% decrease in γ-ray-induced 8-oxoG accumulation compared with hOGG1-siRNA-treated empty vector-transfected cells. These results suggest that MT-III can prevent the γ-ray-induced 8-oxoG accumulation in hOGG1-depleted cells, and the inhibitory effect of MT-III on the 8-oxoG accumulation is higher than MT-I and MT-II.

**Expression of MT-III Prevents γ-Ray-induced Mutant Frequency of the HPRT Gene in hOGG1-depleted Cells—**In order to investigate whether or not MT-III expression may inhibit the γ-irradiation-induced mutation in hOGG1-depleted cells, the γ-radiation-induced mutation in hOGG1-depleted human fibroblast GM00637 cells was initially evaluated. We investigated the γ-irradiation-induced mutagenesis of the HPRT gene, and found that a mutation of this gene leads to 6-TG resistance of mutant cells. The GM00637 cells were transfected with hOGG1-siRNAs. Forty-eight hours after transfection, cells were exposed to 2, 4, 6, and 8 Gy of γ-rays, and the γ-ray-induced mutant frequencies of the HPRT gene were measured. As shown in Fig. 8, the hOGG1 siRNA-transfected cells showed a significantly higher mutant frequency than the mock- and control siRNA-transfected cells. The mutant frequencies of the HPRT were 49 ± 9–51 ± 11 × 10^-6, 98 ± 21–105 ± 24 × 10^-6, 142 ± 28–154 ± 31 × 10^-6, and 206 ± 31–216 ± 34 × 10^-6 in the hOGG1 siRNA-transfected cells exposed to 2, 4, 6, and 8 Gy of γ-rays, respectively. These values were 2–3-fold higher than the HPRT mutant frequency of the γ-irradiated mock- and control siRNA-transfected cells.

The role of MT-III in the inhibition of γ-ray-induced mutation in hOGG1-depleted cells was next investigated. The pcDNA3 clone and MT-III clone-7 cells were treated with hOGG1-siRNAs. Forty-eight hours after treatment, cells were then exposed to either 5 or 10 Gy of γ-radiation, and the HPRT mutant frequencies were then determined. As shown in Table II, the hOGG1-siRNA-treated MT-III clone-7 cells have a decreased mutation frequency at the HPRT locus, compared with that of the hOGG1-siRNA-treated control cells. The HPRT mutant frequencies were 61 ± 29–64 ± 35 × 10^-6 and 124 ± 45–138 ± 57 × 10^-6 in hOGG1-siRNA-treated MT-III clone-7 cells exposed to 5 and 10 Gy of γ-rays, respectively. However,
FIG. 4. Effect of γ-radiation on the hOGG1 protein level and 8-oxoG glycosylase activity. A, Western blot analysis of the hOGG1 protein levels in the γ-irradiated GM00637 cells. The GM00637 cells were treated with various γ-ray doses (20–60 Gy). Cell lysates were then prepared from the γ-irradiated cells, and equal amounts (50 μg of protein) of the cell lysates were separated by 12% SDS-PAGE, and then transferred onto a nitrocellulose membrane. The membrane was immunoblotted with either anti-hOGG1 or anti-α-tubulin antibodies. The hOGG1 and α-tubulin were detected using the enzyme-linked chemiluminescence. The protein expression levels were quantified using a Bio-Rad Versa-Doc imager and Quantity One analysis software. The results are representative of three similar experiments. B, a 21-mer containing an 8-oxoG lesion was incubated with the cell extracts from the γ-irradiated (0–60 Gy) cells, and oligonucleotide cleavage products (lanes 3–6) were analyzed on the DNA sequencing gels and subjected to autoradiography as described under “Experimental Procedures.” Human OGG1 (E, lane 2) and buffer alone (NT, lane 1) serve as positive and negative controls, respectively. The arrow indicates the DNA cleavage products (13-mer).

FIG. 5. siRNA-mediated down-regulation of the hOGG1 mRNA and protein in the GM00637 cells. A, cells were transfected with either the mock, control siRNA (c-siRNA) or hOGG1 siRNAs (OG-siRNA1 and OG-siRNA2). Twenty-four hours after transfection, the total RNA was extracted from cells and analyzed using semiquantitative RT-PCR, as described under “Experimental Procedures.” B, cells were treated with mock, control siRNA or hOGG1 siRNAs. Forty-eight hours later, the cell lysates were prepared from the mock-, control siRNA-, or hOGG1 siRNAs-treated cells. Equal amounts (50 μg of protein) of the cell lysates were separated by 12% SDS-PAGE, and transferred onto a nitrocellulose membrane. The membrane was immunoblotted with either anti-hOGG1 or anti-α-tubulin antibodies. The hOGG1 and α-tubulin were detected using the enzyme-linked chemiluminesence. C, a 21-mer containing an 8-oxoG lesion was incubated with the cell extracts from the mock extracts from the mock-, the control siRNA (c-siRNA)-, or hOGG1 siRNAs (OG-siRNA1, OG-siRNA2)-treated cells for 48 h, and oligonucleotide cleavage products (lanes 3–6) were analyzed on DNA sequencing gels and subjected to autoradiography as described under “Experimental Procedures.” Human OGG1 (E, lane 2) and buffer alone (NT, lane 1) serve as positive and negative controls, respectively. The arrow indicates the DNA cleavage products (13-mer).
Forty-eight hours later, cells were exposed to 50 Gy of -radiation. These results suggest that MT-III is important for suppression of mutation. The induced mutant frequencies in the hOGG1 siRNA-transfected cells treated with -irradiation were significantly higher than those of the mock- and control siRNA-transfected cells. The values are presented as a mean ± S.D. from four separate experiments. ** denotes p < 0.01.

| Table II: Mutation frequencies at the HPRT locus in pcDNA3 transfected cells, MT-III transfected clone-7 cells |
|-------------------------------------------------|
| Dose of \(γ\)-irradiation | Cell line | Mutant frequency \((× 10^{-5})\) | Relative mutant frequency\(^a\) |
|---------------------------|-----------|---------------------------------|-----------------------------|
| 5 Gy                      | pcDNA3    | 39 ± 12                         | 1                           |
|                           | pcDNA3 + OG-siRNA 1 | 139 ± 45                      | 3.6                         |
|                           | pcDNA3 + OG-siRNA 2 | 143 ± 49                      | 3.7                         |
|                           | MTIII-clone-7 + OG-siRNA 1 | 61 ± 29                     | 1.6                         |
|                           | MTIII-clone-7 + OG-siRNA 2 | 64 ± 35                     | 1.7                         |
| 10 Gy                     | pcDNA3    | 94 ± 35                         | 2.4                         |
|                           | pcDNA3 + OG-siRNA 1 | 269 ± 64                      | 6.9                         |
|                           | pcDNA3 + OG-siRNA 2 | 272 ± 69                      | 7.0                         |
|                           | MTIII-clone-7 + OG-siRNA 1 | 124 ± 45                    | 3.2                         |
|                           | MTIII-clone-7 + OG-siRNA 2 | 138 ± 57                    | 3.5                         |

\(^a\) Mutations/cell/generation.  
\(^b\) Frequencies are relative to that observed in 5 Gy-irradiated pcDNA3 cells.

**Fig. 6.** siRNA-mediated down-regulation of the hOGG1 leads to increase in -ray-induced 8-oxoG accumulation. Cells were transfected with the mock, control siRNA, or hOGG1 siRNAs (hOGG1-siRNA1 and hOGG1-siRNA2). Forty-eight hours after transfection, cells were exposed to various doses of -ray, and the amount of 8-oxoG in the DNA was measured as described under “Experimental Procedures.” The values are presented as a mean ± S.D. from four separate experiments. ** denotes p < 0.01.

**Fig. 7.** Effect of MT-III on the -ray-induced 8-oxoG accumulation in hOGG1-depleted cells. The empty vector-transfected cells (pcDNA3), MT-III expressing clone-7 cells (MT-III-clone-7), MT-I expressing clone cells (MT-I-clone), and MT-II expressing clone cells (MT-II-clone) were treated with hOGG1 siRNAs (OG-siRNA1 and OG-siRNA2). Forty-eight hours later, cells were exposed to 50 Gy of -ray, and the amount of 8-oxoG in the DNA were measured as described under “Experimental Procedures.” The values are presented as a mean ± S.D. from four separate experiments. ** denotes p < 0.01.

the -ray-induced mutation was significantly increased in hOGG1-siRNA-transfected pcDNA3 clone cells. The HPRT mutant frequencies were 139 ± 45–143 ± 49 \(× 10^{-6}\) and 269 ± 64–272 ± 69 \(× 10^{-6}\) in the hOGG1-siRNA-treated pcDNA3 clone cells exposed to 5 and 10 Gy of -rays, respectively. These results suggest that MT-III is important for suppressing the -radiation-induced mutation, when the hOGG1 is down-regulated.

Silencing of the MT Expression in Human Neuronal Cells via siRNA Leads to an Increase in the -Irradiation-induced 8-oxoG Accumulation—MT is known to contribute to neuroprotection against oxidative stress (2, 50). Therefore, the MT-III may be important for suppressing the 8-oxoG accumulation in neuronal cells response to the ROS generator, such as -irradiation. In order to determine if the siRNA-mediated attenuation of MT expression results in a subsequent increase in 8-oxoG accumulation after exposure to -irradiation, the human neuroblastoma SKNSH cells were transfected with the mock, the control siRNA oligonucleotide, or the MT-specific siRNA oligonucleotides (i.e. 148–168 sequence), harvested 24 h after transfection, and their mRNA and protein expression levels were determined (Fig. 9, A and B). Semiquantitative RT-PCR analysis revealed that the MT-specific siRNA oligonucleotides decreased by more than 90% in terms of their overall MT mRNA levels in the SKNSH cells, compared with the mock- or control siRNA-transfected cells. We next investigated the effect of MT down-regulation on the -ray-induced 8-oxoG formation in neuronal cells and found that reduced levels of MT exhibited significantly increased -irradiation-induced 8-oxoG accumulation when compared with the mock- or control siRNA-transfected cells (Fig. 9C). The amount of 8-oxoG in the genomic DNA from the mock- or control siRNA-transfected cells exposed to 20, 40, and 60 Gy of -irradiation were 12.2 ± 4.1–13.5 ± 4.4, 21.6 ± 6.5–22.5 ± 6.6, and 29.5 ± 9.9–30.6 ± 10.4 per \(10^7\) dG, respectively. However, the MT-specific siRNA-
transfected cells contains remarkably higher 8-oxoG levels. The amount of 8-oxoG in the genomic DNA from the MT-siRNA-transfected cells exposed to 20, 40, and 60 Gy of γ-irradiation were 28.5 ± 9.2, 48.2 ± 11.2, and 62.5 ± 14.3 per 10⁷ dG, respectively. These results suggest that the transfection and subsequent expression of the MT-targeted siRNAs in the human neuronal cells resulted in a marked increase in the 8-oxoG accumulation in response to γ-irradiation. Therefore, the presence of MT in the neuronal cells is important for the protection against oxidative DNA damage.

**DISCUSSION**

The results of this study demonstrate the essential role of MT-III in inhibiting 8-oxoG formation, the major mutagenic base lesion in DNA caused by exposure to ROS. Ionizing irradiation damages the DNA for the most part by ionizing water to produce ROS. These results suggest that the formation of 8-oxoG in human fibroblast cells is increased as a result of exposure to γ-irradiation, resulting in the dose-dependent accumulation of 8-oxoG, and that the increased 8-oxoG level is suppressed significantly by MT-III expression. In addition, elevated MT-III cells caused a further decrease in the γ-irradiation-induced 8-oxoG formation and γ-irradiation-induced mutation frequency in hOgg1-depleted cells, compared with that of hOGG1-depleted control cells. Moreover, the depleted MT neuronal cells exhibited significantly higher 8-oxoG formation in response to γ-irradiation in a dose-dependent manner. Therefore, it is likely that MT-III is important for suppressing the γ-irradiation-mediated increase in 8-oxoG accumulation and mutation.

Recently, several lines of evidence have suggested that MT has a protective effect against ROS-induced DNA damage, including γ-irradiation. For example, increased MT expression levels led to a significant decrease in the radiation-induced chromosome and DNA damage (51, 52). Although the detailed mechanism of MT in protection against radiation needs to be elucidated, many studies support the hypothesis that MT, acting as ROS scavengers, leads to protection against radiation-induced cellular toxicity in normal and tumor cells (53–55). MT has a high cysteine content, it can freely exchange metals with electrophiles, and thereby prevent Fenton reactions, or react directly with H₂O₂ or hydroxyl radicals (16, 56). It has also been demonstrated that the in vitro oxidation of the MT cysteines induces the release of metals, and that ROS can react directly with MT sulfhydryl groups (16–21). Therefore, MT scavenges ROS before it can react with the DNA. The in vivo and in vitro studies show that the efficiency of the protective effect of MT against DNA degradation from oxidative damage was much higher than that of the reduced glutathione (17, 18, 22). Ionizing radiation generates hydroxyl radicals either directly by the hydrolysis of water or indirectly by forming other ROS, including the superoxide anion, hydrogen peroxide, and peroxyl radicals. These ROS may subsequently be converted to hydroxyl radicals by further reactions during the cellular metabolic processes (34–36). Approximately 90% of cellular DNA damage produced by ionizing radiation is caused by hydroxyl radicals, which generates more than 30 different base adducts as well as various amino acids, proteins, and lipid addition products, strand breaks, and cross-links (57). Of the various types of DNA modifications induced by ionizing radiation, 8-oxoG is one of the most prevalent DNA damage products. 8-oxoG preferentially mispairs with adenosine during replication and thereby gives rise to G:C to T:A transversion muta-
MT-III Prevents γ-Ray-induced 8-oxoG Accumulation

The formation of 8-oxoG that occurs as a result of a reaction of hydroxyl radicals from ionizing radiation with DNA is likely to play a role in DNA mutagenesis and carcinogenesis (33). MT overexpression was found to protect the Chinese hamster G12 cells against mutagenesis by oxidative mutagens, as well as against the oxidative stress induced by the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) (37, 58). Similarly, increased levels of MT in the pancreatic β-cells can prevent streptozotocin-induced DNA damage, which is probably caused by ROS (59). Moreover, the underexpression of MT with antisense RNA led to an increase in spontaneous mutagenesis, which was accompanied by increased levels of oxidative stress (38). Using the DNA strand break assay and comet assay following hydrogen peroxide treatment, we have previously demonstrated that MT-III could prevent ROS-induced DNA damage, and the efficiency of the protective effect of MT-III against DNA degradation from oxidative damage was much higher than that of MT-I/II (39). Therefore, MT-III may play an important role in suppressing mutagenesis through a decrease in 8-oxoG accumulation in response to ROS-induced DNA damage, including ionizing radiation. However, there are no biochemical studies reporting the precise role of MT-III in 8-oxoG accumulation. In order to evaluate the potential role of MT-III in the accumulation of 8-oxoG after ionizing radiation, the coding region of human MT-III was cloned and permanently transfected into the GM00637 cells. As shown in Fig. 2, the 8-oxoG levels in the MT-III-expressing clones following exposure to γ-irradiation, expressed as the ratio of 8-oxoG to dG, were significantly decreased when compared with that in the parental- and empty vector-expressing cells. The inhibitory effect of MT-III to γ-ray-induced 8-oxoG accumulation was higher than MT-I and MT-II. It was also demonstrated that MT-III expression did not affect the regulation of the other antioxidant defense system, such as SOD, catalase, glutathione peroxidase, and glutathione (Table I), and that MT-III expression also did not affect the hOGG1 expression level along with its activity (Fig. 3). These results suggest that MT-III itself effectively suppresses γ-irradiation-induced 8-oxoG formation.

Escherichia coli contains a GO system that prevents the mutagenic effect of 8-oxoG. The bacterial GO system consists of three proteins: MutM, a DNA glycosylase/lyase that recognizes 8-oxoG:C and catalyzes the excision of 8-oxoG; MutY, which is a DNA glycosylase that recognizes 8-oxoG:A and catalyzes the excision of A; and MutT, a specific phosphatase to cleave 8-oxo-dGTP (60). In mammalian cells, the main defense enzyme against the mutagenic effects of 8-oxoG in cellular DNA is OGG1, which is structurally unrelated but functionally similar to Mut M. The inactivation of the OGG1 gene in yeast and mice leads to an increase in the spontaneous mutation frequency in cells. The human OGG1 (hOGG1) gene is found on chromosome 3p26.2, and allelic deletions of this region frequently occur in a variety of human cancers (46–48). In addition, the hOGG1 gene is somatically mutated in some cancer cells and is highly polymorphic among the human populations (61, 62). Recently, Mei et al. (49) reported that the 8-oxoG repair activity was significantly reduced in γ-irradiated cells compared with that in control cells. In this study, it was confirmed that the γ-irradiation appeared to decrease hOGG1 protein expression and its repair activity (Fig. 4). Therefore, the exposure of cells to γ-irradiation may lead to significant increases in 8-oxoG accumulation through both ROS generation and inhibition of the 8-oxoG repair activity. These observations indicate that MT-III plays an important role in the suppression of 8-oxoG formation when the hOGG1 level is decreased. In order to explore this hypothesis, siRNAs in the form of two independent, non-overlapping, 21-base pair RNA duplexes, which target human OGG1 (hOGG1), were used as to inhibit its expression. The transfection of the parent GM00637- and pcDNA3-transfected cells with the hOGG1-siRNA resulted in a significant increase in the 8-oxoG accumulation response to γ-irradiation in a dose-dependent manner compared with the mock- and control siRNA-transfected cells. However, the amount of 8-oxoG formation from the hOGG1-depleted MT-III-expressing cells exposed to γ-irradiation was markedly suppressed compared with that of the hOGG1-depleted control cells (Fig. 7). Furthermore, the γ-ray-induced mutation was significantly increased in the hOGG1-depleted cells, and this increase was also suppressed by MT-III expression (Table II). These results strongly suggest that MT-III can prevent the γ-irradiation-induced accumulation of 8-oxoG as well as the γ-irradiation-induced mutation frequency in normal and hOGG1-depleted cells.

MT-I and MT-II isoforms, which are expressed in most tissues including the central nervous system (CNS), are increased in neurodegenerative disease (2). MT-I and MT-II knockout mice exhibit an impaired inflammatory response (63, 64), and MT-I/II deficiency potentiated the oxidative stress caused by kainic acid, a potent convulsive agent (65). Similarly, MT-I/II-null mice exhibit a delayed wound healing capacity following a focal cryolesion (66), and MT-I/II isoforms are major proteins for protecting the CNS following traumatic brain injury (67). Moreover, SOD1 mutant mice, deficient in either MT-III or MT-I, show significant reductions in survival compared with SOD1 mutant mice, and motor dysfunction was markedly accelerated in SOD1 mutant mice deficient in MT-I and MT-III (68). Therefore, the MT-I and M-II isoforms may also be important in the CNS. However, unlike MT-I and MT-II, MT-III possesses several other unique properties. For example, MT-III is unlikely to be a significant factor for controlling the inflammatory response (69). MT-III, but not MT-I or MT-II, antagonizes both the neurotoxic and neuropharmacologic effect of amyloid β peptide, which is the principal component of neuritic plaques (70, 71). In addition, MT-III prevents aberrant neuronal sprouting and neurofibrillary tangles (4, 72–74). However, neither MT-I nor MT-II exhibit growth inhibitory activity. Similarly, MT-III, but not MT-I or MT-II, normally inhibits peripheral nerve regeneration (75). Moreover, MT-III protects neurons against glutamate (76), hydrogen peroxide (39), high concentration oxygen (77), hypoxia (78), and nitrosative stress (79). These activities are sometimes weak or absent in MT-I and MT-II (39, 76–79). Furthermore, MT-III suppresses zinc-mediated neuronal death in the hippocampal CA1 (80) and the thalamus protects and preserves injured motor neurons after facial nerve (81). How does MT-III have these unique properties? Recently, Chen et al. (79) have demonstrated that MT-III is the most reactive and neuroprotective against S-nitrosothiols, whereas its reactivity and neuroprotection against H2O2, however, are similar to those of MT-I and MT-II. Because S-nitrosothiols are thought to be signaling molecules involved in the action of nitric oxide, this suggests that MT-III could function as a specific nitric oxide scavenger under stress conditions to protect neurons. In contrast, Uchida et al. (77) have demonstrated that MT-III did not scavenge either superoxide anion or nitric oxide, but has scavenging activity for hydroxyl radicals under conditions in which MT-I and MT-II show no scavenging activity for hydroxyl radicals. Therefore, this suggests that MT-III protects cortical neurons against neurotoxicity and neurite sprouting via scavenging intracellular hydroxyl radicals. Such differential effects observed from one study to another could reflect assay system specificity. Accordingly, MT-III acts as a scavenger for hydroxyl radicals and nitric oxide; this may be responsible for the protective action of MT-III.
against neuotoxicity and could bear importantly on the unique properties of MT-III such as neuronal growth inhibitory activity.

Cellular damage as a result of oxidative stress has been suggested to contribute to the pathophysiology of neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases as well as to the normal aging process (82). Oxidative damage results from an impaired oxidative balance in which ROS production exceeds the cellular antioxidant defenses, leading to proteins, lipids, and nucleic acids. The cumulative damage particularly to the DNA is believed to contribute to the progressive neuronal cell loss as unrepaired DNA damage can trigger programmed cell death (83, 84). Additionally, several lines of evidence suggest that there is an association between the accumulation of DNA oxidation and neurodegenerative diseases (85–87). Indeed, the 8-oxoG level in the brain was higher in Alzheimer’s, Parkinson’s, and Huntington’s disease patients as well as in amyotrophic lateral sclerosis (88–90). Moreover, a significant decrease in the base-specific glycosylase activity that excised 8-oxoG has also been observed in all Alzheimer’s disease brain regions examined (91, 92). Endogenous and exogenous triggers may cause either the overproduction of ROS or the impairment of these antioxidant defense systems, thereby, leading to oxidative DNA damage. The brain contains several defense systems that balance the ratio between the generation and detoxification of ROS. However, the central nervous system is particularly susceptible to oxidative damage because of its higher energy requirement, high oxygen consumption rate, and low antioxidant content compared with other organs (93). MT-III levels are reduced in neurodegenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis (26, 94). In addition, the expression level of MT-III was significantly increased after brain injury (27). Moreover, MT-III is not induced by typical promotors of the MT-I and MT-II such as increased zinc, kainic acid, dexamethasone, or lipopolysaccharides, but is induced by dopamine, which is known to generate ROS, and this induction is abrogated by a group of antioxidants (glutathione, vitamin E, and ascorbic acid) known to scavenge reactive oxygen species (95–97). These observations suggest that MT-III expression in the neuronal cells might be important for suppressing the oxidative DNA damage in response to oxidative stress. In order to test this hypothesis, human neuronal cells were transfected with the MT-specific siRNA, which targets the MT and inhibits its expression, and it was found that the transfection of cells with the MT-specific siRNA resulted in an increase in 8-oxoG accumulation response to γ-irradiation compared with the mock- and control siRNA-transfected cells (Fig. 9). Thus, the results of the neuroblastoma study and those of fibroblast studies suggest that MT-III contributes to the suppression of γ-irradiation-induced 8-oxoG accumulation in normal and hOGG1-depleted cells. Because oxidative DNA damage has been implicated in the pathogenesis of a variety of neuronal diseases (82–90), the inhibitory effect of MT on the accumulation of 8-oxoG might contribute to its neuroprotective activity.

In summary, oxidative DNA damage is the result of an imbalance between the production of ROS, which are believed to be responsible for most of the initial DNA damage caused by oxidative stress, and the capability of cellular antioxidant defense systems. Cells have developed elaborate networks to deal with potentially damaging radicals, which include enzymatic and non-enzymatic antioxidative systems. Although antioxidant enzymes including SOD, catalase, and glutathione peroxidase are important cellular antioxidant defense systems; they do not contribute to the protection of oxidative DNA damage because the nucleus lacks these antioxidant enzymes (98). In contrast to the antioxidant enzymes, MT are present in both the nucleus and cytoplasm in the normal circumstances. Moreover, DNA-damaging agents such as UV-irradiation lead to an increase in MT expression level as well as an increase in the stimulation of the nuclear translocation of MT (99–101). This nuclear accumulation of MT is an important factor that determines the role of MT in the protection from ROS-induced DNA damage, because DNA is quite sensitive to oxidative stress, whereas the nucleus does not contain antioxidant enzymes.

MT-null mice are high incidence to tumor formation caused by chemical carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA), TPA and N-butyl-N-(4-hydroxybutyl)nitrosamine (58, 102). In addition, the carcinogenicity caused by γ-rays and anticancer drugs such as cisplatin and melphalan have been prevented by a pretreatment with bismuth and zinc, both of which are well known MT inducers (103, 104). We demonstrate that MT-III is important for suppressing the level of γ-ray-induced 8-oxoG and mutations in the normal and hOGG1-depleted cells. These results strongly suggest that MT-III protect against oxidative DNA damage, and this protection may, at least in part, contribute to the anticarcinogenic and neuroprotective role of MT-III.

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Metallothionein-III Prevents γ-Ray-induced 8-Oxoguanine Accumulation in Normal and hOGG1-depleted Cells

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