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Review

Oxidative Damage in Nucleic Acids and Parkinson's Disease

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Oxidative DNA lesions, such as 8-oxoguanine (8-oxoG), accumulate in nuclear and mitochondrial genomes during aging, and such accumulation can increase dramatically in patients with Parkinson's disease (PD). To counteract oxidative damage to nucleic acids, human and rodents are equipped with three distinct enzymes. One of these, MTH1, hydrolyzes oxidized purine nucleoside triphosphates, such as 8-oxo-2′-deoxyguanosine triphosphate and 2-hydroxy-2′-deoxyadenosine triphosphate, to their monophosphate forms. The other two enzymes are 8-oxoG DNA glycosylase encoded by the *OGG1* gene and adenine/2-hydroxyadenine DNA glycosylase encoded by the *MUTYH* gene. We have shown a significant increase in 8-oxoG in mitochondrial DNA as well as an elevated expression of MTH1, OGG1, and MUTYH in nigrostriatal dopaminergic neurons of PD patients, suggesting that the buildup of these lesions may cause dopamine neuron loss. We established MTH1-null mice and found that MTH1-null fibroblasts were highly susceptible to cell death caused by H_2O_2 characterized by pyknosis and electron-dense deposits in the mitochondria, and that this was accompanied by an ongoing accumulation of 8-oxoG in nuclear and mitochondrial DNA. We also showed that MTH1-null mice exhibited an increased accumulation of 8-oxoG in striatal mitochondrial DNA, followed by more extreme neuronal dysfunction after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration than that of wild-type mice. In conclusion, oxidative damage in nucleic acids is likely to be a major risk factor for Parkinson's disease, indicating that a solid understanding of the defense mechanisms involved will enable us to develop new strategies for protecting the brain against oxidative stress.   © 2007 Wiley-Liss, Inc.

Key words: oxidative damage; nucleic acids; Parkinson's disease

The adult human brain represents only about 2% of total body weight; however, it consumes about 20% of the resting total body O_2 consumption, which totals about 360 liters per day. In children, the brain takes up an even larger fraction, as much as 50% in the middle of the first decade of life. About 90% of total oxygen consumption occurs in mitochondria, where oxidative phosphorylation allows cells to produce ATP as energy to ensure viability and function. The brain requires an extraordinarily large amount of energy to maintain active transport of the ions required for neuronal excitation and neurotransmission, and the adult brain utilizes about 10 mol of ATP per day (approximately 5 kg/day; Clarke and Sokoloff, 1999).

In mitochondria, about 1–4% of consumed oxygen molecules are partially reduced by electrons that have leaked from the respiratory chain, generating reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals (Kang et al., 1998). Reflecting its extraordinarily large consumption of oxygen, the brain is thought to produce large amounts of ROS continuously. ROS are so highly reactive that they can readily oxidize macromolecules in living cells, including lipids, proteins, and nucleic acids, thereby leading to various types of cellular dysfunction, including cell death and mutagenesis (Ames et al., 1993).

Among the various types of oxidative damage in cellular macromolecules, damage to nucleic acids is particularly hazardous, because it can alter the genetic information present in both nuclear and mitochondrial genomic DNA (Cooke et al., 2003). Neurons are postmitotic cells and thus undergo no replication of nuclear genomes, however, we assume that genetic information contained in nuclear genomes would be expected to be

well maintained in order to support proper expression of genes whose functions are essential to brain activity. Moreover, mitochondrial genomes, which are continuously replicated even in the postmitotic neurons under an increased oxidative stress, have to be maintained so that the production of energy is secure.

We recently established that brains are equipped with defense mechanisms against oxidative damage to nucleic acids and found that Parkinson's disease (PD) brains contained a large buildup of oxidative DNA damage, which was accompanied by an altered expression of the genes involved in defense mechanisms (Nakabeppu et al., 2006b). In this Mini-Review, our discussion centers on the implications of oxidative DNA damage in neurodegeneration, especially with regard to an animal model of PD.

DAMAGE TO NUCLEIC ACIDS UNDER OXIDATIVE STRESS

Among the five normal nucleobases—uracil, thymine, cytosine, adenine, and guanine—guanine is the most susceptible to oxidation, and, among guanine residues in nucleic acids, the C8 position of deoxyguanosine (dG) or dGTP is the most effectively oxidized by hydroxyl radicals. The lesion formed is 8-oxo-dG or 8-oxo-dGTP (Kasai and Nishimura, 1984), and, in fact, eight to nine times more 8-oxoguanine (8-oxoG) is formed in nucleotide dGTP than in DNA. In the case of dATP, however, the C2 position is under attack, and, once it is effectively oxidized, the lesion formed is 2-hydroxy-dATP (2-OH-dATP). However, the formation of 2-OH-A residues in DNA is only about 1.5% of the level of 2-OH-A residues that are formed from dATP (Kamiya and Kasai, 1995). It is known that 8-oxo-dATP is generated by γ-ray irradiation (Fujikawa et al., 1999). Free nucleotides are thus more susceptible to oxidation by ROS than is DNA; however, it is very difficult to detect these oxidized nucleotides in vivo, probably because the dNTP precursors are newly synthesized just prior to DNA replication. Ribonucleotides such as ATP and GTP are also similarly oxidized to 2-OH-ATP and 8-oxo-GTP, respectively (Fujikawa et al., 2001).

8-OxoG generated in DNA by its direct oxidation can pair with an incoming adenine as well as cytosine during DNA replication, thus resulting in a G:C to T:A transversion mutation after two rounds of replication (Fig. 1; Shibutani et al., 1991). It has been established that 8-oxo-dGTP and 2-OH-dATP are frequently misinserted opposite an incorrect base in the template DNA by various replicative DNA polymerases ranging from bacterial to human (Maki and Sekiguchi, 1992; Kamiya and Kasai, 2000). As summarized in Figure 1, 8-oxodGTP is misinserted opposite adenine as well as cytosine in template DNA, generally causing an A:T to C:G transversion after two rounds of replication. 2-OH-dATP tends mostly to be misinserted opposite guanine, generally inducing a G:C to T:A transversion. It is also known that 8-oxoGTP or 2-OH-ATP can be incorpo-rated into RNA by RNA polymerases, thereby generating mutant proteins through translational errors (Taddei et al., 1997; Hayakawa et al., 1999).

THE OXIDIZED PURINE NUCLEOSIDE TRIPHOSPHATASE MTH1 SANITIZES NUCLEOTIDE POOLS

In human and rodent cells, an oxidized purine nucleoside triphosphatase termed "MTH1" efficiently hydrolyzes two forms of oxidized dATP, namely, 2-OH-dATP and 8-oxo-dATP, to their monophosphates, in addition to 8-oxo-dGTP. These monophosphates are converted to nucleosides such as 8-oxo-dG, thus avoiding their incorporation into DNA (Fig. 1B; Fujikawa et al., 1999; Nakabeppu, 2001a). MTH1 also hydrolyzes the oxidized ribonucleotides 2-OH-ATP, 8-oxo-ATP, and 8-oxo-GTP (Fujikawa et al., 2001). Among substrates, MTH1 has its highest affinity for 2-OH-ATP (Km = 4.3 µM), whereas its highest catalytic efficiency is toward 2-OH-dATP (kcat/Km = $1.68 \text{ sec}^{-1} \text{ µm}^{-1}$). We recently determined the structure of MTH1 in solution by multidimensional heteronuclear NMR spectroscopy (Mishima et al., 2004). We generated models for the substrate recognition of MTH1, based on the arrangement of the pocket-forming residues, combined with the mutagenesis data, and found that Asn-33 and Asp-119 play pivotal roles in discriminating the oxidized forms of purine, namely, 8-oxoG and 2-OH-A, whereas Trp-117 is important in determining affinity with the purine rings (Sakai et al., 2002).

The human *MTH1* gene is located on chromosome 7p22, and consists of five major exons; two alternative exon 1 sequences, namely, exons 1a and 1b, and three contiguous exon 2 segments (exons 2a, 2b, and 2c), which are alternatively spliced. Thus, the *MTH1* gene produces seven types of mRNA that encode three different human MTH1 isoforms, hMTH1b (p22), hMTH1c (p21), and hMTH1d (p18; Oda et al., 1997). There are two major single nucleotide polymorphisms (SNPs) in the *MTH1* gene: one (GC/GT) is located at the beginning of exon 2c, whereas the other (GTG/ATG) is located on exon 4. The GC/GT polymorphism modifies patterns of alternative splicing and encodes a fourth isoform, hMTH1a (p26), in addition to the three known isoforms. The GTG/ATG polymorphism replaces amino acid residue valine 83 (Val83) in hMTH1d with methionine 83 (Met83; Oda et al., 1999). The substitution of Val83 with Met83 in hMTH1d was found to increase the thermolability of this enzyme as well as its α-helix content (Yakushiji et al., 1997). Furthermore, molecular epidemiological studies have revealed that the allele frequencies of Met83 with the GC polymorphism in patients with hepatocellular carcinoma, gastric cancer, and lung cancer were higher than those of healthy volunteers, supporting the suggestion that hMTH1d (Met83) may represent a functional defect (Nakabeppu, 2001a; Kimura et al., 2004; Kohno et al., 2006).
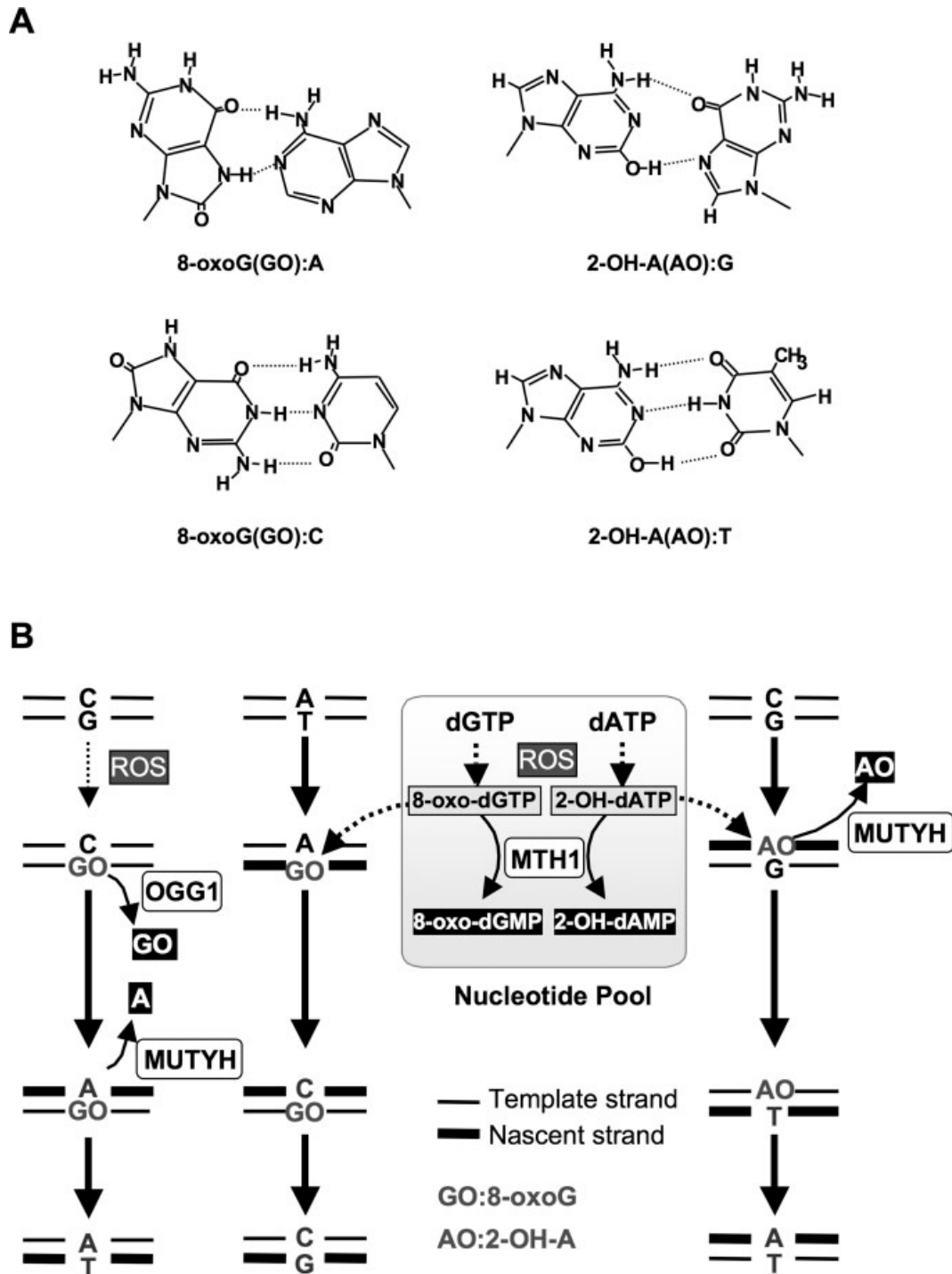
Fig. 1. Mutagenesis caused by the oxidation of nucleic acids and the defense mechanisms in mammals. **A:** Altered base pairing of 8-oxo-guanine and 2-hydroxyadenine. During DNA replication, 8-oxoG and 2-OH-A can pair with adenine and guanine, respectively, in template DNA. **B:** Mutagenesis caused by 8-oxoG and 2-OH-A. 8-OxoG is accumulated in DNA as a result of the incorporation of 8-oxo-dGTP from nucleotide pools or because of the direct oxidation of DNA, and this buildup increases the likelihood of an A:T to C:G or G:C to T:A transversion. On the other hand, 2-OH-A is derived mainly from the incorporation of 2-OH-dATP from nucleotide pools. The accumulation of 8-oxoG or 2-OH-A in DNA is minimized through the coordinated actions of MTH1, OGG1, and MUTYH. See text for details. GO, 8-oxoguanine (8-oxoG); AO, 2-hydroxyadenine (2-OH-A). Bold lines: Nascent strands of DNA.

We reported earlier that most of the 18-kDa hMTH1 protein is localized in the cytoplasm, with about 5% in the mitochondrial matrix (Kang et al., 1995). We recently demonstrated that all hMTH1 isoforms are capable of hydrolyzing both 2-OH-dATP and 8-oxo-dGTP to their monophosphates and that the 18-amino acid sequence in the amino (N)-terminal region of hMTH1a functions as a mitochondria-targeting signal. hMTH1a is thus considered to be localized in the mitochondria to the same extent as hMTH1d. When the latter contains a Met83 substitution, it is less likely to localize in mitochondria than is MTH1d with Val83 (Sakai et al., 2006). These observations support the idea that hMTH1 plays an important role in maintaining the quality of the nucleotide pools of both nuclear and mitochondrial genomes, as well as that of ribonucleotide pools.

8-OXOGUANINE DNA GLYCOSYLASE, OGG1, PREVENTS ACCUMULATION OF 8-OXOGUANINE IN BOTH NUCLEAR AND MITOCHONDRIAL GENOMES

Once 8-oxoG has formed in DNA, 8-oxoG DNA glycosylase encoded by the OGG1 gene removes this oxidized base to initiate base excision repair (BER). The DNA glycosylase activity of OGG1 preferentially excises 8-oxoG or 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) opposite cytosine, and to a lesser extent thymine, but not when either is opposite guanine or adenine. In addition, OGG1 also possesses AP lyase activity (Fig. 1B; Boiteux and Radicella, 2000).

The eight exons of the human OGG1 gene are located on chromosome 3p25, a region showing a frequent loss of heterozygosity in lung and kidney tumors (Aburatani et al., 1997; Boiteux and Radicella, 2000). There are more than seven alternatively spliced forms of OGG1 mRNA, and these have been classified into two types based on their last exons (type 1 with exon 7: 1a and 1b; type 2 with exon 8: 2a to 2e; Nishioka et al., 1999). Types 1a and 2a mRNA are the major OGG1 transcripts in various human tissues and encode hOGG1-1a and hOGG1-2a isoforms of human OGG1 protein, respectively. hOGG1-1a protein has a nuclear localization signal (NLS) at its C-terminal end and thus is located in the nucleus, whereas hOGG1-2a protein, which has a unique C-terminal region consisting of two distinct regions, namely, an acidic region (amino acid residues from Ile^{345} to Asp^{381}) and a hydrophobic region (the last 20 residues), is located exclusively in the mitochondria. Both hOGG1-1a and hOGG1-2a carry a relatively poor mitochondrial targeting sequence (MTS) at their N-terminal region. This sequence consists of residues 9–26, whose activity is not sufficient for localizing nuclear hOGG1-1a with NLS within the mitochondria. On the other hand, hOGG1-2a is likely associated with the mitochondrial inner membrane and other BER machinery dependent on the unique C-terminal region (Nishioka et al., 1999; Stuart et al., 2005).

MUTYH FUNCTIONS AS A BIFUNCTIONAL DNA GLYCOSYLASE FOR 2-HYDROXYADENINE OPPOSITE GUANINE AND ADENINE OPPOSITE 8-OXOGUANINE

DNA polymerases may insert adenine into the nascent strand when they encounter 8-oxoG in the template strand during DNA replication, thus increasing the likelihood of a G:C to T:A transversion (Fig. 1B; Miller, 1996; Maki, 2002). A DNA glycosylase encoded by the MUTYH gene excises the adenine inserted opposite 8-oxoG in the template strand (Slupska et al., 1999; Tominaga et al., 2004). MUTYH protein also has the ability to excise 2-OH-A incorporated opposite guanine in the template (Fig. 1B; Ohtsubo et al., 2000; Ushijima et al., 2005). It has been shown that the adenine base in DNA is barely oxidized, whereas adenine nucleotides are easily oxidized in vitro, suggesting that 2-OH-A is derived mostly through the incorporation of 2-OH-dATP during DNA replication (Kamiya and Kasai, 1995). It is therefore likely that MUTYH has to recognize specifically adenine or 2-OH-A incorporated into the nascent strand at this time. MUTYH has been demonstrated to have a functional proliferating cell nuclear antigen (PCNA) binding motif (Parker et al., 2001). We have already shown that, in cultured cells, MUTYH repair activity directed toward adenine incorporated opposite 8-oxoG in transfected plasmid DNA is dependent on this motif (Hayashi et al., 2002). However, we recently found that the PCNA-binding motif in MUTYH is not essential for suppressing the increased spontaneous mutation rate observed in MUTYH-null cells (Hirano et al., 2003). MUTYH has been shown to interact with other replication-associated proteins such as RPA or MSH2/MSH6, which also can interact with PCNA, thus suggesting that the interaction of MUTYH with these proteins may support MUTYH function in the absence of PCNA (Gu et al., 2002).

The human MUTYH gene is located on the short arm of chromosome 1, between p32.1 and p34.3, and consists of 16 exons (Slupska et al., 1996). We previously reported that there are three major MUTYH transcripts in human cells, namely, types α, β, and γ. Each transcript has a different 5′ sequence or first exon, and each is alternatively spliced, so multiple forms of human MUTYH proteins are present in nuclei and mitochondria (Ohtsubo et al. 2000). Human MUTYH protein encoded by type α mRNA possesses a mitochondrial targeting sequence (MTS) consisting of 14 amino-terminal residues, which are required for its localization in the mitochondria (Takao et al. 1998), whereas those encoded by type β and γ mRNAs lack the MTS and are localized in the nuclei (Ohtsubo et al., 2000). As a result, the subcellular localization of MUTYH in human cells indicates that mitochondrial DNA is an important target for BER initiated by MUTYH, as well as OGG1, probably because of increased oxidative stress (Nakabeppu, 2001b).

CELLULAR DYSFUNCTION CAUSED BY THE ACCUMULATION OF OXIDIZED NUCLEOTIDES AND ITS PREVENTION BY MTH1

We reported that lung adenoma/carcinoma developed spontaneously in OGG1-null mice at about 1.5 years after birth and that 8-oxoG had accumulated in their genomes because of the absence of BER of 8-

oxoG initiated by 8-oxoG DNA glycosylase encoded by the *Ogg1* gene (Sakumi et al., 2003). We then found that no tumors had formed in the lungs of mice lacking both OGG1 and MTH1 proteins, despite their increased accumulation of 8-oxoG. This suggests that *Mth1* gene disruption resulted in suppression of the tumorigenesis caused by OGG1 deficiency. We hypothesized, based on these facts, that, because of these combined deficiencies, a large accumulation of oxidized purine nucleoside triphosphates in nucleotide pools and/or a buildup of oxidized purine bases such as 8-oxoG or 2-OH-A in cellular DNA and RNA would necessarily result in cell death. If this is the case, one could expect that cancer stem cells lacking both OGG1 and MTH1 proteins might not survive to produce progenitors with mutations in either protooncogenes or tumor suppressor genes, as was seen in a study in which carcinogenesis was suppressed in mice lacking both OGG1 and MTH1 proteins (Nakabeppu et al., 2004).

Indeed, MTH1-null mouse embryo fibroblasts (MEF) were found to be highly susceptible to cell dysfunction and death caused by exposure to H_2O_2 and showed morphological features of pyknosis and electron-dense deposits in their mitochondria (Fig. 2; Yoshimura et al., 2003). The cell death observed was not dependent on either poly(ADP-ribose)-polymerase or caspases. High-performance liquid chromatography-tandem mass spectrometry analysis and immunofluorescence microscopy revealed an ongoing accumulation of 8-oxoG in nuclear and mitochondrial DNA after exposure to H_2O_2. All of the H_2O_2-induced changes observed in MTH1-null MEF were effectively suppressed by the expression of wild-type hMTH1, but were only partially suppressed by the expression of mutant hMTH1 defective in either 8-oxo-dGTPase or 2-OH-dATPase activity. Thus, hMTH1 protects cells from H_2O_2-induced cell death by hydrolyzing oxidized purine nucleotides,
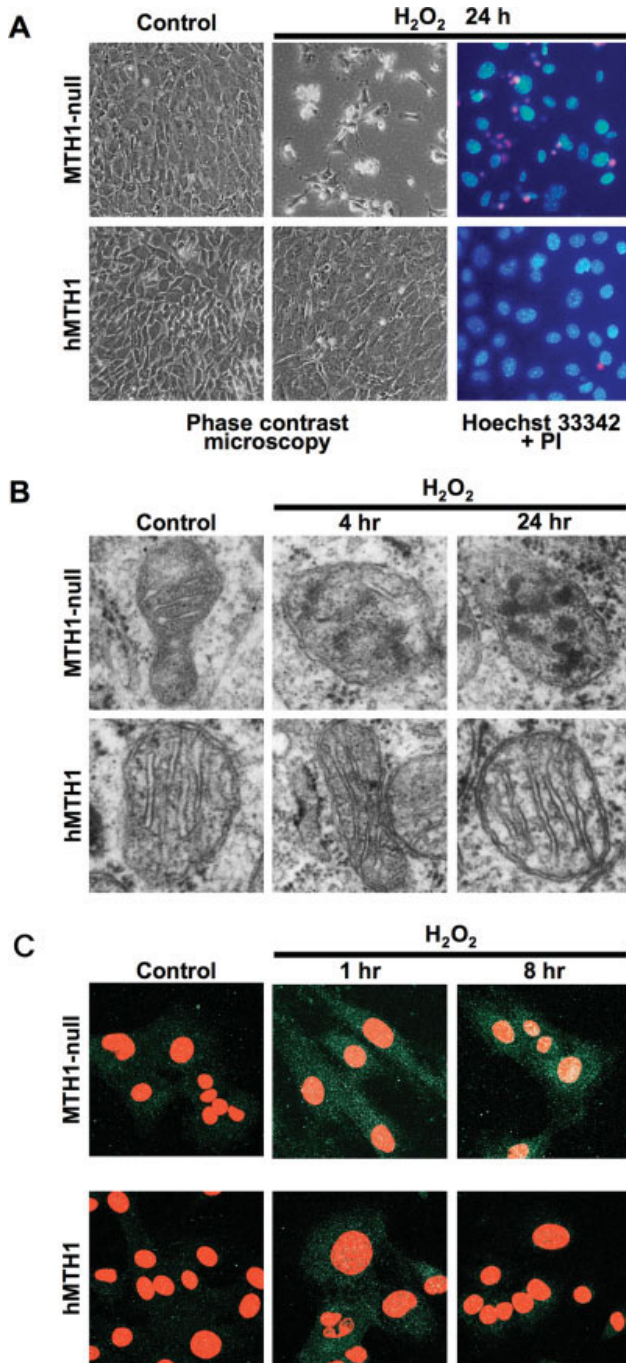

Fig. 2. hMTH1 suppressed the H_2O_2-induced cell death of MTH1-null MEF preceded by pyknosis and mitochondrial dysfunction. **A:** MTH1-null MEF (MTH1-null) or hMTH1-expressing cells (hMTH1) were examined by phase-contrast microscopy either with or without (control) exposure to 500 μM H_2O_2 for 24 hr. These cells were also stained with Hoechst 33342 and propidium iodide (PI) and examined under a fluorescence microscope. **B:** hMTH1 suppressed the morphological alterations of mitochondria in MTH1-null MEF induced by exposure to H_2O_2. Mitochondria in MTH1-null MEF (MTH1-null) and hMTH1-expressing cells (hMTH1) were examined with an electron microscope 4–24 hr after exposure to 500 μM H_2O_2. Control: untreated cells. **C:** The accumulation of 8-oxoG in cellular DNA of MTH1-null MEF after exposure to H_2O_2 was suppressed by hMTH1. 8-OxoG that accumulated in MTH1-null MEF (MTH1-null) and hMTH1-expressing cells (hMTH1) exposed to 300 μM H_2O_2 for 1 and 8 hr was detected by laser scanning fluorescence microscopy with an anti-8-oxoG antibody (green). The nuclei were counterstained with PI (red). Control, cells not exposed to H_2O_2. (Adapted from Yoshimura et al., 2003).

including 8-oxo-dGTP and 2-OH-dATP, a cell death that may be partially attributed to a dysfunction following the buildup of electron-dense deposits in mitochondria (Fig. 2B). These results would explain why mice lacking both OGG1 and MTH1 developed dramatically fewer lung tumors than OGG1-null mice (Nakabeppu et al., 2004).

ALTERED EXPRESSION OF MTH1, OGG1, AND MUTYH IN PARKINSON'S DISEASE BRAINS ASSOCIATED WITH 8-OXOGUANINE ACCUMULATION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. PD is characterized primarily by a loss of dopaminergic neurons in the nigrostriatal system. PD patients suffer from various motor impairments, including bradykinesia, tremors, and rigidity, and the appearance of such symptoms is thought to require a 70–80% loss of striatal nerve terminals, a 50–60% loss of dopaminergic neurons in the substantia nigra (SN), and a 70–90% dopamine deficiency (Riederer and Wuketich, 1976; Mayeux, 2003).

Oxidative damage and partial deficiencies in mitochondrial complex I in the nigrostriatal pathway have been suggested by many investigators to contribute to the selective loss of dopaminergic neurons in this disease (Lotharius and Brundin, 2002; Jenner, 2003). Indeed, several types of oxidative damage have been demonstrated in midbrain tissue from PD patients, including increased levels of iron, decreased levels of reduced glutathione, and the increased appearance of oxidative products of lipids, proteins, and DNA. We and others have reported that PD patients show a significant increase in 8-oxoG in mitochondrial DNA or cytoplasmic RNA in the dopaminergic neurons remaining in the SN (Alam et al., 1997; Shimura-Miura et al., 1999; Zhang et al., 1999), compared with age-matched controls or patients with multiple system atrophy (MSA) who also have degenerated dopaminergic neurons in the SN as well as in other parts of the brain (Fig. 3A–D; Wenning et al., 2004). In addition, we observed that the level of MTH1 protein localized in mitochondria was significantly increased in dopaminergic neurons of the SN of PD patients, but not in other related neurodegenerative disorders, such as MSA (Fig. 3E–H; Shimura-Miura et al., 1999). In SN of the control brains, MTH1 expression was hardly detectable in dopaminergic neurons, suggesting that the increased SN expression of MTH1 in the surviving dopaminergic neurons of PD brains may have a neuroprotective role.

As for the control subjects, hOGG1-2a immunoreactivity was rarely observed in any of the brain regions examined, including cortex, basal ganglia, SN, and pontine nuclei, and was never observed in glial cells (Fig. 4A,C; Fukae et al., 2005). However, the number of hOGG1-2a-positive neurons in the SN increased with age (Fig. 4K). Western blot analysis showed that expression of hOGG1-2a also increased with age in control subjects. When the duration of PD was less than 10 years, the dopaminergic neurons remaining in the SN

showed intense cytoplasmic immunostaining and a granular pattern for hOGG1-2a, which was colocalized with cytochrome oxidase I in mitochondria. However, those who had had PD for longer than 10 years did not exhibit intense cytoplasmic immunostaining (Fig. 4B,E–H; Fukae et al., 2005). Semiquantitative analysis showed that the expression of hOGG1-2a was significantly
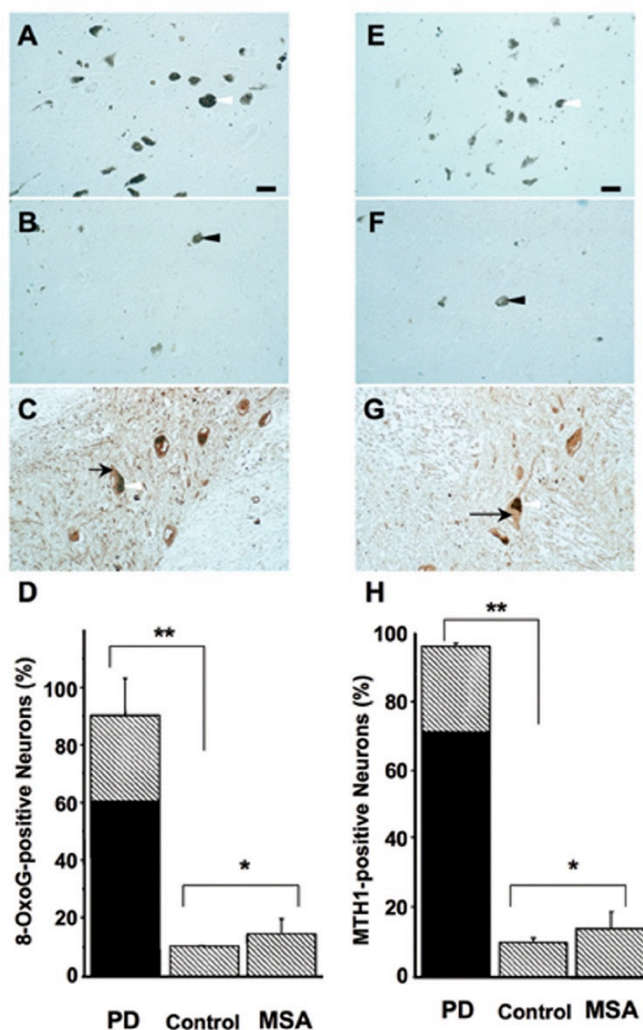

Fig. 3. Accumulation of 8-oxoG and altered expression of hMTH1 in Parkinson's brain. **A–D:** Immunohistochemistry of 8-oxoG. **E–H:** Immunohistochemistry of hMTH1. Control (A,E), multiple system atrophy (MSA) patient (B,F), PD patient (C,G). The cytoplasm of SN neurons in the PD patient shows homogeneous staining for both 8-oxo-dG (C) and hMTH1 (G), but the control and MSA patient show no staining. The arrows indicate neuronal cytoplasm, and the arrowheads indicate neuromelanin. D: Quantitative assessment of 8-oxoG accumulation in SN neurons. H: Quantitative assessment of hMTH1 expression in SN neurons. Data are expressed as a percentage of total neurons (mean ± SEM). **$P < 0.01$ in Parkinson's disease vs. control and MSA cases. *$P < 0.05$ in controls vs. MSA cases. Solid bars indicate intense staining; hatched bars indicate weak staining. Scale bars = 50 μm. (Adapted from Shimura-Miura et al., 1999).
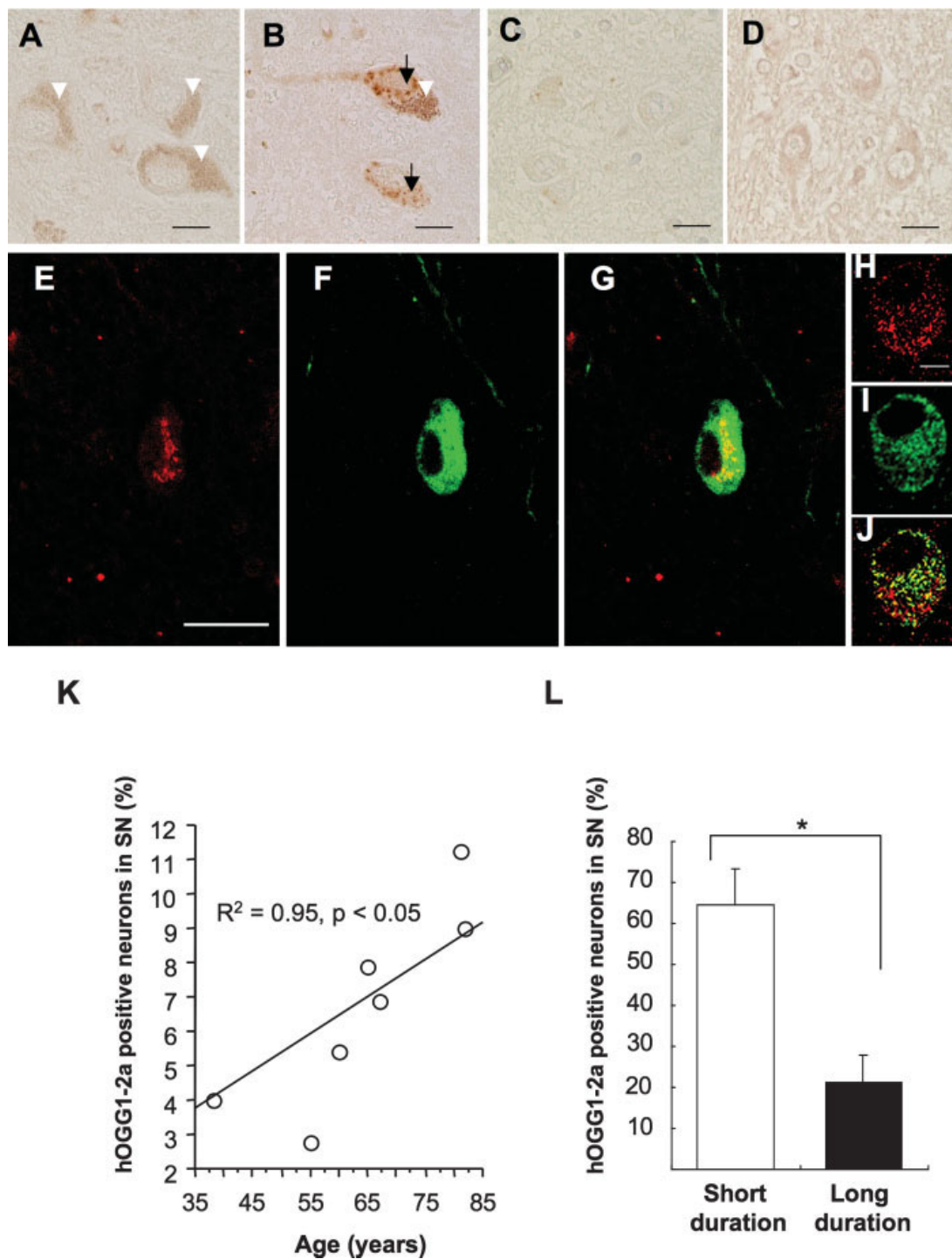
Fig. 4. Altered expression of the mitochondrial form of hOGG1 in PD brain. **A–D:** Immunohistochemistry for hOGG1-2a in the SN (A,B) and pontine nuclei (PN; C,D) of representative subjects. Control (A,C), PD patient (B,D). Note the granular staining of hOGG1-2a (arrow) in the cytoplasm of SN neurons of the PD patient. Arrowheads indicate neuromelanin. **E–J:** Neurons in the SN of a PD patient doubly stained with anti-hOGG1-2a antibody (red in E) and antityrosine hydroxylase (TH) antibody (green in F) and merged (G), or with anti-hOGG1-2a antibody (red in H) and anticytochrome oxidase subunit I antibody (green in I) and merged (J). Note the granular hOGG1-2a expression pattern in the cytoplasm of TH-positive neurons and colocalization with cytochrome oxidase subunit 1. **K:** Age-dependent increase in the percentage of hOGG1-2a-positive neurons in the SN of control subjects. There was a significant correlation with age ($P < 0.05$). **L:** Results of semiquantitative analysis of hOGG1-2a in the SN of PD patients. Percentage of hOGG1-2a-positive neurons in short- and long-duration PD groups (mean ± SEM). The percentage of hOGG1-2a-positive neurons was significantly higher in the short-duration group than in the long-duration group (★$P < 0.05$). Scale bars = 10 μm in A–D; 20 μm in E (applies to E–G); 10 μm in H (applies to H–J). (Adapted from Fukae et al., 2005, with permission from Springer.)
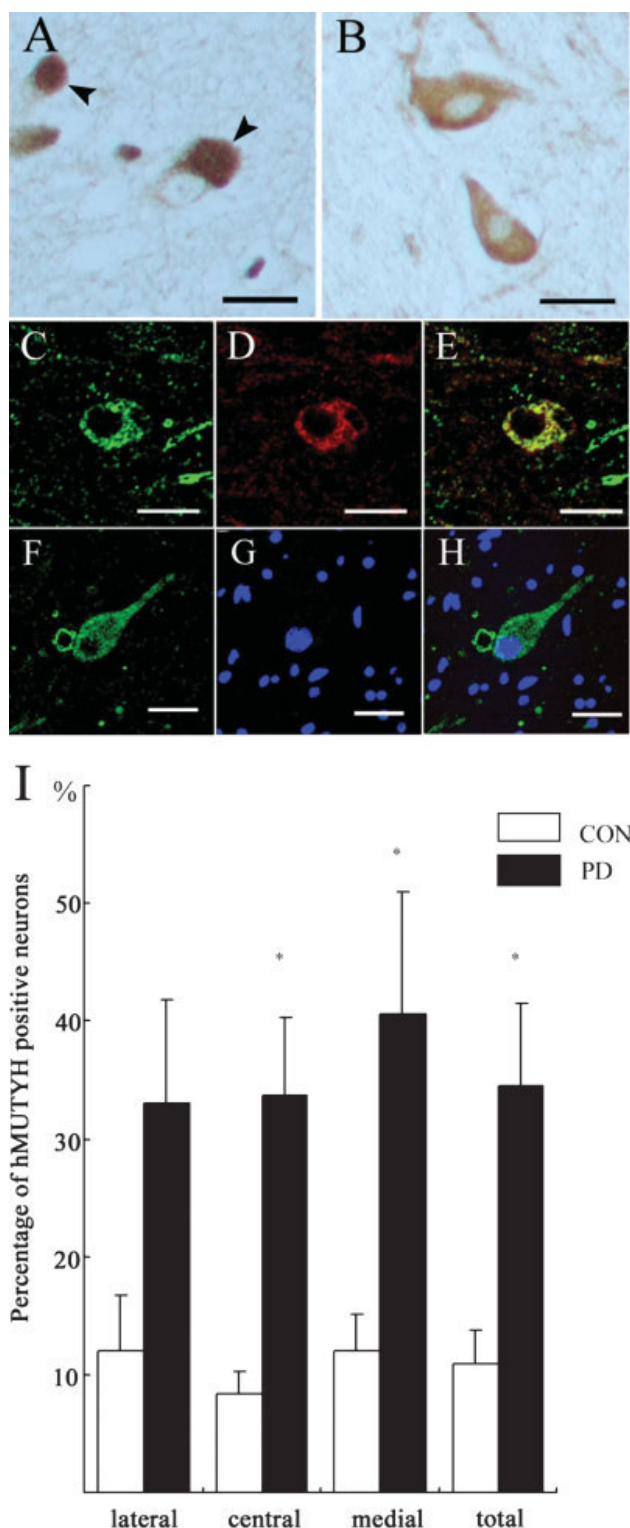
higher in the short-duration group relative to the age-matched controls (Fig. 4L). In the long-duration group, the number of hOGG1-2a-positive neurons was slightly higher, although this was statistically insignificant compared with the aged-matched controls. Western blot analysis demonstrated that the level of hOGG1-2a (43 and 40 kDa) in the SN of PD brain was 1.6–2.9-fold higher than that of the age-matched controls.

We also demonstrated up-regulation of MUTYH in the mitochondria of the SN of PD patients (Fig. 5; Arai et al., 2006). The dopaminergic neurons remaining in the SN showed intense and diffuse immunostaining for MUTYH in the cytoplasm but none in nuclei or Lewy bodies (Fig. 5). On the other hand, glial cells, including oligodendrocytes and astrocytes, were barely immunoreactive. Western blot analysis of PD patients revealed high MUTYH levels and the expression in PD brains of a 47-kDa molecule as the major MUTYH isoform. The 47-kDa molecule was localized within the mitochondria as confirmed by double staining with a mitochondrial marker (Fig. 5C–E).

To confirm the expression of the 47-kDa MUTYH isoform, we performed RT-PCR with total RNA isolated from the SN of PD and control brains (Arai et al., 2006), and we detected four types of MUTYH mRNA. Among these, type $\beta4$ mRNA encoding the 47-kDa MUTYH isoform was identified for the first time, although its existence had already been theoretically considered. The level of type $\alpha3$ mRNA encoding a 54-kDa MUTYH isoform with an MTS, was similar to that of type $\alpha4$ mRNA also encoding the 47-kDa isoform. In contrast, the level of type $\beta3$ mRNA, encoding a 53-kDa nuclear form of MUTYH, and that of type $\beta4$ mRNA were lower than those of type $\alpha3$ and $\alpha4$ mRNAs. Given the size of the molecules (47-kDa), the main MUTYH proteins detected in the PD brains may be derived from type $\alpha4$ mRNA. On Western blot analysis, we could not detect the common forms of MUTYH, such as the 53- and/or 54-kDa molecules encoded by $\beta3$ and $\alpha3$ type mRNAs, suggesting that these two molecules are unstable in these brains.

Fig. 5. Altered expression of MUTYH in Parkinson's brain. **A,B:** Immunohistochemistry of MUTYH in the SN of a representative control (A) and PD patient (B). Note the diffuse staining pattern for MUTYH in the cytoplasm of SN neurons of PD. Arrowheads indicate neuromelanin. **C–H:** Double-immunofluorescence study for MUTYH. Neurons in the SN of PD brain were stained with anti-MUTYH antibody (green in C) and anticytochrome oxidase subunit Vb antibody (red in D; and E is the merged image) or were stained with anti-MUTYH antibody (green in F) and DAPI (blue in G; and H is the merged image). The dot-like signals of MUTYH in the cytoplasm are colocalized with the cytochrome oxidase subunit Vb(E), but not with DAPI (H). **I:** Semiquantitative analysis of MUTYH-expressing neurons. The percentage of MUTYH-positive neurons (mean ± SEM) was significantly higher in the SN of PD brain than in the control (CON; $\star P < 0.05$). Scale bars = 20 μm. (Adapted from Arai et al., 2006, with permission from Springer.)
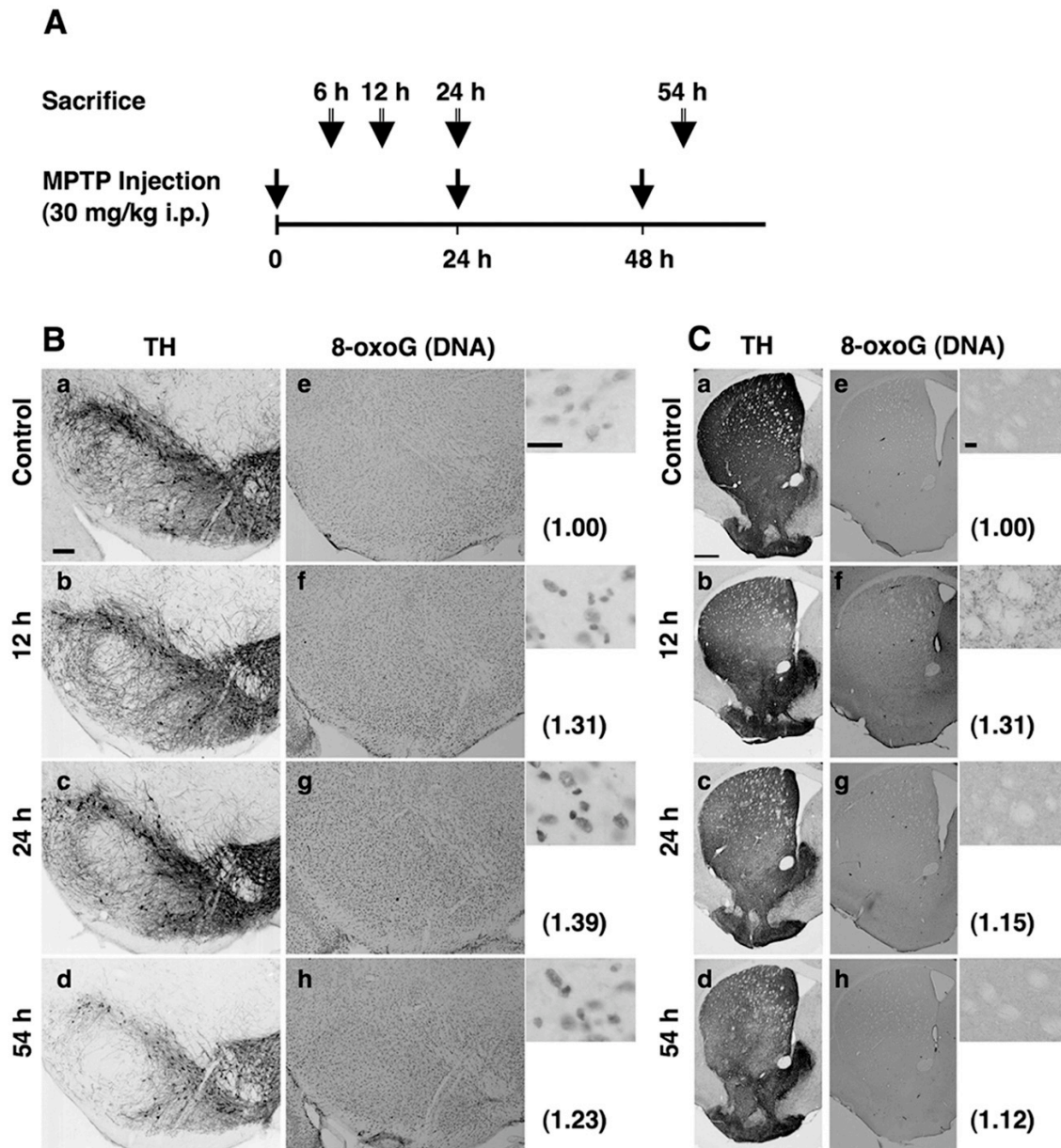
Fig. 6. Systemic administration of MPTP increased the accumulation of 8-oxoG in the SN and striatum. **A:** Ten-week-old C57BL/6J male mice were injected i.p with MPTP (30 mg/kg). Mice that received a single injection of MPTP were sacrificed 6, 12, and 24 hr after the injection, and those that received three injections of MPTP were sacrificed 54 hr after the first injection. **B:** Immunohistochemical detection of TH (a–d) and 8-oxoG (e–h) in the SN. *Insets* in e–h show enlarged views of each section. Relative densities of 8-oxoG

immunoreactivity in TH-positive cells to that of the control (e) are shown in parenthesis. **C:** Immunohistochemical detection of TH (a–d) and 8-oxoG (e–h) in the striatum. *Insets* in e–h show enlarged views of each section. Relative densities of 8-oxo-dG immunoreactivitiy in the striatum compared with that of the control (e) are shown in parenthesis. Scale bars = 200 μm in Ba–h; 20 μm in B,C insets; 500 μm in Ca–h. (Adapted from Yamaguchi et al., 2006).

Because the 47-kDa isoform of MUTYH lacks the DNA minor groove-reading motif, it is not yet clear whether this molecule retains the full function of MUTYH, such as adenine/2-OH-A DNA glycosylase activities. It is worth noting that expression of similar isoforms has been identified in rodent brains (Englander et al., 2002; Ichinoe et al., 2004), suggesting

that the 47-kDa isoform might have a neuron-specific function.

MOUSE MODEL OF PD

Results of epidemiological and animal model studies have suggested that environmental or synthetic toxins

have a causative role in PD, and some of these toxins in-hibit the mitochondrial respiratory chain (Mayeux, 2003). Among them, the 1-methyl-4-phenyl-1,2,3,6-tet-rahydropyridine (MPTP) model constitutes the best-characterized toxin paradigm for PD, faithfully replicat-ing most of its clinical and pathological hallmarks (Javitch et al., 1985; Nicklas et al., 1985; Speciale, 2002; Przedborski and Vila, 2003).

Increased lipid peroxidation and chemical modifi-cation of proteins are common features of the MPTP-induced PD model, but there have been only a few published studies reporting that MPTP induces the accu-mulation of 8-oxoG in this model (Chen et al., 2005; Yamaguchi et al., 2006). We clearly showed that the amount of 8-oxoG in cellular DNA increased signifi-cantly in both the SN and the striatum 12–24 hr after a single administration of MPTP (30 mg/kg, ip; Fig. 6; Yamaguchi et al., 2006). Of particular interest, 8-oxoG accumulation in mitochondrial DNA of striatal dopami-nergic nerve terminals was noted prior to their degener-ation and earlier than the 8-oxoG buildup in nuclear DNA of the SN, in which a loss of dopaminergic neu-rons became apparent after the repeated administration of MPTP. These observations strongly suggest that MPTP triggers degeneration of the dopaminergic neu-rons from their terminals in the striatum together with mitochondrial dysfunction, and that this progresses in a retrograde manner, as previously reported (Mitsumoto et al., 1998; Nakai et al., 2003). We further observed that microglia and astrocytes were significantly activated in the striatum or SN 12–24 hr after a single administra-tion of MPTP. It has been shown that the activated microglia or astrocytes produce large amounts of extra-cellular superoxide as a result of NADPH oxidase activity, further increasing oxidative stress in the nigrostriatal pathway (Kurkowska-Jastrzebska et al., 1999; Song et al., 2004).

MTH1 DEFICIENCY INCREASED DEGENERATION OF STRIATAL NERVE TERMINALS OF DOPAMINE NEURONS AFTER MPTP ADMINISTRATION

Mth1 mRNA was detected in neurons throughout mouse brain by in situ hybridization, and neurons in the SN, including substantia nigra pars compacta (SNc), sub-stantia nigra pars reticulata (SNr), and ventral tegmental area (VTA), exhibited substantial levels of *Mth1* mRNA expression, as did neurons in the cerebral cortex and hippocampus (Fig. 7; Yamaguchi et al., 2006).

To examine the role of MTH1 in the nigrostriatal pathway, we assessed the neuroprotective role of MTH1 in the degeneration of dopaminergic neurons based on a comparison of wild-type and MTH1-null mice. MPTP (30 mg/kg, ip) or saline was administered to wild-type and MTH1-null mice once a day for 5 consecutive days, and then the degeneration of dopaminergic nerve termi-nals in the striatum and the loss of dopaminergic neurons in the SN were compared (Fig. 8A; Yamaguchi et al.,
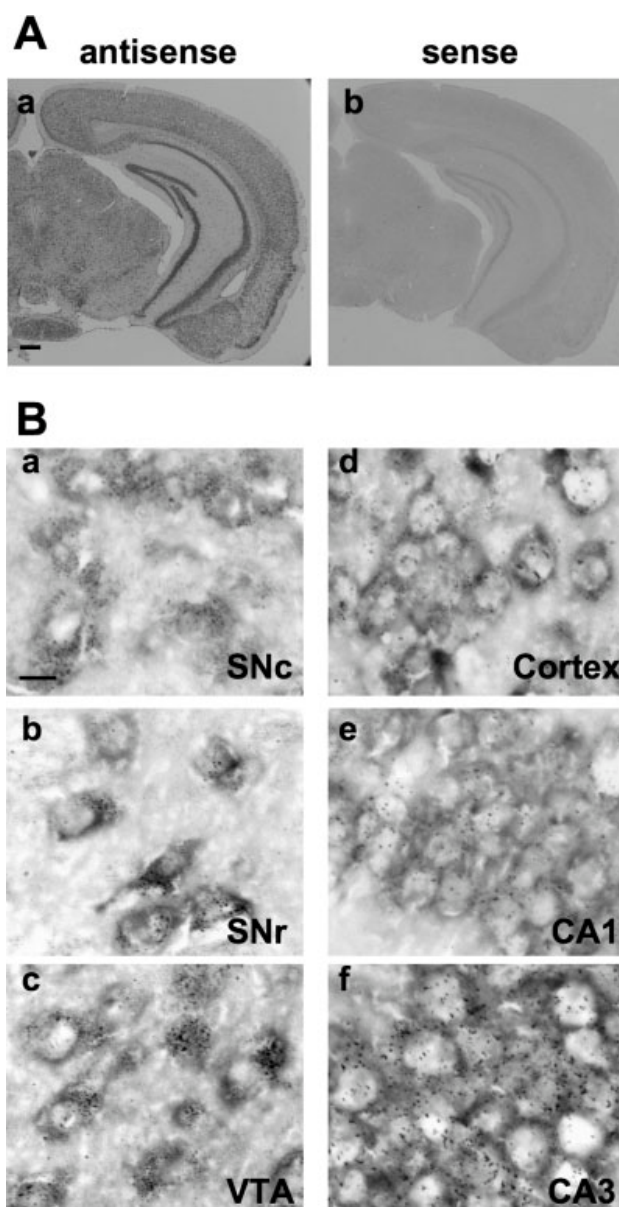

Fig. 7. Expression of *Mth1* mRNA in the normal mouse brain. **A:** The level of *Mth1* mRNA in coronal midbrain sections prepared from a C57BL/6J male mouse was visualized by in situ hybridization with an antisense (a) and sense probe (b). Hybridization signals were detected only with the antisense probe, and they were present in neurons throughout the brain, including those of the SN and hippo-campus. **B:** Hybridization signals for *Mth1* mRNA were present in neurons of the SNc (a), SNr (b), and VTA (c) as well as in neurons scattered throughout the cerebral cortex (d), hippocampal CA1 (e), and CA3 subfields (f). Scale bars = 500 μm in A; 10 μm in B. (Adapted from Yamaguchi et al., 2006).

2006). About a 30% reduction in the number of dopa-minergic neurons across several levels of the SN was observed in both wild-type and MTH1-null mice after MPTP treatment, and there was no apparent difference
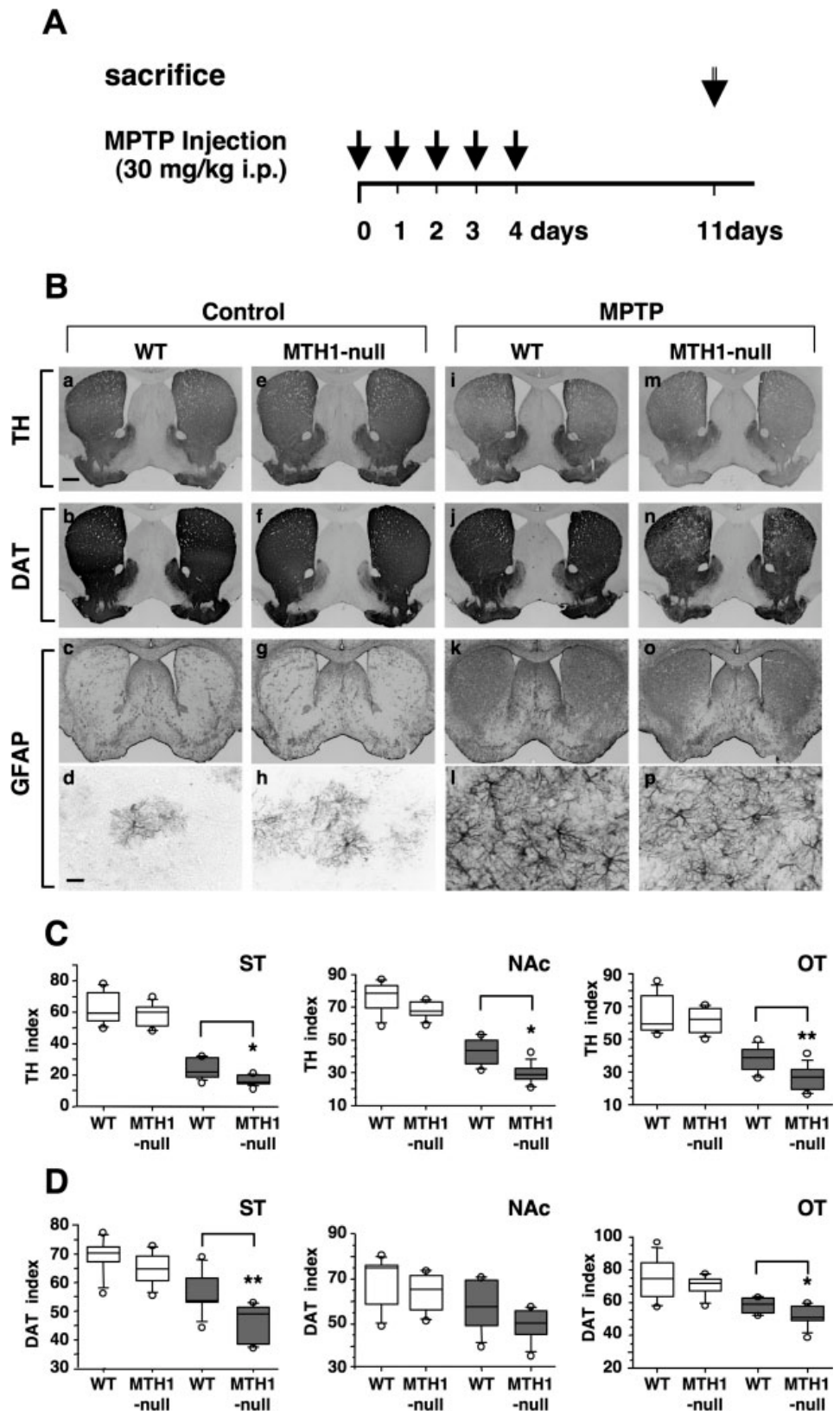
Fig. 8. MTH1-null mice display an increased reduction in TH and DAT immunoreactivities in striatal terminal fibers of dopaminergic neurons after chronic exposure to MPTP. **A:** MPTP (30 mg/kg) or saline was administered i.p. to wild-type and MTH1-null mice once per day for 5 consecutive days. Seven days after the last injection, the mice were sacrificed for analyses. **B:** Striatal immunoreactivities for TH, DAT, and glial fibrillary acidic protein (GFAP) were examined in sections prepared from mice administered saline (control, a–h) or MPTP (MPTP, i–p). Wild type: a–d, i–l; MTH1-null mice: e–h, m–p. The images d, h and i, p are magnified images of c, g and k, o, respectively. **C:** TH immunoreactivities in the striatum, nucleus accumbens (NAc), and olfactory tubercle (OT) were measured in the sections shown in B, and TH indexes are shown in a box-and-whisker plot. In each plot, the boxes are drawn with the ends at the quartiles, and the statistical median is shown as a horizontal line within the box. The whiskers extend to the farthest points that are not outliers (circles). TH indexes in striatum, NAc, and OT of MTH1-null mice after MPTP injection were significantly lower than those of the wild type (WT; $\star\star P < 0.01$, $\star P < 0.02$). Control, open boxes; MPTP-treated, shaded boxes. **D:** DAT immunoreactivities in the striatum, NAc and OT were measured in the sections shown in B, and DAT indexes are shown in a box-and-whisker plot (n = 4 ∼ 5), as in C. DAT indexes in striatum and OT of MTH1-null mice after MPTP injection were also significantly lower than those of the wild type ($\star\star P < 0.01$, $\star P < 0.05$). Scale bars = 500 μm in Ba (applies to Ba–c,e–g,i–k,m–o); 20 μm in Bd (applies to Bd,h,l,p). (Adapted from Yamaguchi et al., 2006).
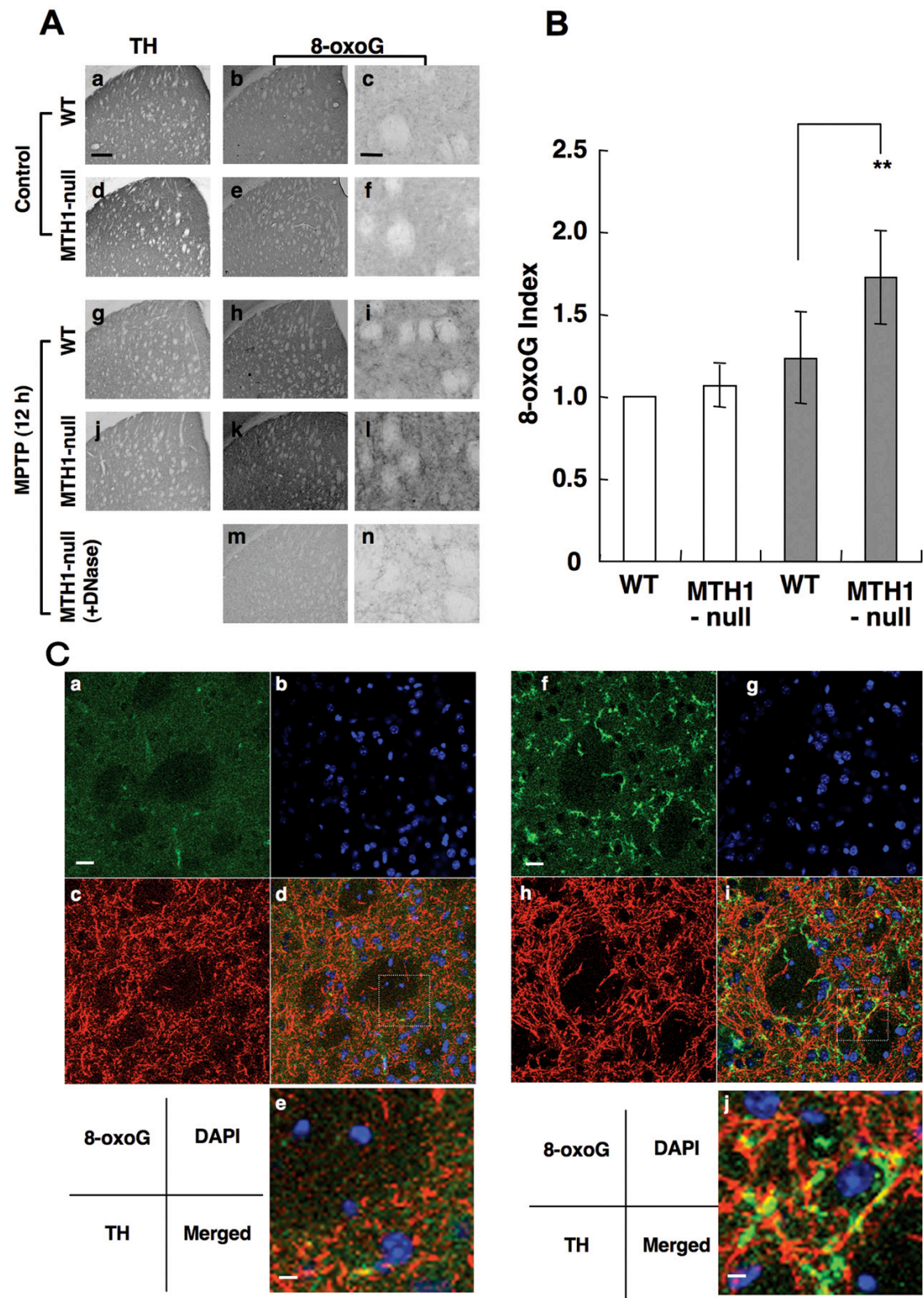
Fig. 9. Increased accumulation of 8–oxoG in the striatum of MTH1–null mice after MPTP administration. **A:** Striatal immunoreactivities for TH (a,d,g,j) and 8–oxoG in DNA (b,c,e,f,h,i,k,l) were examined in the sections prepared 12 hr after the administration of saline (control) or MPTP. To detect 8–oxoG in DNA, the sections were pretreated only with RNase and reacted with anti-8–oxoG. One section was further pretreated with DNase and was then subjected to the immunohistochemistry using the same antibody (m,n). Wild type: a–c, g–i; MTH1–null mice: d–f, j–n. The images c, f, i, l, n are magnified images of b, e, h, k, m, respectively. **B:** Immunoreactivities for 8–oxoG in the sections shown in A were digitized, and the 8–oxoG index of each sample was determined. The relative value of each 8–oxoG index to that of the control is shown as a bar graph with the mean ± SD. Open bar: control; shaded bar, MPTP-treated. The 8–oxoG index increased more significantly in MTH1–null mice than in the wild type (**$P < 0.01$). **C:** The localization of 8–oxoG in the striatal terminal fibers of dopaminergic neurons determined by laser scanning confocal microscopy. Sections prepared from wild-type (a–e) and MTH1–null mice (f–j) 12 hr after MPTP injection (30 mg/kg, i.p.) were stained for 8–oxoG in DNA (green, a,f), nuclear DNA with DAPI (blue, b,g), and TH (red, c,h), and their merged images are shown (d,i). The sections were pretreated only with RNase. Scale bars = 300 μm in Aa (applies to Aa,b,d,e,g,h, j,k,m); 40 μm in Ac (applies to Ac,f,i,l,n); 20 μm in Ca,f (applies to Ca–d,f–i); 5 μm in Ce,j. Each portion of images shown in the dotted boxes (d,i) was magnified (e,j). (Adapted from Yamaguchi et al., 2006).
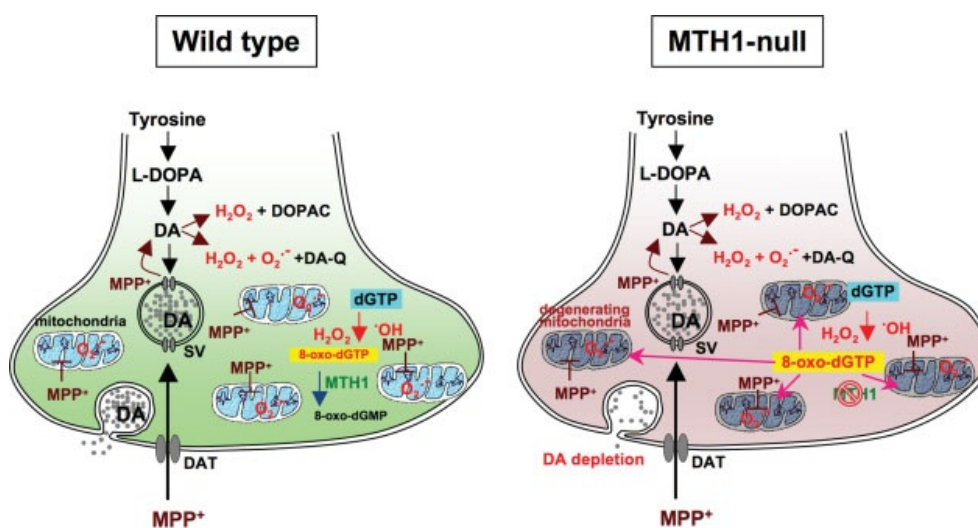
Fig. 10. MTH1 deficiency augments MPTP-induced accumulation of 8-oxoG in mitochondrial DNA of striatal dopaminergic nerve terminals and causes their dysfunction. Systemically administered MPTP is converted to MPP$^+$, which can be specifically taken up by dopaminergic neurons through DAT. MPP$^+$ in dopaminergic neurons binds to complex I of the respiratory chain in mitochondria and blocks electron transport, resulting in the formation of ROS, which oxidize various macromolecules, including nucleotides. MPP$^+$ further increases ROS through the release of dopamine (DA) from synaptic vesicles (SV). In wild-type mice, MTH1 efficiently sanitizes the nucleotide pool by hydrolyzing 8-oxo-dGTP or other oxidized purine nucleotides in the nucleotide pool, thereby avoiding accumulation of 8-oxoG in the mitochondrial DNA of the striatal dopaminergic nerve terminals. On the other hand, 8-oxo-dGTP or other oxidized purine nucleotides accumulated in MTH1-null mice is incorporated into mitochondrial DNA in striatal dopaminergic nerve terminals, resulting in mitochondrial dysfunction and dopamine depletion (DA depletion). L-DOPA, levodopa; DOPAC, 3,4-dihydroxyphenylacetic acid; DA-Q, dopamine quinone. (Adapted from Nakabeppu et al., 2006b, with permission from Elsevier.)

between the two groups. On the other hand, MTH1-null mice displayed a more severe degeneration of the dopaminergic nerve terminals in the striatum after chronic exposure to MPTP, which was revealed by tyrosine hydroxylase (TH) and dopamine transporter (DAT) immunostaining (Fig. 8B–D). Furthermore, 12 hr after MPTP injection, the accumulation of 8-oxoG in mitochondrial DNA of the striatum was significantly increased in MTH1-null mice compared with the wild-type mice (Fig. 9A,B), and, at the same time, the striatum of the former also showed strong 8-oxoG immunoreactivities (Fig. 9Ca,f). The 8-oxoG signals exhibited a fiber-like shape and were distributed with TH-positive fibers, and most of these were colocalized in MTH1-null mice (Fig. 9Ci,j). It is noteworthy that there was very little 8-oxoG immunoreactivitiy in the cytoplasm of postsynaptic spiny neurons (their nuclei are in blue in Fig. 9C). We thus concluded that, 12 hr after MPTP injection, 8-oxoG in the MTH1-null mice accumulated mostly in mitochondrial DNA in striatal nerve terminals of dopaminergic neurons.

MPTP is converted to 1-methyl-4-phenylpyridinium ion (MPP$^+$) in the brain, mostly by monoamine oxidase B in glial cells, and MPP$^+$ can then be specifically taken up by dopaminergic neurons through DAT, which is mostly localized in dopaminergic nerve terminals, as shown in Figure 10. MPP$^+$ that accumulates in dopaminergic nerve terminals binds to complex I of the respiratory chain in mitochondria and blocks electron transport. This leads to an energy failure and ATP depletion as well as to an increase in electron leakage from the respiratory chain, which results in the formation of ROS (Nicklas et al., 1985; Ramsay et al., 1991). In addition, dopaminergic nerve terminals are more likely to be exposed to increased levels of oxidative stress caused by the metabolic products of dopamine than are other parts of the brain. MPP$^+$ itself induces a massive release of vesicular dopamine into the cytosol (Lotharius and O'Malley, 2000) or increased dopamine turnover. Therefore, oxidative stress in dopaminergic nerve terminals is suspected to increase significantly and specifically in MPTP-administered animals (Schmidt and Ferger, 2001; Przedborski and Vila, 2003). The oxidation of dopamine stoichiometrically produces hydrogen peroxide, which reacts with Fe^{2+} ion to form hydroxyl radicals as a result of the Fenton reaction. Hydroxyl radicals are the most powerful forms of ROS and easily oxidize almost all biomolecules, such as lipids, proteins, and nucleic acids, resulting in cellular dysfunction or cell death (Karam et al., 1991; Halliwell, 1992).

As indicated earlier, MPTP triggers mitochondrial dysfunction and degeneration of the dopaminergic neurons from their terminals in the striatum, and this degeneration progresses in a retrograde manner. Although we have examined neither 8-oxoG accumulation nor expression of MTH1, OGG1, and MUTYH in striatal regions of PD brains, it is likely that such retrograde degeneration of dopaminergic neurons also takes place in

PD brain (Riederer and Wuketich, 1976; Mayeux, 2003). As shown in Figure 3, loss of dopaminergic neurons in the SN is more evident in brains of chronic PD patients and is accompanied by an increased accumulation of 8-oxoG and expression of MTH1 in perikaryal mitochondria. This indicates that these damaged mitochondria further contribute to the loss of dopaminergic neurons in the SN, as in striatal nerve terminals, and cause their degeneration.

In addition to 8-oxo-dGTP, MTH1 efficiently hydrolyzes other oxidized purine nucleoside triphosphates (Nakabeppu et al., 2006a), so MTH1-null mice accumulated a larger amount of these triphosphates in dopaminergic nerve terminals after MPTP administration, resulting in an increased accumulation of oxidized purines in mitochondrial DNA (Fig. 10). As a result, an MTH1 deficiency augments MPTP-provoked degeneration of dopaminergic nerve terminals in the striatum. Take together, these findings strongly suggest that increased levels of various oxidized purine nucleoside triphosphates, including 8-oxo-dGTP, induce a dysfunction of dopaminergic nerve terminals (Yamaguchi et al., 2006).

FUTURE PERSPECTIVES

Observations in human PD patients together with experimental analyses of a mouse model of PD indicated that oxidative damage in nucleic acids might be a major risk factor for a loss of dopaminergic neurons in the nigrostriatal system. We suggest that oxidative damage initially builds up in mitochondrial genomes of striatal nerve terminals and triggers their dysfunction and that, later, the damage is further accumulated in perikaryal mitochondria of dopamine neurons in the SN, thereby resulting in their loss.

It has been widely accepted that neurodegeneration is tightly associated with mitochondrial dysfunction as well as DNA damage (Ames et al., 1993). Our studies reveal that oxidized purine deoxyribonucleotides are misincorporated into mitochondrial DNA, causing mitochondrial dysfunction, which results in a loss of dopaminergic nerve terminals, as observed in human PD patients (Yoshimura et al., 2003; Yamaguchi et al., 2006). In this regard, we are very much interested in what the 47-kDa isoform of MUTYH, which is highly expressed in PD brain, actually does in the mitochondria of dopamine neurons (Arai et al., 2006). Adenine can be misinserted opposite 8-oxoG that has accumulated in mitochondrial DNA of the PD brain. Therefore, it is likely that MUTYH can excise the adenine opposite 8-oxoG in DNA to initiate the process of base excision repair. This BER may protect dopamine neurons from the deleterious effects of 8-oxoG buildup in mitochondrial DNA, although there is no evidence showing whether the 47-kDa isoform of MUTYH retains its repair capacity.

MTH1 efficiently hydrolyzes oxidized purine ribonucleotides such as 2-OH-ATP, 8-oxo-ATP, and to a lesser extent 8-oxo-GTP (Nakabeppu et al., 2006a). As a result, cellular dysfunction might also be caused by their incorporation into RNA. Furthermore, an increasing concentration of 8-oxo-dGTP itself has been reported to reduce the amounts of newly synthesized DNA in a cell-free DNA replication system derived from *Xenopus* egg lysates (Kai et al., 2002), indicating the possibility that free forms of oxidized purine nucleotides independently exert a certain degree of cytotoxicity. To elucidate further the biological significance of these oxidized nucleotides in vivo, we are now developing transgenic mice expressing human MTH1 protein. Extended analyses of MTH1 using these materials will hopefully shed some light on the molecular mechanisms of neurodegeneration as well as carcinogenesis.

REFERENCES

Aburatani H, Hippo Y, Ishida T, Takashima R, Matsuba C, Kodama T, Takao M, Yasui A, Yamamoto K, Asano M. 1997. Cloning and characterization of mammalian 8-hydroxyguanine-specific DNA glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. Cancer Res 57:2151–2156.

Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B. 1997. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. J Neurochem 69:1196–1203.

Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 90:7915–7922.

Arai T, Fukae J, Hatano T, Kubo SI, Ohtsubo T, Nakabeppu Y, Mori H, Mizuno Y, Hattori N. 2006. Up-regulation of hMUTYH, a DNA repair enzyme, in the mitochondria of substantia nigra in Parkinson's disease. Acta Neuropathol 112:136–145.

Boiteux S, Radicella JP. 2000. The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 377:1–8.

Chen LJ, Gao YQ, Li XJ, Shen DH, Sun FY. 2005. Melatonin protects against MPTP/MPP+-induced mitochondrial DNA oxidative damage in vivo and in vitro. J Pineal Res 39:34–42.

Clarke DD, Sokoloff L. 1999. Circulation and energy metabolism in the brain. In: Siegel GJ, Arganoff BW, Albers RW, Fisher SK, Uhler MD, editors. Basic neurochemistry: molecular, cellular and medical aspects, 6th ed. Philadelphia: Lippincott-Raven Publishers. p 637–669.

Cooke MS, Evans MD, Dizdaroglu M, Lunec J. 2003. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J 17:1195–1214.

Englander EW, Hu Z, Sharma A, Lee HM, Wu ZH, Greeley GH. 2002. Rat MYH, a glycosylase for repair of oxidatively damaged DNA, has brain-specific isoforms that localize to neuronal mitochondria. J Neurochem 83:1471–1480.

Fujikawa K, Kamiya H, Yakushiji H, Fujii Y, Nakabeppu Y, Kasai H. 1999. The oxidized forms of dATP are substrates for the human MutT homologue, the hMTH1 protein. J Biol Chem 274:18201–18205.

Fujikawa K, Kamiya H, Yakushiji H, Nakabeppu Y, Kasai H. 2001. Human MTH1 protein hydrolyzes the oxidized ribonucleotide, 2-hydroxy-ATP. Nucleic Acids Res 29:449–454.

Fukae J, Takanashi M, Kubo S-I, Nishioka K-I, Nakabeppu Y, Mori H, Mizuno Y, Hattori N. 2005. Expression of 8-oxoguanine DNA glycosylase (OGG1) in Parkinson's disease and related neurodegenerative disorders. Acta Neurophathol 109:256–262.

Gu Y, Parker A, Wilson TM, Bai H, Chang DY, Lu AL. 2002. Human MutY homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human MutS homolog 2/human MutS homolog 6. J Biol Chem 277:11135–11142.

Halliwell B. 1992. Reactive oxygen species and the central nervous system. J Neurochem 59:1609–1623.

Hayakawa H, Hofer A, Thelander L, Kitajima S, Cai Y, Oshiro S, Yakushiji H, Nakabeppu Y, Kuwano M, Sekiguchi M. 1999. Metabolic fate of oxidized guanine ribonucleotides in mammalian cells. Biochemistry 38:3610–3614.

Hayashi H, Tominaga Y, Hirano S, McKenna AE, Nakabeppu Y, Matsumoto Y. 2002. Replication-associated repair of adenine: 8-oxoguanine mispairs by MYH. Curr Biol 12:335–339.

Hirano S, Tominaga Y, Ichinoe A, Ushijima Y, Tsuchimoto D, Honda-Ohnishi Y, Ohtsubo T, Sakumi K, Nakabeppu Y. 2003. Mutator phenotype of MUTYH-null mouse embryonic stem cells. J Biol Chem 278:38121–38124.

Ichinoe A, Behmanesh M, Tominaga Y, Ushijima Y, Hirano S, Sakai Y, Tsuchimoto D, Sakumi K, Wake N, Nakabeppu Y. 2004. Identification and characterization of two forms of mouse MUTYH proteins encoded by alternatively spliced transcripts. Nucleic Acids Res 32:477–487.

Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH. 1985. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. Proc Natl Acad Sci U S A 82:2173–2177.

Jenner P. 2003. Oxidative stress in Parkinson's disease. Ann Neurol 53(Suppl 3):S26–S36; discussion S36–S38.

Kai T, Matsunaga R, Eguchi M, Kamiya H, Kasai H, Suzuki M, Izuta S. 2002. An oxidized nucleotide affects DNA replication through activation of protein kinases in Xenopus egg lysates. Nucleic Acids Res 30:569–573.

Kamiya H, Kasai H. 1995. Formation of 2-hydroxydeoxyadenosine triphosphate, an oxidatively damaged nucleotide, and its incorporation by DNA polymerases. Steady-state kinetics of the incorporation. J Biol Chem 270:19446–19450.

Kamiya H, Kasai H. 2000. 2-Hydroxy-dATP is incorporated opposite G by Escherichia coli DNA polymerase III resulting in high mutagenicity. Nucleic Acids Res 28:1640–1646.

Kang D, Nishida J, Iyama A, Nakabeppu Y, Furuichi M, Fujiwara T, Sekiguchi M, Takeshige K. 1995. Intracellular localization of 8-oxo-dGTPase in human cells, with special reference to the role of the enzyme in mitochondria. J Biol Chem 270:14659–14665.

Kang D, Takeshige K, Sekiguchi M, Singh KK. 1998. Introduction. In: Singh KK, editor. Mitochondrial DNA mutations in aging, disease and cancer. New York: Springer Verlag. p 1–15.

Karam LR, Bergtold DS, Simic MG. 1991. Biomarkers of OH radical damage in vivo. Free Radic Res Commun 12–13:11–16.

Kasai H, Nishimura S. 1984. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. Nucleic Acids Res 12:2137–2145.

Kimura Y, Oda S, Egashira A, Kakeji Y, Baba H, Nakabeppu Y, Maehara Y. 2004. A variant form of hMTH1, a human homologue of the E. coli mutT gene, correlates with somatic mutation in the p53 tumour suppressor gene in gastric cancer patients. J Med Genet 41:e57.

Kohno T, Sakiyama T, Kunitoh H, Goto K, Nishiwaki Y, Saito D, Hirose H, Eguchi T, Yanagitani N, Saito R, Sasaki-Matsumura R, Mimaki S, Toyama K, Yamamoto S, Kuchiba A, Sobue T, Ohta T, Ohki M, Yokota J. 2006. Association of polymorphisms in the MTH1 gene with small cell lung carcinoma risk. Carcinogenesis (in press).

Kurkowska-Jastrzebska I, Wronska A, Kohutnicka M, Czlonkowski A, Czlonkowska A. 1999. The inflammatory reaction following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine intoxication in mouse. Exp Neurol 156:50–61.

Lotharius J, Brundin P. 2002. Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat Rev Neurosci 3:932–942.

Lotharius J, O'Malley KL. 2000. The parkinsonism-inducing drug 1-methyl-4-phenylpyridinium triggers intracellular dopamine oxidation. A novel mechanism of toxicity. J Biol Chem 275:38581–38588.

Maki H. 2002. Origins of spontaneous mutations: specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses. Annu Rev Genet 36:279–303.

Maki H, Sekiguchi M. 1992. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature 355:273–275.

Mayeux R. 2003. Epidemiology of neurodegeneration. Annu Rev Neurosci 26:81–104.

Miller JH. 1996. Spontaneous mutators in bacteria: insights into pathways of mutagenesis and repair. Annu Rev Microbiol 50:625–643.

Mishima M, Sakai Y, Itoh N, Kamiya H, Furuichi M, Takahashi M, Yamagata Y, Iwai S, Nakabeppu Y, Shirakawa M. 2004. Structure of human MTH1, a Nudix family hydrolase that selectively degrades oxidized purine nucleoside triphosphates. J Biol Chem 279:33806–33815.

Mitsumoto Y, Watanabe A, Mori A, Koga N. 1998. Spontaneous regeneration of nigrostriatal dopaminergic neurons in MPTP-treated C57BL/6 mice. Biochem Biophys Res Commun 248:660–663.

Nakabeppu Y. 2001a. Molecular genetics and structural biology of human MutT homolog, MTH1. Mutat Res 477:59–70.

Nakabeppu Y. 2001b. Regulation of intracellular localization of human MTH1, OGG1, and MYH proteins for repair of oxidative DNA damage. Prog Nucleic Acid Res Mol Biol 68:75–94.

Nakabeppu Y, Tsuchimoto D, Furuichi M, Sakumi K. 2004. The defense mechanisms in mammalian cells against oxidative damage in nucleic acids and their involvement in the suppression of mutagenesis and cell death. Free Radic Res 38:423–429.

Nakabeppu Y, Behmanesh M, Yamaguchi H, Yoshimura D, Sakumi K. 2006a. Prevention of the mutagenecity and cytotoxicity of oxidized purine nucleotides. In: Evans MD, Cooke MS, editors. Oxidative damage to nucleic acids. Georgetown, TX: Landes Bioscience (http://eurekah.com/abstract.php?chapid = 2920&bookid = 214&catid=78).

Nakabeppu Y, Kajitani K, Sakamoto K, Yamaguchi H, Tsuchimoto D. 2006b. MTH1, an oxidized purine nucleoside triphosphatase, prevents the cytotoxicity and neurotoxicity of oxidized purine nucleotides. DNA Repair 5:761–772.

Nakai M, Mori A, Watanabe A, Mitsumoto Y. 2003. 1-Methyl-4-phenylpyridinium (MPP$^+$) decreases mitochondrial oxidation-reduction (REDOX) activity and membrane potential ($\Delta\Psi_m$) in rat striatum. Exp Neurol 179:103–110.

Nicklas WJ, Vyas I, Heikkila RE. 1985. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. Life Sci 36:2503–2508.

Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K, Nakabeppu Y. 1999. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. Mol Biol Cell 10:1637–1652.

Oda H, Nakabeppu Y, Furuichi M, Sekiguchi M. 1997. Regulation of expression of the human MTH1 gene encoding 8-oxo-dGTPase. Alternative splicing of transcription products. J Biol Chem 272:17843–17850.

Oda H, Taketomi A, Maruyama R, Itoh R, Nishioka K, Yakushiji H, Suzuki T, Sekiguchi M, Nakabeppu Y. 1999. Multi-forms of human MTH1 polypeptides produced by alternative translation initiation and single nucleotide polymorphism. Nucleic Acids Res 27:4335–4343.

Ohtsubo T, Nishioka K, Imaiso Y, Iwai S, Shimokawa H, Oda H, Fujiwara T, Nakabeppu Y. 2000. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. Nucleic Acids Res 28:1355–1364.

Parker A, Gu Y, Mahoney W, Lee SH, Singh KK, Lu AL. 2001. Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair. J Biol Chem 276:5547–5555.

Przedborski S, Vila M. 2003. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: a tool to explore the pathogenesis of Parkinson's disease. Ann N Y Acad Sci 991:189–198.

Ramsay RR, Krueger MJ, Youngster SK, Gluck MR, Casida JE, Singer TP. 1991. Interaction of 1-methyl-4-phenylpyridinium ion (MPP$^+$) and its analogs with the rotenone/piericidin binding site of NADH dehydrogenase. J Neurochem 56:1184–1190.

Riederer P, Wuketich S. 1976. Time course of nigrostriatal degeneration in parkinson's disease. A detailed study of influential factors in human brain amine analysis. J Neural Transm 38:277–301.

Sakai Y, Furuichi M, Takahashi M, Mishima M, Iwai S, Shirakawa M, Nakabeppu Y. 2002. A molecular basis for the selective recognition of 2-hydroxy-dATP and 8-oxo-dGTP by human MTH1. J Biol Chem 277: 8579–8587.

Sakai Y, Oda H, Yoshimura D, Furuichi M, Kang D, Iwai S, Hara T, Nakabeppu Y. 2006. The GT to GC single nucleotide polymorphism at the beginning of an alternative exon 2C of human MTH1 gene confers an amino terminal extension that functions as a mitochondrial targeting signal. J Mol Med 84:660–670.

Sakumi K, Tominaga Y, Furuichi M, Xu P, Tsuzuki T, Sekiguchi M, Nakabeppu Y. 2003. Ogg1 knockout-associated lung tumorigenesis and its suppression by Mth1 gene disruption. Cancer Res 63:902–905.

Schmidt N, Ferger B. 2001. Neurochemical findings in the MPTP model of Parkinson's disease. J Neural Transm 108:1263–1282.

Shibutani S, Takeshita M, Grollman AP. 1991. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 349:431–434.

Shimura-Miura H, Hattori N, Kang D, Miyako K, Nakabeppu Y, Mizuno Y. 1999. Increased 8-oxo-dGTPase in the mitochondria of substantia nigral neurons in Parkinson's disease. Ann Neurol 46:920–924.

Slupska MM, Baikalov C, Luther WM, Chiang JH, Wei YF, Miller JH. 1996. Cloning and sequencing a human homolog (hMYH) of the Escherichia coli mutY gene whose function is required for the repair of oxidative DNA damage. J Bacteriol 178:3885–3892.

Slupska MM, Luther WM, Chiang JH, Yang H, Miller JH. 1999. Functional expression of hMYH, a human homolog of the Escherichia coli MutY protein. J Bacteriol 181:6210–6213.

Song DD, Shults CW, Sisk A, Rockenstein E, Masliah E. 2004. Enhanced substantia nigra mitochondrial pathology in human alpha-synuclein transgenic mice after treatment with MPTP. Exp Neurol 186:158–172.

Speciale SG. 2002. MPTP: insights into parkinsonian neurodegeneration. Neurotoxicol Teratol 24:607–620.

Stuart JA, Mayard S, Hashiguchi K, Souza-Pinto NC, Bohr VA. 2005. Localization of mitochondrial DNA base excision repair to an inner membrane-associated particulate fraction. Nucleic Acids Res 33:3722–3732.

Taddei F, Hayakawa H, Bouton M, Cirinesi A, Matic I, Sekiguchi M, Radman M. 1997. Counteraction by MutT protein of transcriptional errors caused by oxidative damage. Science 278:128–130.

Takao M, Aburatani H, Kobayashi K, Yasui A. 1998. Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. Nucleic Acids Res 26:2917–2922.

Tominaga Y, Ushijima Y, Tsuchimoto D, Mishima M, Shirakawa M, Hirano S, Sakumi K, Nakabeppu Y. 2004. MUTYH prevents OGG1 or APEX1 from inappropriately processing its substrate or reaction product with its C-terminal domain. Nucleic Acids Res 32:3198–3211.

Ushijima Y, Tominaga Y, Miura T, Daisuke Tsuchimoto, Sakumi K, Nakabeppu Y. 2005. A functional analysis of the DNA glycosylase activity of mouse MUTYH protein excising 2-hydroxyadenine opposite guanine in DNA. Nucleic Acids Res 33:672–682.

Wenning GK, Colosimo C, Geser F, Poewe W. 2004. Multiple system atrophy. Lancet Neurol 3:93–103.

Yakushiji H, Maraboeuf F, Takahashi M, Deng ZS, Kawabata S, Nakabeppu Y, Sekiguchi M. 1997. Biochemical and physicochemical characterization of normal and variant forms of human MTH1 protein with antimutagenic activity. Mutat Res 384:181–194.

Yamaguchi H, Kajitani K, Dan Y, Furuichi M, Ohno M, Sakumi K, Kang D, Nakabeppu Y. 2006. MTH1, an oxidized purine nucleoside triphosphatase, protects the dopamine neurons from oxidative damage in nucleic acids caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Cell Death Differ 13:551–563.

Yoshimura D, Sakumi K, Ohno M, Sakai Y, Furuichi M, Iwai S, Nakabeppu Y. 2003. An oxidized purine nucleoside triphosphatase, MTH1, suppresses cell death caused by oxidative stress. J Biol Chem 278: 37965–37973.

Zhang J, Perry G, Smith MA, Robertson D, Olson SJ, Graham DG, Montine TJ. 1999. Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 154:1423–1429.