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OXIDATIVE DAMAGE IN BRAIN GENOME AND NEUROPROTECTION

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Abstract Oxidative DNA lesions, such as 8-oxoguanine (8-oxoG), accumulate in nuclear and mitochondrial genomes during aging, and such accumulation is known to dramatically increase in patient brains with Parkinson's disease (PD) or Alzheimer's disease (AD). To counteract oxidative damage to nucleic acids, human and rodents are equipped with three distinct enzymes, MTH1, OGG1 and MUTYH. MTH1 hydrolyzes oxidized purine nucleoside triphosphates, such as 8-oxo-dGTP to their monophosphate forms. OGG1 and MUTYH are DNA glycosylases excising 8-oxoG opposite cytosine and adenine opposite 8-oxoG in DNA, respectively. We showed a significant increase in 8-oxoG in cellular DNA as well as altered expression of MTH1, OGG1 and MUTYH in PD and AD brains, suggesting that the buildup of 8-oxoG may cause neurodegeneration. We have shown that buildup of 8-oxoG in either nuclear or mitochondrial DNA causes MUTYH-dependent cell death through two distinct pathways, and that accumulation of oxidized nucleotides in nucleotide pools also causes MUTYH-dependent cell death. MTH1-null mice exhibited an increased buildup of 8-oxoG in striatal mitochondrial DNA followed by more extreme neuronal dysfunction after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration, while hMTH1-transgenic mice are resistant to a mitochondrial neurotoxin, 3-nitropropionic acid (3-NP)-induced striatal degeneration, in comparison to wild-type mice. We found that double-knockout (DKO) mice lacking OGG1 and MTH1, and to a lesser extent OGG1-KO mice, are significantly sensitive to 3-NP-induced striatal degeneration, in comparison to MTH1-KO or wild-type mice, while MUTYH deficiency increases resistance to 3-NP in OGG1-KO or wild-type background. We thus demonstrated that 8-oxoG accumulated in brain genomes causes neurodegeneration in a MUTYH-dependent manner, and which is efficiently suppressed by MTH1 and OGG1.

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Key words: 8-oxoguanine; MTH1; OGG1; MUTYH.

Introduction

For living organisms, the most fundamental biological function is maintaining the integrity of their genomic DNAs harboring the genetic information and transmitting them precisely from cell to cell, as well as from parents to their offspring. The genomic DNA and its precursor nucleotides are always in danger of oxidation by reactive oxygen species (ROS) which are generated both as byproducts of oxidative metabolism and as a consequence of exposure to pathogens, ionizing radiation, chemicals and other environmental factors[1,2]. Various oxidized bases and nucleotides are formed in DNA or nucleotide pools by ROS, and such oxidative lesions may cause mutations or cell death if they are not efficiently eliminated or repaired. Mutations may induce cancers, and cell death may be related to various degenerative diseases, during aging[3].

8-Oxoguanine (8-oxoG) is one of the major oxidative base lesions in DNA or nucleotides, and is highly mutagenic because it can pair with adenine as well as cytosine (Figure 1A)[4]. 8-OxoG accumulates in both nuclear and mitochondorial DNA during aging, and the level is known to increase in patient brains with various neurodegenerative diseases, such as Parkinson's disease (PD) or Alzheimer's disease (AD)[5,6],

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suggesting that a buildup of 8-oxoG in cellular DNA contributes to cell death. However, it is not known which form of DNA was responsible for such cell death, whether nuclear DNA or mitochondrial DNA or both, nor is it known how the death order was executed[7].

In this review, we first summarized the defense mechanisms against oxidative damage in nucleic acids, and then presented the two distinct pathways in which cell death is executed when 8-oxoG accumulates in either nuclear or mitochondrial DNA, and finally discussed their involvement in neurodegeneration when 8-oxoG accumulates in brain genomes.

1. The oxidized purine nucleoside triphosphatase, MTH1, sanitizes nucleotide pools.

In human and rodent cells, MTH1, a homolog of *Escherichia coli* MutT protein, efficiently hydrolyzes oxidized purine nucleoside triphosphates such as 8-oxo-(d)GTP and

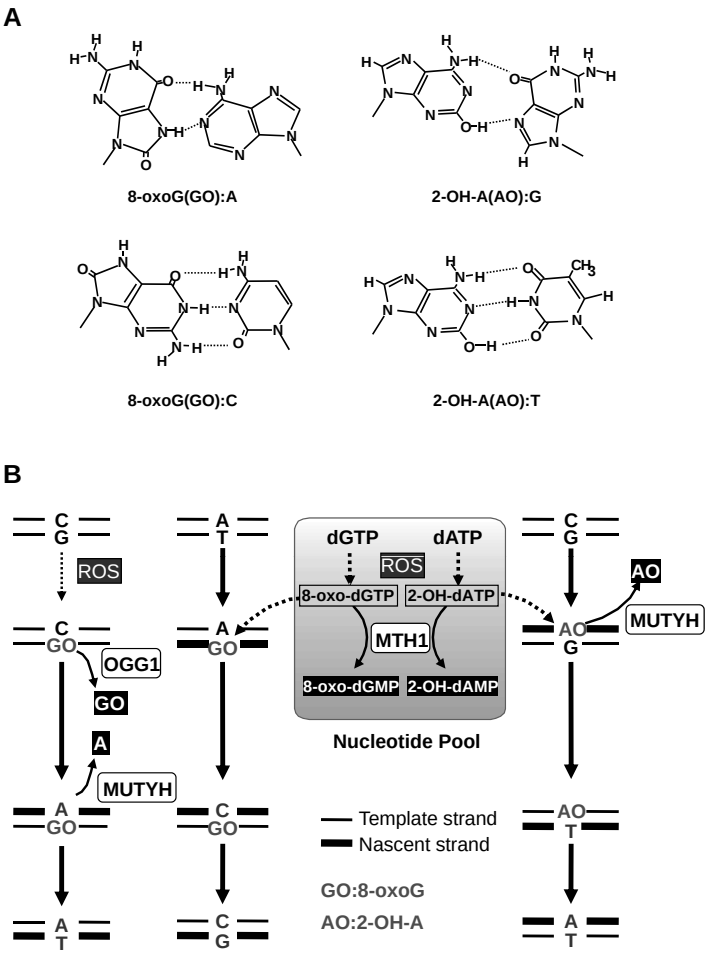

Figure 1 Altered base pairing and mutagenesis caused by the oxidation of nucleic acids and the defense mechanisms in mammals. A: Altered base pairing of 8-oxoguanine and 2-hydroxyadenine. During DNA replication, 8-oxoG and 2-OH-A can pair with adenine and guanine, respectively, in template DNA. B: Mutagenesis 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. (Adapted from reference 8)

2-hydroxy-(d)ATP (2-OH-(d)ATP) to the corresponding monophosphates and pyrophosphates. These monophosphates are further converted to nucleosides such as 8-oxo-dG, thus avoiding their incorporation into DNA (Figure 1B)[8]. We determined the structure of MTH1 in solution by multi-dimensional heteronuclear NMR spectroscopy[9]. Based on the arrangement of the pocket-forming residues, combined with the mutagenesis data, we generated models for the substrate recognition of MTH1, and found that Asn-33 and Asp-119 play pivotal roles in discriminating the oxidized forms of purine, namely 8-oxoG and 2-hydroxyadenine (2-OH-A), while Trp-117 is important in determining affinity with the purine rings[10].

The human *MTH1* gene is located on chromosome 7p22, and consists of 5 major exons; two alternative exon 1 sequences, namely exon 1a and 1b, and three contiguous exon 2 segments (exon 2a, 2b, and 2c) which are alternatively spliced. Thus, the *MTH1* gene produces 7 types of mRNA which encode three different human MTH1 isoforms, hMTH1b (p22), hMTH1c (p21) and hMTH1d (p18). Most of the major form of hMTH1 (p18) protein is localized in the cytoplasm with about 5% in the mitochondrial matrix, suggesting that hMTH1 plays an important role in maintaining the quality of the nucleotide pools of both nuclear and mitochondrial genomes[11].

2. 8-oxoguanine DNA glycosylase, OGG1, prevents buildup 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 opposite cytosine. In addition, OGG1 also possesses a weak AP lyase activity (Figure 1B)[12].

The 8 exons of the human *OGG1* gene are located on chromosome 3p25, a region showing a frequent loss of heterozygosity in lung and kidney tumors[12]. 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)[13]. Types 1a and 2a mRNA are the major *OGG1* transcripts in various human tissues, and encode OGG1-1a and OGG1-2a isoforms of human *OGG1* protein, respectively. OGG1-1a protein, also known as OGG1α, has a nuclear localization signal (NLS) at its C-terminal end, and thus is located in the nucleus, while OGG1-2a protein (OGG1β), 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 OGG1-1a and OGG1-2a carry a relatively poor mitochondrial targeting sequence (MTS) at their N-terminal region. This sequence consists of residues 9 to 26, whose activity is not sufficient for localizing nuclear OGG1-1a with NLS within the mitochondria. On the other hand, OGG1-2a is likely associated with the mitochondrial inner membrane and other BER machinery dependent on the unique C-terminal region[13,14].

3. MUTYH functions as a DNA glycosylase for 2-hydroxyadenine opposite guanine and adenine opposite 8-oxoguanine.

A DNA glycosylase encoded by the *MUTYH* gene excises the adenine inserted opposite 8-oxoG in the template strand (Figure 1B)[15,16]. MUTYH protein also has the ability to excise 2-OH-A incorporated opposite guanine in the template[17,18]. It has been shown that the adenine base in DNA is barely oxidized, while adenine nucleotides are easily oxidized *in vitro*, suggesting that 2-OH-A in DNA is mostly

derived through the incorporation of 2-OH-dATP during DNA replication (Figure 1B)[19]. MUTYH thus has to recognize selectively adenine or 2-OH-A incorporated into the nascent DNA strands. MUTYH has a functional proliferating cell nuclear antigen (PCNA) binding motif[20] and we have shown that MUTYH repairs adenine incorporated opposite 8-oxoG in transfected plasmid DNA in cultured cells dependent on this PCNA binding motif[21]. However, we found that the PCNA-binding motif in MUTYH is not essential for suppressing the increased spontaneous mutation rate observed in MUTYH-null cells[22]. MUTYH has been shown to interact with other replication-associated proteins such as RPA or MSH2 which also can interact with PCNA, thus suggesting that the interaction of MUTYH with these proteins support its proper function[20].

The human *MUTYH* gene is located on the short arm of chromosome 1 between p32.1 and p34.3, and consists of 16 exons[23]. We previously reported that there are 3 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, thus multiple forms of human MUTYH proteins are present in nuclei and mitochondria[17]. Human *MUTYH* protein encoded by type α mRNA possesses an MTS consisting of 14 amino terminal residues which are required for its localization in the mitochondria[24], while those encoded by type β and γ mRNAs lack the MTS, and are localized in the nuclei[17]. The subcellular localization of MUTYH in human cells thereby indicates that mitochondrial DNA is an important target for BER initiated by MUTYH, as well as OGG1, probably because of increased oxidative stress[25].

4. Cell death triggered by oxidized purine nucleotides accumulated in nucleotide pool.

Mth1-deficient mouse embryo fibroblasts (MEFs) are highly susceptible to cellular dysfunction and cell death caused by H_2O_2, with morphological features of pyknosis and electron dense deposits accumulated in mitochondria. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis and immunofluorescence microscopy revealed a continuous buildup of 8-oxo-G both in nuclear and mitochondrial DNA after exposure to H_2O_2. All H_2O_2-induced alterations observed in *Mth1*-deficient MEFs were effectively suppressed by the expression of human MTH1 (hMTH1)[26].

We then examined whether hMTH1 prevents cellular dysfunction induced by sodium nitroprusside, a spontaneous NO donor. Exposure to sodium nitroprusside caused an 8-oxoG buildup in DNA of proliferating MTH1- null cells which underwent mitochondrial degeneration and subsequently died. Quiescent MTH1-null cells also died with 8-oxoG buildup but only when the buildup affected mitochondrial and not nuclear DNA. In both proliferative and quiescent conditions, the accumulation of 8-oxoG in DNA and cell death were effectively prevented by hMTH1. Knockdown of MUTYH in quiescent MTH1-null cells significantly prevented the cell death, indicating that 8-oxoG incorporated into mitochondrial DNA is a main cause of this form of cell death[27], as described below.

To verify this possibility, an artificially modified hMTH1, namely mTP-EGFP-hMTH1, which localizes exclusively in mitochondria, was expressed in MTH1-null cells. mTP-EGFP- hMTH1 selectively prevented buildup of 8-oxoG in mitochondrial but not nuclear DNA after exposure of proliferating cells to sodium nitroprusside, and also efficiently prevented cell death. We thus concluded that exposure of cells to sodium nitroprusside causes oxidation of mitochondrial deoxynucleotide pools, and that buildup of oxidized bases in mitochondrial DNA initiates cell death[27].

5. Cell death triggered by 8-oxoguanine accumulated in nuclear DNA.

We established cell lines which selectively accumulate 8-oxoG in nuclear DNA by expression of a mitochondrial form of human OGG1 (hOGG1-2a) in OGG1-null mouse cells. These cell lines are deficient in BER of 8-oxoG in their nuclear DNA but not in their mitochondrial DNA. Therefore, only the nuclear DNA showed a buildup of 8-oxoG after exposure to oxidative stress, as well as a massive buildup of single strand DNA breaks (SSBs), followed by poly(ADP-ribose)-polymearse (PARP) activation with increased poly-ADP-ribosylation of cellular proteins. Thus these MEFs underwent caspase- independent cell death after nuclear translocation of apoptosis inducing factor (AIF). In the presence of PARP inhibitor or *Mutyh*-siRNA, the nuclear translocation of AIF was efficiently diminished, resulting in the suppression of the cell death command. Furthermore, *Mutyh*-siRNA suppressed the massive buildup of SSBs in the nuclear DNA (Figure 2)[7].

PARP is a known molecular nick-sensor that binds specifically to SSBs and its specific activity catalyzing poly-ADP-ribosylation of cellular proteins or of PARP itself can increase approximately 500-fold[28]. Our data clearly indicate that MUTYH is responsible for the generation of SSBs in nuclear DNA with buildup of 8-oxoG. Since MUTYH functions as an adenine DNA glycosylase, by excising adenine inserted opposite 8-oxoG in template DNA during replication, many abasic sites can be generated opposite 8-oxoG[16]. During BER, abasic sites are concomitantly converted to SSBs by the action of AP endonuclease or AP lyases, thereby activating PARP.

Recently, it has been reported that poly-ADP-ribose polymer induces mitochondrial AIF release and translocation to the nucleus[29], thus executing the cell death command, leading us to conclude that buildup of 8-oxoG in nuclear DNA

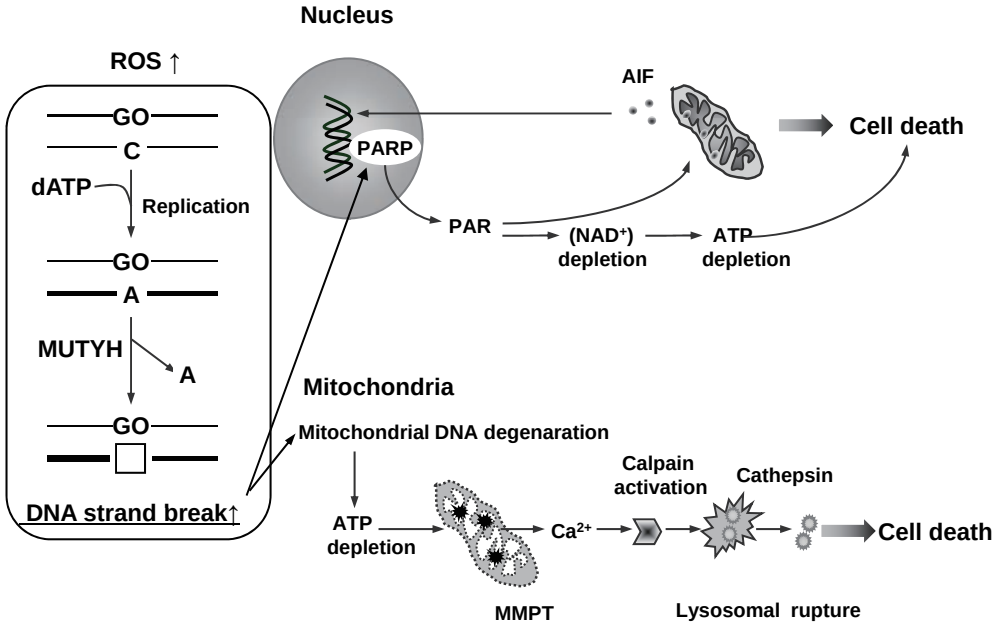

Figure 2 MUTYH-dependent programmed cell death triggered by buildup of 8-oxoguanine in nuclear and mitochondrial DNA. ROS, reactive oxygen species, GO: 8-oxoG, BER, base excision repair, SSBs: single strand breaks, AIF: apoptosis inducing factor, PARP:poly(ADP-ribose) polymerase, PAR:poly(ADP-ribosyl)ation, NAD^+: nicotinamide adenine dinucleotide. (Adapted from reference 7)

causes massive buildup of SSBs through MUTYH-initiated BER, resulting in PARP-dependent cell death. PARP catalyzes poly-ADP-ribosylation using nicotinamide adenine dinucleotide[28]. Excessive activation of PARP leads to its auto-consumption resulting in delayed depletion of ATP, as observed with 8-oxoG accumulation in nuclear DNA, thus an energy crisis may also contribute to cell death.

6. Cell death triggered by 8-oxoguanine accumulated in mitochondrial DNA.

We established cell lines which selectively accumulate 8-oxoG in mitochondrial DNA by expression of a nuclear form of human OGG1 (hOGG1-1a) in OGG1-null mouse cells[7,30]. These cell lines are deficient in BER of 8-oxoG in mitochondrial DNA but not nuclear DNA, thereby causing 8-oxoG to accumulate only in mitochondrial DNA after exposure to menadione. These cells exhibited depletion of mitochondrial DNA and ATP after the treatment, followed by mitochondrial membrane permeability transition (MMPT) and Ca^{2+} release from mitochondria, thus activating calpains to execute caspase-independent cell death.

Buildup of 8-oxoG in mitochondrial DNA rapidly resulted in the depletion of mitochondrial DNA which was efficiently suppressed in the presence of *Mutyh*-siRNA, therefore we again conclude that MUTYH- initiated BER is responsible for the depletion of mitochondrial DNA. This depletion results in the simultaneous depletion of ATP, probably because of a decreased supply of mitochondrially-encoded proteins, as well as tRNAs and rRNAs which are all essential for mitochondrial respiratory function (Figure 2).

Depletion of ATP is known to open the MMPT pore allowing Ca^{2+} to leave mitochondria[31], indicating that mitochondrial dysfunction initiated by buildup of 8-oxoG in mitochondrial DNA causes MMTP, thereby increasing the cytoplasmic concentration of Ca^{2+} which in turn activates calpains. Activated calpains are known to induce lysosomal rupture causing the release of cathepsin which ultimately executes the cell death command[32].

7. Altered expression of MTH1, OGG1 and MUTYH in Parkinson's disease brains accompanied by a buildup of 8-oxoguanine.

Oxidative damage and partial deficiencies in mitochondrial complex I in the nigro-striatal pathway have been suggested by many investigators to contribute to the selective loss of dopaminergic neurons in PD brains[33,34]. Indeed, several types of oxidative damage have been demonstrated in midbrain tissue from PD brains, 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 brains show a significant increase in 8-oxoG in mitochondrial DNA or cytoplasmic RNA in the dopaminergic neurons remaining in the substantia nigra (SN)[6,35,36], compared to 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[37]. 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[6]. In SN of the control brains, MTH1 expression was hardly detectable in dopaminergic neurons, suggesting that the increased expression of MTH1 in the surviving dopaminergic neurons of PD brains may have a neuroprotective role.

We showed that in PD brains, OGG1-2a immunoreactivity is rarely observed in any of the brain regions including cortex, basal ganglia, SN, and pontine nuclei, and is never observed in glial cells as well as in control subjects, how-

ever, the number of OGG1-2a-positive neurons in the SN increases with age[38]. Western blot analysis showed that expression of OGG1-2a also increases 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 OGG1-2a which was colocalized with cytochrome oxidase I in mitochondria. However, those who had PD for longer than 10 years did not exhibit intense cytoplasmic immunostaining[38]. Semiquantitative analysis showed that the expression of OGG1-2a is significantly higher in the short-duration group relative to the aged-matched controls. In the long-duration group, the number of OGG1-2a-positive neurons is slightly higher. Western blot analysis demonstrated that the level of OGG1-2a (43- and 40-kDa) in the SN of PD brain was 1.6 to 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[39]. The dopaminergic neurons remaining in the SN showed intense and diffuse immunostaining for MUTYH in the cytoplasm but none in nuclei or Lewy bodies. 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.

8. MUTYH-initiated programmed cell death is responsible for neurodegeneration.

We found that levels of 8-oxoG in cellular DNA and RNA increased in the mouse nigrostriatal system during the tyrosine hydroxylase (TH)-positive dopamine neuron loss induced by the administration of 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP). MTH1-null mice exhibited a greater accumulation of 8-oxoG in mitochondrial DNA accompanied by a more significant decrease in TH and dopamine transporter immunoreactivities in the striatum after MPTP administration, than in wild-type mice. We thus demonstrated that MTH1 protects the dopamine neurons from oxidative damage in the nucleic acids, especially in the mitochondrial DNA of striatal nerve terminals of dopamine neurons[40].

To confirm the neuroprotective function of MTH1, we constructed a transgenic mouse in which the hMTH1 is expressed[41]. hMTH1 expression protected embryonic fibroblasts and mouse tissues against the effects of oxidants. Wild-type mice exposed to 3-nitropropionic acid (3-NP), a naturally occurring mycotoxin which inhibits succinate dehydrogenase, develop neuropathological and behavioural symptoms that resemble those of Huntington's disease. hMTH1 transgene expression conferred a dramatic protection against these Huntington's disease–like symptoms, including weight loss, dystonia and gait abnormalities, striatal degeneration, and death. In a complementary approach, an *in vitro* genetic model for Huntington's disease was also used. hMTH1 expression protected progenitor striatal cells containing an expanded CAG repeat of the *huntingtin* gene from toxicity associated with expression of the mutant *huntingtin*. The findings implicate oxidized nucleic acid precursors in the neuropathological features of Huntington's disease and identify the utilization of oxidized nucleoside triphosphates by striatal cells as a significant contributor to the pathogenesis of this disorder[41].

We then examined effects of chronic administration of 3-nitropropionic acid, to various mutant mice lacking MTH1, OGG1 or MUTYH (Sheng *et al.*, in preparation). We found that double-knockout (DKO) mice lacking OGG1 and MTH1, and to a lesser extent OGG1-KO

mice, significantly decreased their locomotor activity in comparison to MTH1-KO or wild-type. While OGG1/MUTYH DKO mice exhibited much higher locomotor activity than OGG1-KO or wild-type mice. 3-NP administration to MTH1/OGG1 but not OGG1/MUTYH DKO mice caused a severe striatal degeneration, and early accumulation of 8-oxoG in mitochondrial DNA was observed in the striatal medium spiny neurons accompanied by calpin activation, while delayed accumulation of 8-oxoG in nuclear DNA was observed in microglias accompanied by increased poly-ADP-ribosylation and nuclear translocation of AIF. These results indicate that 3-NP induces MUTYH-dependent striatal degeneration through the two distinct death pathways for the medium spiny neurons and microglias. Our results provide new mechanistic insights into neurodegenerative disorders with medium spiny neuron loss in striatum such as Huntington's disease. We further observed that administration of inhibitors for PARP or calpain ameliorate the decreased locomotor activity in OGG1/MTH1 DKO mice induced by 3-NP administration. We thus concluded that 8-oxoG accumulated in brain genomes causes neurodegeneration through the two distinct MUTYH-dependent programmed cell death pathways (Figure 2), and which is efficiently suppressed by MTH1 and OGG1.

References

1) Ames BN, Gold LS. Endogenous mutagens and the causes of aging and cancer. Mutat Res 1991;250:3-16.

2) Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-22.

3) Nakabeppu Y, Sakumi K, Sakamoto K, et al. Mutagenesis and Carcinogenesis Caused by the Oxidation of Nucleic Acids. Biol Chem 2006; 387:373-9.

4) Kasai H. Chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis. Free Radic Biol Med 2002;33:450-6.

5) Moreira PI, Nunomura A, Nakamura M, et al. Nucleic acid oxidation in Alzheimer disease. Free Radic Biol Med 2008;44:1493-505.

6) Shimura-Miura H, Hattori N, Kang D, et al. Increased 8-oxo-dGTPase in the mitochondria of substantia nigral neurons in Parkinson's disease. Ann Neurol 1999;46:920-4.

7) Oka S, Ohno M, Tsuchimoto D, et al. Two distinct pathways of cell death triggered by oxidative damage to nuclear and mitochondrial DNAs. EMBO J 2008;27:421-32.

8) Nakabeppu Y, Tsuchimoto D, Yamaguchi H, Sakumi K. Oxidative Damage in Nucleic Acids and Parkinson's Disease. J Neurosci Res 2007;85: 919-34.

9) Mishima M, Sakai Y, Itoh N, et al. Structure of human MTH1, a Nudix family hydrolase that selectively degrades oxidized purine nucleoside triphosphates. J Biol Chem 2004;279:33806-15.

10) Sakai Y, Furuichi M, Takahashi M, et al. A molecular basis for the selective recognition of 2-hydroxy-dATP and 8-Oxo-dGTP by human MTH1. J Biol Chem 2002;277:8579-87.

11) Nakabeppu Y. Molecular genetics and structural biology of human MutT homolog, MTH1. Mutat Res 2001;477:59-70.

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

13) Nishioka K, Ohtsubo T, Oda H, et al. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. Mol Biol Cell 1999;10:1637-52.

14) Stuart JA, Mayard S, Hashiguchi K, Souza-Pinto NC, Bohr VA. Localization of mitochondrial DNA base excision repair to an inner membrane-associated particulate fraction. Nucleic Acids Res 2005;33:3722-32.

15) Slupska MM, Luther WM, Chiang JH, Yang H, Miller JH. Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY

protein. J Bacteriol 1999;181:6210-3.

16) Tominaga Y, Ushijima Y, Tsuchimoto D, et al. MUTYH prevents OGG1 or APEX1 from inappropriately processing its substrate or reaction product with its C-terminal domain. Nucleic Acids Res 2004;32:3198-211.

17) Ohtsubo T, Nishioka K, Imaiso Y, et al. 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 2000;28:1355-64.

18) Ushijima Y, Tominaga Y, Miura T, et al. A functional analysis of the DNA glycosylase activity of mouse MUTYH protein excising 2-hydroxyadenine opposite guanine in DNA. Nucleic Acids Res 2005;33:672-82.

19) Kamiya H, Kasai H. Formation of 2-hydroxydeoxyadenosine triphosphate, an oxidatively damaged nucleotide, and its incorporation by DNA polymerases. Steady-state kinetics of the incorporation. J Biol Chem 1995; 270:19446-50.

20) Parker A, Gu Y, Mahoney W, et al. Human homolog of the MutY repair protein (hMYH) physically interacts with proteins involved in long patch DNA base excision repair. J Biol Chem 2001;276:5547-55.

21) Hayashi H, Tominaga Y, Hirano S, et al. Replication-Associated Repair of Adenine:8-Oxoguanine Mispairs by MYH. Current Biol 2002;12:335-9.

22) Hirano S, Tominaga Y, Ichinoe A, et al. Mutator Phenotype of MUTYH-null Mouse Embryonic Stem Cells. J Biol Chem 2003;278:38121-4.

23) Slupska MM, Baikalov C, Luther WM, et al. 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 1996;178:3885-92.

24) Takao M, Aburatani H, Kobayashi K, Yasui A. Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. Nucleic Acids Res 1998;26:2917-22.

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

26) Yoshimura D, Sakumi K, Ohno M, et al. An oxidized purine nucleoside triphosphatase, MTH1, suppresses cell death caused by oxidative stress. J Biol Chem 2003;278:37965-73.

27) Ichikawa J, Tsuchimoto D, Oka S, et al. Oxidation of mitochondrial deoxynucleotide pools by exposure to sodium nitroprusside induces cell death. DNA Repair 2008;7:418-30.

28) de Murcia G, Ménissier de Murcia J. Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends Biochem Sci 1994;19:172-6.

29) Yu SW, Andrabi SA, Wang H, et al. Apoptosis-inducing factor mediates poly(ADP- ribose) (PAR) polymer-induced cell death. Proc Natl Acad Sci USA 2006;103:18314-9.

30) Oka S, Ohno M, Nakabeppu Y. Construction and characterization of a cell line deficient in repair of mitochondrial, but not nuclear, oxidative DNA damage. Methods Mol Biol 2009;554:251-64.

31) Simbula G, Glascott PJ, Akita S, Hoek J, Farber J. Two mechanisms by which ATP depletion potentiates induction of the mitochondrial permeability transition. Am J Physiol 1997;273: 479-88.

32) Yamashima T. Ca2+-dependent proteases in ischemic neuronal death: a conserved 'calpain-cathepsin cascade' from nematodes to primates. Cell Calcium 2004;36:285-93.

33) Lotharius J, Brundin P. Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. Nat Rev Neurosci 2002;3:932-42.

34) Jenner P. Oxidative stress in Parkinson's disease. Ann Neurol 2003;53 Suppl 3:S26-36;discussion S36-28.

35) Alam ZI, Jenner A, Daniel SE, et al. Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. J Neurochem 1997;69: 1196-203.

36) Zhang J, Perry G, Smith MA, et al. Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. Am J Pathol 1999;154:1423-9.

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

38) Fukae J, Takanashi M, Kubo S-i, et al. Expression of 8-oxoguanine DNA glycosylase (OGG1) in Parkinson's disease and related neurodegenerative disorders. Acta Neurophathologica 2005;109: 256-62.

39) Arai T, Fukae J, Hatano T, et al. Up-regulation of hMUTYH, a DNA repair enzyme, in the mitochondria of substantia nigra in Parkinson's disease. Acta Neuropathol 2006;112:136-45.

40) Yamaguchi H, Kajitani K, Dan Y, et al. 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 2006;13:551-63.

41) De Luca G, Russo MT, Degan P, et al. A Role for Oxidized DNA Precursors in Huntington's Disease-Like Striatal Neurodegeneration. PLoS Genet 2008;4:e1000266.