Neurodegeneration Caused by Accumulation of an Oxidized Base Lesion, 8-oxoguanine, in Nuclear and Mitochondrial DNA: From Animal Models to Human Diseases

Nakabeppu, Yusaku
Division of Neurofunctional Genomics, Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, Kyushu University

出版情報：The Base Excision Repair Pathway : Molecular Mechanisms and Role in Disease Development and Therapeutic Design, pp.523-556, 2017-01. World Scientific
バージョン：
権利関係：

The Base Excision Repair Pathway

Molecular Mechanisms and Role in Disease Development and Therapeutic Design

Edited by David M Wilson III,

Published by World Scientific,

(https://www.worldscientific.com/worldscibooks/10.1142/9776)

ISBN: 978-981-4719-74-2(ebook)

Chapter 14

(pp. 523-556)

Neurodegeneration caused by accumulation of an oxidized base lesion, 8-oxoguanine, in nuclear and mitochondrial DNA: from animal models to human diseases

Yusaku Nakabeppu

Division of Neurofunctional Genomics,

Department of Immunobiology and Neuroscience,

Medical Institute of Bioregulation, Kyushu University,

3-1-1 Maidashi Higashi-ku, Fukuoka 812-8582, Japan

E-mail: yusaku@bioreg.kyushu-u.ac.jp

A large amount of energy, in the form of ATP, is required to maintain basic brain functions, such as maintenance or re-establishment of membrane potential, signaling and essential cellular activities. This ATP is supplied by consuming large amounts of oxygen and glucose via oxidative phosphorylation in mitochondria (MacKenna et al., 2012). However, highly reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide or hydroxyl radicals are generated by reduction of oxygen or by the leakage of electrons from the electron transport chain in mitochondria (Kang et al., 1999; Nunnari and Suomalainen, 2012). Reactive nitrogen species (RNS), such as nitric oxide (NO), are physiologically generated upon neural excitation or during the inflammatory responses of glial cells, which are activated during infection or inflammation in the brain. In the presence of superoxide anions, NO can be converted into peroxynitrite, a highly RNS. RO/NS are highly reactive and attack various biomolecules, such as lipids, proteins, carbohydrates, and nucleic acids. This outcome induces various chemical modifications in these molecules, including oxidation, nitration, S-nitrosylation, deamination, and glutathionylation (Cobb and Cole, 2015). There are many defense mechanisms that minimize the toxic effects of RO/NS, including antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase; however, the brain is constantly exposed to endogenous RO/NS produced during normal brain functions (Dasuri et al., 2013).

The toxicity of endogenous RO/NS has been demonstrated in the brains of SOD- and NO synthase (NOS)-deficient mice. Mice deficient in the mitochondrial form of SOD (SOD2) in the brain exhibit spongiform neurodegeneration in the motor cortex, hippocampus, and brainstem, accompanied by gliosis, severe growth retardation and perinatal death. In addition, these mutant mice had markedly decreased mitochondrial complex II activity in the brain, demonstrating that superoxide buildup, generated by mitochondrial respiration, is highly toxic (Izuo et al., 2015). However, deficiency in either inducible NOS or neuronal NOS protects against 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) toxicity in the brain (Dehmer et al., 2000; Hoang et al., 2009), indicating that neuronal damage by ROS is exacerbated by NO, whose mitochondrial production is known to be enhanced in

dopaminergic neurons after administration of MPTP.

Despite the many defense mechanisms that eukaryotic cells have evolved, various oxidized lipids, proteins, carbohydrates and nucleic acids accumulate in the brains of humans and experimental animals during aging or under pathological conditions. Lipids in neuronal membranes are considered to be the principal targets of oxidation, and accumulating evidence indicates that lipid peroxidation products, such as acrolein, 4-hydroxy-2-nonenal or malondialdehyde, are highly accumulated in neurodegenerated brains, such as in Alzheimer disease (AD), Parkinson disease (PD) and Huntington disease (HD) (Cobb and Cole, 2015). Peroxidation of membrane lipids results in increased membrane rigidity and affects a variety of functions, such as decreased activity of membrane-bound enzymes, impairment of membrane receptors, and altered permeability (Sultana et al., 2013). Also, lipid peroxidation products cause modification of membrane proteins and DNA, and induce lipid–protein and protein–protein crosslinking, and oxidative DNA damage, all of which are highly accumulated in brains of various neurodegenerative disease patients (Coppede and Migliore, 2015).

Among the nucleobases, guanine is the most susceptible to oxidation by hydroxyl radicals or one-electron oxidants, and is modified to 8-oxo-7,8-dihydro-guanine (8-oxoG) or 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-G). The former is one of the major oxidized bases in the nucleotide pool or DNA. Under conditions of oxidative stress, 8-oxoG can be further oxidized to spiroiminodihydantoin (Sp) and guanidinohydantoin (Gh) (Cadet et al., 2010; Cooke et al., 2003; Dizdaroglu and Jaruga, 2012; Kasai and Nishimura, 1984).

Among these oxidized guanine derivatives, 8-oxoG is highly accumulated in AD, PD and HD patient brains, as will be discussed in detail below. This raises the question of whether 8-oxoG is a cause or consequence of the neurodegenerative process. There is accumulating evidence for a causative association, especially from experiments with genetically-manipulated animals, in which levels of 8-oxoG in the brain are artificially altered, suggesting the possible mechanism(s) that underlies the neurodegeneration. In this Chapter, I address these issues and discuss the roles of 8-oxoG and the enzymes involved in

processing 8-oxoG in the nucleotide pool and DNA during the process of neurodegeneration. I also discuss possible therapeutic targets.

1. 8-oxo-G accumulation in the brains of patients with major neurodegenerative diseases

1.1. *AD*

More than 35 million people worldwide suffer from dementia, and this number is expected to exceed 115 million by 2050 because of the rapid increase in the elderly population. Sporadic AD, also known as late-onset AD, is the most common dementia subtype, accounting for between 60 to 80% of all dementia cases (Prince et al., 2013; Sosa-Ortiz et al., 2012). It is thus important to identify the risk factors and to understand the roles of these factors in the development of sporadic AD.

AD is characterized by accumulation in the brain of both senile plaques containing aggregated amyloid β (Aβ) and neurofibrillary tangles (NFTs) consisting of aggregated highly phosphorylated TAU protein. Aβ plaques can be present more than 20 years before the onset of dementia in patients with dominantly inherited AD. Deposition of Aβ is considered to be an early event in sporadic AD cases, although the mechanism of how Aβ is deposited is not yet known. Aβ is known to cause mitochondrial dysfunction as well as a neuroinflammatory response in the brain, thus increasing ROS or RNS production in the AD brain (Cobb and Cole, 2015; Yan et al., 2013).

As markers for oxidative damage in the AD brain, various oxidized bases in DNA and lipid peroxidation products have been detected using high-performance liquid chromatography with electrochemical detection or gas chromatography-mass spectrometry (Bradley-Whitman et al., 2014; Gabbita et al., 1998; Gabbita et al., 2002; Lyras et al., 1997; Lyras et al., 2002; Mecocci et al., 1994; Wang et al., 2006; Wang et al., 2005). Among the various oxidized bases, such as 8-oxoG, 8-oxoadenine, 2-hydroxyadenine, Fapy-G, Fapy-A,

5-hydroxycytosine, 5-hydroxyuracil and thymine glycol, which have been detected in either nuclear or mitochondrial DNA prepared from postmortem AD brains, 8-oxoG is recognized as the most pronounced marker in AD brain, accumulating in both nuclear and mitochondrial DNA. Immunohistochemical examination of postmortem AD brains revealed that cytoplasmic accumulation of 8-oxoG is evident in hippocampal CA1 and CA3 pyramidal neurons (Song et al., 2011), and in neurons of the temporal cortex (de la Monte et al., 2000), where Aβ is also highly accumulated. Accumulation of 8-oxoG in the AD brain is likely to be an early event, occurring before onset of dementia (Coppede and Migliore, 2015; Lovell and Markesbery, 2007).

1.2. *PD*

PD is the second most common age-related neurodegenerative disease after AD, with about 95% of PD cases being sporadic and the rest inheritable. It has been estimated that the number of individuals with PD over the age of 50 in Western Europe's five most populous and the world's 10 most populous nations, which in 2014 account for 62.7% of the world's population, was between 4.1 and 4.6 million in 2005 and will double to between 8.7 and 9.3 million by 2030 (Dorsey et al., 2007; Wirdefeldt et al., 2011). PD is primarily characterized by a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), and the presence of intracytoplasmic proteinaceous inclusions termed Lewy bodies and dystrophic neurites (Lewy neurites). PD patients suffer from various motor impairments, including bradykinesia, tremors and rigidity, and the appearance of such symptoms is thought to require the loss of 50–60% of SNc dopaminergic neurons and an 80–85% dopamine deficiency in the putamen (Dauer and Przedborski, 2003; Wirdefeldt et al., 2011).

To date, 17 genes or loci have been associated with PD (http://www.genenames.org/genefamilies/PARK), and individuals with disease alleles at these loci often exhibit early-onset PD. However, up to 95% of PD cases are sporadic and late onset, indicating that there must be other factors, including occupational and environmental factors, that trigger PD pathology. Studies of postmortem brains show that

both inherited and sporadic PD brains exhibit oxidative damage and partial deficiencies in mitochondrial complex I in the nigrostriatal pathway, and thus many investigators propose that oxidative damage and mitochondrial dysfunction contribute to the selective loss of dopaminergic neurons in PD (Dauer and Przedborski, 2003; Moore et al., 2005). 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 lipid, protein, and DNA oxidation products (Jenner, 2003). We and others have reported that PD patients have significantly increased levels of 8-oxoG in mitochondrial DNA and cytoplasmic RNA in the remaining dopaminergic neurons of the substantia nigra (SN) (Alam et al., 1997; Shimura-Miura et al., 1999; Zhang et al., 1999). Recently, it has also been shown that apurinic/apyrimidinic (AP or abasic) sites are highly accumulated in mitochondrial DNA, especially in the dopaminergic neurons of the SN, but not in cortical neurons from postmortem PD specimens (Sanders et al., 2014), implying high accumulation of base damage in the mitochondrial DNA of SN dopaminergic neurons.

1.3. *HD*

HD is an autosomal dominant and progressive neurodegenerative disorder caused by an expanded CAG repeat in the *huntingtin* gene, which encodes an abnormally long polyglutamine repeat in the huntingtin (HTT) protein (Ross and Tabrizi, 2011). Meta-analysis revealed an overall prevalence of HD in Europe, North America, and Australia of 5.70 per 100,000, while the prevalence is significantly lower in Asia (0.4 per 100,000) (Pringsheim et al., 2012). The clinical and pathophysiological hallmarks of HD are spontaneous choreiform and dystonic movements, frontal-type cognitive deficits, and progressive heterogeneous striatal degeneration with cytoplasmic aggregates and nuclear inclusions of mutant HTT protein throughout the brain (Ross and Tabrizi, 2011). Although it is not clear how mutant HTT protein causes pathology, mitochondrial dysfunction and increased oxidative modification of proteins, as well as increased accumulation of 8-oxoG in

both nuclear and mitochondrial DNA have been demonstrated in the HD brain (Browne et al., 1997; Polidori et al., 1999; Siddiqui et al., 2012; Sorolla et al., 2008).

2. Defense mechanisms minimizing 8-oxoG accumulation in DNA

There are two pathways for the accumulation of 8-oxoG in DNA (see also Chapter 2). One is the result of incorporation of 8-oxo-dGTP from the nucleotide pool and the other is the result of direct oxidation of guanine in DNA (Figure 1A). Recent studies of the sanitization of the nucleotide pool, as well as DNA repair, have revealed that the impact of dGTP oxidation is unexpectedly large, in comparison with the direct oxidation of DNA (Nakabeppu, 2014; Nakabeppu et al., 2010). 8-OxoG pairs with adenine and cytosine with equal efficiency, because it prefers the *syn*-configuration, while guanine mostly takes the *anti*-configuration and exclusively pairs with cytosine (Figure 1B). 8-Oxo-dGTP can therefore be inserted opposite template adenine as well as cytosine in DNA (Figure 1C).

2.1. *MTH1, an 8-oxo-dGTPase*

Human and rodent cells have strong 8-oxo-dGTP hydrolyzing activities, and the major enzyme involved is MTH1 (also known as NUDT1), a homolog of *Escherichia coli* MutT. MTH1 efficiently hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate. 8-Oxo-dGMP is further converted to the nucleoside, 8-oxo-2′-deoxyguanosine (8-oxo-dG), thus avoiding incorporation of 8-oxo-dG into DNA (Sakumi et al., 1993) (Figure 1C).

The human *MTH1* gene is located on chromosome 7p22, and consists of five 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 (Furuichi et al., 1994; Oda et al., 1997). This gene structure enables *MTH1* to produce seven mRNAs encoding three different human MTH1 isoforms, namely MTH1b (p22), MTH1c (p21) and MTH1d (p18). Most of the major form of MTH1 (p18) protein is localized in the cytoplasm with about 5% in the mitochondrial matrix, and thus MTH1 plays an important role in maintaining the

quality of the nucleotide pool for both the nuclear and mitochondrial genomes. There are two major single nucleotide polymorphisms (SNPs) in *MTH1*: rs1139425 [G<u>T</u>/G<u>*C*</u>, global minor allele frequency (GMAF)=0.0112, http://www.ncbi.nlm.nih.gov/snp/] is located at the beginning of exon 2c, while rs4866 (<u>G</u>TG/<u>*A*</u>TG, GMAF = 0.0206) is located in exon 4. The G<u>T</u>/G<u>*C*</u> polymorphism modifies patterns of alternative splicing and the minor allele (G<u>*C*</u>) encodes a fourth isoform, MTH1a (p26), in addition to the three known isoforms. The <u>G</u>TG/<u>*A*</u>TG polymorphism replaces amino acid residue valine 83 in MTH1d with methionine (Oda et al., 1999). This substitution was found to increase the thermolability of the enzyme and its α-helix content (Yakushiji et al., 1997); however, MTH1d(Val83) exhibits less efficient mitochondrial localization compared with MTH1d(Met83). Interestingly, MTH1a (p26), which carries an additional 41 residues in its amino terminal region, is more efficiently localized in mitochondria than MTH1d (Sakai et al., 2006). Furthermore, molecular epidemiological studies have revealed that the allele frequencies of Met83 with the GC polymorphism were higher in patients with hepatocellular carcinoma, gastric cancer and lung cancer compared with healthy volunteers (Kimura et al., 2004; Kohno et al., 2006), supporting the suggestion that MTH1d(Met83) harbors a functional defect.

2.2 *OGG1, an 8-oxoG DNA glycosylase*

Once 8-oxoG has been formed or incorporated into DNA, 8-oxoG opposite cytosine is mainly excised by a DNA glycosylase/AP lyase encoded by *OGG1*, thus initiating base excision repair (BER) (Figure 1C). The DNA glycosylase activity of OGG1 preferentially excises 8-oxoG or Fapy-G opposite cytosine, and to a lesser extent, thymine, but not when either is opposite guanine or adenine (Boiteux and Radicella, 2000). The human *OGG1* gene, containing eight exons, is located on chromosome 3p25, a region frequently showing 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 are the major *OGG1*

transcripts in various human tissues, and encode OGG1-1a (also known as α–OGG1) and OGG1-2a (β–Ogg1) isoforms, respectively (Boiteux and Radicella, 2000). OGG1-1a has a nuclear localization signal at its C-terminal end and is therefore located in the nucleus, whereas OGG1-2a, which has a unique C-terminal region consisting of two distinct regions, an acidic region (amino acid residues Ile^{345} to Asp^{381}) and a hydrophobic region (the last 20 residues), is located exclusively in mitochondria. Both OGG1-1a and OGG1-2a carry a relatively weak mitochondrial targeting sequence (MTS) in their N-terminal region. This sequence consists of residues 9–26, and its targeting capacity is not sufficient to localize nuclear OGG1-1a within mitochondria. Conversely, OGG1-2a is dependent on its unique C-terminal region for association with the mitochondrial inner membrane and BER machinery (Nishioka et al., 1999; Stuart et al., 2005).

rs1052133 is a high-frequency polymorphism in *OGG1* (GMAF=0.3021), which causes a Ser326 to Cys326 (minor allele) substitution in OGG1-1a (Dherin, 1999). OGG1-1a(Cys326) is prone to form dimers via disulfide bonding between Cys326 and other cysteines in the polypeptide especially under oxidative conditions, resulting in decreased OGG1 repair activity *in vivo* (Hill and Evans, 2006; Morreall et al., 2015; Simonelli et al., 2013). In addition, the repair activity of the Cys326 form is more vulnerable to NO exposure than the Ser326 form (Jaiswal et al., 2001; Moritz et al., 2014). Thus, the *OGG1* Ser326Cys polymorphism may be a risk factor for several cancers, including of the lung, digestive system and head and neck (Zhou et al., 2015).

2.3. *MUTYH, an adenine DNA glycosylase*

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 G:C to T:A transversions. A DNA glycosylase encoded by the *MUTYH* gene excises adenine inserted opposite 8-oxoG in the template strand (Figure 1C), and MUTYH appears to possess no AP lyase activity (Slupska et al., 1999; Tominaga et al., 2004). MUTYH interacts with replication-associated proteins, such as RPA, MSH2, and PCNA (Parker et al., 2001).

Moreover, MUTYH also interacts with the Rad9-Rad1-Hus1 checkpoint clamp (Hwang et al., 2015) and the BER enzyme, APE1 (Luncsford et al., 2013).

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). 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, thus multiple forms of human MUTYH are present in nuclei and mitochondria (Ohtsubo et al., 2000). Human MUTYH encoded by the type α mRNA possesses a MTS consisting of 14 N-terminal residues, which are required for its localization in mitochondria (Takao et al., 1998), while MUTYH encoded by type β and γ mRNAs lacks the MTS and is localized in the nucleus (Ohtsubo et al., 2000). The subcellular distribution of MUTYH in human cells indicates that mitochondrial DNA is an important target for BER initiated by MUTYH, as well as OGG1 (see Chapter 19), particularly in response to increased oxidative stress (Oka and Nakabeppu, 2011).

Familial mutations in *MUTYH* are causative for certain types of autosomal recessive colorectal adenomatous polyposis (Al-Tassan et al., 2002; Jones et al., 2002). The colorectal phenotype of MUTYH-associated polyposis (MAP) resembles that of attenuated familial adenomatous polyposis; usually from 10 to a few hundred adenomas occur, and the mean age at diagnosis is 45 years. Although patients with MAP have no germ-line mutation in the adenomatous polyposis coli (*APC*) gene, somatic mutations in the *APC* gene were found exclusively in tumor tissue and most of them were G:C to T:A transversions, a hallmark of 8-oxoG driven mutagenesis. A similar spectrum of *KRAS* mutations was also found in patients with MAP, indicating that MUTYH prevents such mutational events (Knopperts et al., 2013; Sieber et al., 2003).

3. Alterations of MTH1, OGG1 and MUTYH in neurodegenerative diseases

3.1. *MTH1*

Immunohistochemical examination of human postmortem brains from non-neurological subjects revealed that MTH1 protein is most highly expressed in the stratum lucidum of the CA3 hippocampal subfield corresponding to mossy fiber synapses, followed by perikarya of granular neurons of the dentate gyrus (DG) and pyramidal neurons of the entorhinal cortex (Furuta et al., 2001). Weaker expression is seen in the cytoplasm of CA1 and CA3 pyramidal neurons (Song et al., 2011).

In AD subjects, MTH1 synaptic immunoreactivity in CA3 as well as cytoplasmic immunoreactivity in CA1 and CA3 neurons were significantly decreased, whereas increased immunoreactivity was observed in the entorhinal cortex (Furuta et al., 2001; Song et al., 2011); decreased MTH1 levels in CA1 and CA3 neurons correlated with increased 8-oxoG levels in these neurons (Song et al., 2011). It is noteworthy that expression profiles of DNA repair and stress response genes in lymphocytes of AD patients revealed that MTH1 levels were significantly decreased in AD samples, suggesting that protective responses against oxidative damage in DNA may be compromised even in peripheral lymphocytes in AD patients (Leandro et al., 2013).

In contrast, significantly increased levels of MTH1 were seen in the SN neurons of PD patients, a brain region that also accumulated high levels of 8-oxoG in mitochondrial DNA (Shimura-Miura et al., 1999). Because MTH1 expression was barely detectable in over 90% of SN neurons in non-neurological subjects or patients with multiple system atrophy, increased oxidative stress in the PD brain may cause significant induction of *MTH1* expression as a protective response, and thus only neurons with high levels of MTH1 can survive.

It has been suggested that the MTH1 Ile45Thr polymorphism might be associated with sporadic PD in Chinese males (Jiang et al., 2008). These patients exhibit a higher frequency of the Thr45 allele than control subjects (0.073 vs. 0.019, corrected p value = 0.050); however, there has been no functional analysis of this allele or replication of this finding.

3.2. *OGG1*

Western blot analysis of nuclear and mitochondrial fractions prepared from non-neurological postmortem human brains revealed that substantial levels of both nuclear and mitochondrial forms of OGG1 are expressed in frontal, temporal and parietal lobes and cerebellum. Protein levels of nuclear OGG1 in the frontal lobes from patients with late-stage AD (LAD) were significantly decreased, while nuclear OGG1 in temporal lobe and cerebellum from patients with mild cognitive impairment (MCI) were significantly increased. There was no significant difference in mitochondrial OGG1 levels among control, MCI and LAD cases. Irrespective of the alteration in OGG1 protein levels in MCI or LAD brains, 8-oxoG DNA glycosylase activity was significantly decreased in nuclear fractions and to a lesser extent in mitochondrial fractions from MCI and LAD brains (Shao et al., 2008). Shao *et al.* (Shao et al., 2008) also found that both nuclear and mitochondrial forms of OGG1 are modified by 4-hydroxynonenal, a neurotoxic by-product of lipid peroxidation in aged brains. This modification of mitochondrial OGG1 is likely to be elevated in MCI, perhaps underlying the decreased mitochondrial OGG1 activity in MCI.

In preclinical brains from subjects showing no overt clinical manifestations of AD, but with significant AD pathology at autopsy, quantification of OGG1 showed significantly elevated levels of mRNA in superior and middle temporal gyri and a trend toward elevated protein levels in hippocampus/parahippocampal gyri (Lovell et al., 2011). These observations suggest that an increase in *OGG1* expression occurs early in the pathogenesis of AD to counteract accumulation of 8-oxoG, which is also observed as an early event before the onset of dementia (Coppede and Migliore, 2015; Lovell and Markesbery, 2007).

Immunohistochemical examination of non-diseased postmortem human brains using an antibody specific to the mitochondrial form of OGG1 (OGG1-2a or β–OGG1) revealed that OGG1-2a is strongly expressed in the superior occipital gyrus, orbitofrontal gyrus and entorhinal cortex, is at much lower levels in CA1, CA3 and CA4, and is absent from the DG. In LAD brains, immunoreactivity to OGG1-2a was associated with NFTs, dystrophic neurites and reactive astrocytes. suggesting that OGG1-2a in part, may not be

properly targeted into mitochondria in LAD brains (Iida et al., 2002).

It is noteworthy that levels of *OGG1* mRNA are not altered between LAD and control brains (Hokama et al., 2014), suggesting that the levels of OGG1 proteins might be post-translationally regulated, especially in late stages of the disease. It has been shown that NO or peroxynitrite, for example, inhibits OGG1 activity by the formation of S-nitrosothiol adducts and loss/ejection of zinc ions from its core zinc finger motif (Jaiswal et al., 2001). Moreover, Hill *et al.* (Hill et al., 2008) showed that OGG1 is degraded by calpain, a cysteine protease shown to be highly activated in AD brains (McBrayer and Nixon, 2013; Yamashima, 2013).

Mutations in *OGG1* (C796 deletion, Ala53Thr, Ala288Vval) specific to AD patients have been reported (Mao et al., 2007). Mutant OGG1-1a protein with the C756 deletion has an altered carboxy terminal sequence (267aa to 345aa), resulting in complete loss of 8-oxoG DNA glycosylase activity. The two other missense mutations (Ala53Thr and Ala288Val), which are likely to be rare polymorphic variants, conferred significantly reduced repair capacity to OGG1-1a, as well as reduced binding capacity to its partner proteins, poly(ADP-ribose) polymerase 1 (PARP-1) and X-ray repair cross-complementing protein 1 (XRCC1) (Jacob et al., 2013). All three mutations also alter the amino acid sequence of OGG1-2a (C796 deletion alters 267aa to 424aa). This suggests that both the nuclear form (OGG1-1a) and the mitochondrial form (OGG1-2a) lose repair capacity. The most frequent polymorphic variant, OGG1-1a (Ser326Cys), is not associated with AD (Coppede et al., 2007), despite the fact that the protein has been demonstrated to have impaired functionality (Hill and Evans, 2006; Morreall et al., 2015; Simonelli et al., 2013). The SNP rs1052133 (Ser326Cys) only affects the nuclear form, OGG1-1a, and not the mitochondrial form, OGG1-2a; therefore, impairment of mitochondrial OGG1-2a may be responsible for AD.

Expression levels of mitochondrial OGG1-2a in SN neurons are significantly higher in the short-duration group of PD patients (less than 10 years) compared with aged matched controls, but not in PD patients in the long-duration groups (Fukae et al., 2005).

Up-regulation of OGG1-2a in the early phase of PD is therefore likely to be a protective response to the neurodegenerative process. The increased oxidative stress in mitochondria causes modification and degradation of OGG1 proteins as described above, thus leading to a decreased level in the late phase of PD.

In HD patients, bearers of the polymorphic Ser326Cys allele tend to have an increased number of CAG repeats in the expanded *huntingtin* allele. Moreover, bearers of at least one copy of the mutant Ser326Cys allele, mainly heterozygous subjects, showed a significantly earlier disease onset than Ser326 homozygous individuals, suggesting a possible role of the OGG1 Ser326Cys polymorphism in the HD phenotype (Coppede et al., 2010). Expansion of the CAG trinucleotide repeat sequences within the coding region of the *huntingtin* gene located within the nuclear genome is likely to be affected by 8-oxoG and its repair reactions (Ayala-Pena, 2013; Kovtun et al., 2007) (see Chapter 14), and the Ser326Cys polymorphism only alters the function of nuclear OGG1-1a as noted above. This observation suggests that the roles of the OGG1 proteins in HD pathology are different from those in AD and PD.

3.3. *MUTYH*

To date, no association of a *MUTYH* polymorphism with any neurodegenerative disorder has been reported. Only in PD patients has the expression of MUTYH protein been studied (Arai et al., 2006). Up-regulation of MUTYH expression was observed in the mitochondria of SN neurons of PD brains. Western blot analysis detected a 47 kDa MUTYH protein in midbrain samples from both controls and PD patients; however, its level was significantly increased in PD patients. RNA analysis revealed *MUTYH* transcript variant α4 as the major *MUTYH* mRNA. Interestingly, this α4 *MUTYH* transcript encodes a MUTYH protein lacking more than 100 residues from its N-terminus, which harbors a DNA minor groove reading motif and a MTS. This MTS is present in the MUTYH proteins (54–57 kDa) encoded by transcript variants α1–3. These results suggest that the 47 kDa MUTYH protein expressed in the midbrain may have a different function from the other variants, although further study is

needed.

4. 8-OxoG and its repair in animal models of neurodegenerative disease

4.1. *AD models*

Many mouse models for familial AD have been established (Puzzo et al., 2015). Some of the more commonly employed are the transgenic (Tg)-APP$^{Arc/Swe}$ mice which carry three substitutions (Arctic mutation, APP^{Arc}: Glu693Gly, Swedish mutation, APP^{swe}: Lys670Asn and Met671Leu), and the APP/PS1 mice, which carry APP^{swe} and .PS1 lacking exon 9 (PS1ΔE9). In many of these models, increased cytoplasmic immunoreactivity for 8-oxoG has been observed in brain samples (Aliev et al., 2003; Duffy and Holscher, 2013; Song et al., 2011; Xiong et al., 2011). In most cases, it is likely that 8-oxoG is being detected in mitochondrial DNA or cytoplasmic RNA. These observations support the existence of oxidative stress as a feature of the current mouse models of AD, consistent with what has been observed in AD patient brains.

In the Tg-APP$^{Arc/Swe}$ mouse model, *OGG1* mRNA levels are transiently increased 4-fold or more in the hippocampus, frontal cortex, cerebellum and other regions in 4-month-old mice compared to the levels measured in 6-week-old Tg-APP$^{Arc/Swe}$ and wild-type mice; and the levels are 2–3-fold higher than those detected in 4-month-old wild-type mice. The *OGG1* mRNA levels in 12-month-old Tg-APP$^{Arc/Swe}$ mouse brains are significantly decreased in all brain regions examined and are equivalent to the levels in 6-week-old mouse brains (Lillenes et al., 2013). The Tg-APP$^{Arc/Swe}$ model has early onset senile plaque formation (4–6 months) and increased intraneuronal Aβ aggregation (1 month) prior to extracellular Aβ deposition, suggesting that the increased expression of *OGG1* is likely to be a protective response to oxidative damage caused by the accumulation of intraneuronal Aβ aggregation, as seen in preclinical AD brains. Moreover, such a protective response is likely to be diminished in late stages of AD pathology, as found in LAD patient

brains.

It has been shown that exercise induces neuroprotection of the hippocampus in APP/PS1 transgenic mice, and the protection is associated with increased levels and repair activity of mitochondrial OGG1 (Bo et al., 2014), suggesting a protective role of OGG1 in AD pathology. This possibility should be examined using AD mouse models with OGG1-deficiency.

MTH1 and MUTYH have not yet been investigated in any AD mouse model; however, it would be interesting to examine their expression levels in AD mouse models and to determine whether progression of AD pathology can be altered in AD mouse models with MTH1 or MUTYH deficiency.

4.2. *PD models*

The loss of dopamine neurons in the SNc, a PD specific pathology, can be induced in rodents and primates by the administration of several neurotoxins, such as 6-hydroxydopamine, paraquat, rotenone or MPTP (Dauer and Przedborski, 2003). Systemic administration of MPTP to wild-type mice (10-week-old) induces the accumulation of 8-oxoG in nuclear DNA in dopamine neurons in the SN and also in mitochondrial DNA in the SN nerve terminals in the striatum. These changes are associated with decreased MTH1 protein levels in the striatum. MTH1 deficiency did not enhance MPTP-induced loss of dopamine neurons; however, a significantly exacerbated degeneration of striatal nerve terminals of dopamine neurons, associated with significantly increased 8-oxoG accumulation in mitochondrial DNA in the striatal nerve terminals of dopamine neurons, was observed (Yamaguchi et al., 2006).

Systemically administered MPTP is converted to 1-methyl-4-phenylpryridinium (MPP^+), mostly in glial cells, and can be specifically taken up by dopaminergic neurons through the dopamine transporter (DAT). MPP^+ in dopaminergic neurons binds to complex I of the respiratory chain in mitochondria and blocks electron transport, and also releases dopamine from synaptic vesicles, thus increasing ROS levels especially in striatal nerve

terminals of dopamine neurons. In MTH1-deficient mice, it is likely that 8-oxo-dGTP, at increased levels in the nucleotide pool due to the oxidative stress, is incorporated into replicating mitochondrial DNA in striatal dopaminergic nerve terminals, resulting in mitochondrial dysfunction and dopamine depletion (Nakabeppu et al., 2007).

Aged OGG1-deficient mice (26-month-old) show decreased spontaneous locomotor behavior, a decrease in striatal dopamine levels, a loss of dopamine neurons in the SN, and an increase in ubiquitin-positive inclusions in their remaining SN neurons compared with age-matched wild-type mice. In addition, young OGG1-deficient mice (3-month-old) are more susceptible to MPTP-induced dopamine depletion and loss of dopamine neurons than wild-type mice (Cardozo-Pelaez et al., 2012). These observations in mouse models of PD, taken together with the altered expression of mitochondrial OGG1 in PD patient brains, indicate that age-related accumulation of 8-oxoG in dopamine neurons with decreased OGG1 expression may be critical in the progression of PD pathology.

MUTYH has not been investigated in PD mouse models; however, it would be interesting to examine its expression levels in these models, and also whether progression of PD pathology can be altered in PD mouse models with MUTYH deficiency, because increased levels of a MUTYH variant were observed in PD patient brains.

4.3. *HD models*

An irreversible inhibitor of mitochondrial succinate dehydrogenase, 3-nitropropionic acid (3-NP), a naturally occurring plant or fungal toxin, causes striatal degeneration and motor impairments in humans and animals similar to those seen in HD. Systemic administration of 3-NP to rodents and non-human primates replicates most of the clinical and pathophysiological hallmarks of HD, including spontaneous choreiform and dystonic movements, frontal-type cognitive deficits, and progressive heterogeneous striatal degeneration involving apoptosis (Brouillet, 1999).

Mitochondrial DNA damage with 8-oxoG accumulation is a hallmark of the 3NP-induced HD model and is also seen in the HD transgenic model, R6/2, which carries a

transgenic *huntingtin* gene containing 115–150 CAG repeats (Acevedo-Torres et al., 2009). Increased expression of human MTH1 in mouse striatum efficiently suppresses 8-oxoG accumulation in mitochondrial DNA in the striatum induced by 3-NP, resulting in a dramatic protection against the HD-like symptoms of weight loss, dystonia and gait abnormalities, striatal degeneration, and death (De Luca et al., 2008; Ventura et al., 2013). This finding indicates that 8-oxo-dGTP accumulation in the nucleotide pool in striatal neurons induces mitochondrial dysfunction resulting in striatal degeneration.

In mouse striatum, MTH1 and OGG1 are mainly expressed in medium spiny neurons and localized in nuclei and cytoplasm or mitochondria. Mutant mice lacking MTH1 and/or OGG1 exhibit severe striatal neurodegeneration after 3-NP administration, with significantly increased 8-oxoG accumulation in the mitochondrial DNA of striatal neurons, indicating that OGG1 and MTH1 are protective for 3-NP-induced striatal neurodegeneration (Sheng et al., 2012). Mutant mice lacking MUTYH, which is also localized in mitochondria of medium spiny neurons, and OGG1/MUTYH-double deficient mice are resistant to 3-NP-induced striatal neurodegeneration, indicating that MUTYH promotes neurodegeneration. MUTYH excises adenine inserted opposite 8-oxoG in template DNA, thus initiating a BER response to reduce 8-oxoG-driven mutagenesis (Figure 1C). Thus, 8-oxoG accumulation in mitochondrial DNA causes neurodegeneration through a MUTYH-mediated BER mechanism (Sheng et al., 2012), which will be described in the next section.

4.4. *Models for other neurodegenerative diseases*

Cerebral ischemia and reperfusion is known to be associated with brain damage induced by ROS (Chan, 1994; Chan, 2001), and can be experimentally reproduced in rodents. Liu et al (Liu et al., 1996) have shown that levels of 8-oxoG in mouse cortical DNA are increased 2–4-fold during a 10–20 min reperfusion, after 30 min of forebrain ischemia. Furthermore, OGG1 protein together with OGG1 repair activity is up-regulated in mouse cortex after forebrain ischemia, while *OGG1* mRNA remained unchanged (Lin et al., 2000).

OGG1-deficient mice develop larger cortical infarcts and behavioral deficits after permanent middle cerebral artery occlusion compared with wild-type mice, with increased accumulation of oxidative DNA base lesions (8-oxoG, Fapy-G) in nuclear DNA of the brain (Liu et al., 2011), indicating that OGG1 protects neurons under ischemic conditions.

OGG1-deficient mice exhibit enhanced post-natal neurodevelopmental deficits, including a deficit in learning and memory, and moreover, when exposed *in utero* to ethanol, OGG1-deficient progeny exhibit higher levels of 8-oxoG in fetal brain and more severe postnatal neurodevelopmental deficits than wild-type littermates. These results suggest that increased 8-oxoG accumulation in fetal brain contributes to neurodevelopmental deficits caused by *in utero* ethanol exposure, and that OGG1 plays a role in protecting the developing brain against such insults (Miller-Pinsler et al., 2015).

5. Molecular mechanisms of neurodegeneration induced by 8-oxoG accumulated in DNA

Observations in neurodegenerative postmortem brains and studies using animal models for various neurodegenerative diseases have revealed that 8-oxoG accumulation in nuclear or mitochondrial DNA in neurons under oxidative conditions somehow results in neurodegeneration, and that MTH1 and OGG1, but not MUTYH, protect neurons by preventing 8-oxoG build-up. Instead, MUTYH promotes neuronal cell death, especially during 3-NP-induced striatal degeneration in MTH1 and/or OGG1-deficient animals (Sheng et al., 2012).

Oka *et al*. (Oka et al., 2008) demonstrated that accumulation of 8-oxoG in nuclear and mitochondrial DNA triggers two distinct cell death pathways that are independent of each other. Both pathways are initiated by the accumulation of MUTYH-generated single-strand breaks (SSBs) in nuclear or mitochondrial DNA (Figure 2A). When 8-oxoG accumulates to high levels in nuclear DNA, PARP binds to the SSBs generated by MUTYH-initiated BER. This increases poly(ADP-ribose) polymer resulting in nicotinamide

adenine dinucleotide (NAD^+) and ATP depletion, followed by nuclear translocation of apoptosis-inducing factor (AIF). AIF then executes apoptotic cell death. Thus, 8-oxoG accumulation at high levels in mitochondrial DNA causes degradation of mitochondrial DNA through MUTYH-initiated BER, resulting in mitochondrial dysfunction and activation of calpains, which in turn cause lysosomal rupture and cell death (Oka and Nakabeppu, 2011).

In the early phase of 3-NP-induced striatal degeneration, MTH1 and/or OGG1-deficient medium spiny neurons accumulate high levels of 8-oxoG and SSBs in mitochondrial DNA in an MUTYH-dependent manner, resulting in calpain activation and neuronal death. In the later phase, dead neurons or damaged neurons activate microglia, which produce ROS, and activated microglia consequently accumulate high levels of 8-oxoG and SSBs in nuclear DNA. In activated microglia, accumulated SSBs in nuclear DNA cause activation of the PARP-AIF pathway in a MUTYH-dependent manner, thus exacerbating microgliosis and neurodegeneration (Sheng et al., 2012) (Figure 2B).

It has been observed that under conditions of oxidative stress, 8-oxoG significantly builds-up in mitochondrial DNA, but not nuclear DNA, in neurons, and that 8-oxoG accumulation in mitochondrial DNA is efficiently suppressed by increased expression of MTH1 (De Luca et al., 2008; Leon et al., 2016; Sheng et al., 2012). These observations indicate that 8-oxoG accumulation in mitochondrial DNA is derived from high levels of 8-oxo-dGTP in the nucleotide pool under oxidative conditions, and not from direct oxidation of guanine in DNA, because only mitochondrial DNA and not nuclear DNA is replicating in post-mitotic neurons. Nonetheless, microglial proliferation can be induced during inflammatory responses in the brain with increased production of ROS, such that microglia accumulate 8-oxoG in nuclear DNA.

Administration of a calpain or PARP inhibitor significantly ameliorated 3-NP-induced striatal degeneration and decreased microgliosis in MTH1/OGG1-deficient mice, indicating that calpain-dependent neuronal death causes microgliosis, and that microgliosis indeed exacerbates neurodegeneration (Sheng et al., 2012). It is noteworthy that

activation of calpain and PARP is a hallmark of neurodegeneration under oxidative conditions, not only in animal models, but also in brains of patients with neurodegeneration (Esteves et al., 2010; Gladding et al., 2012; Kauppinen and Swanson, 2007; Martire et al., 2015; Saito et al., 1993; Yamashima, 2013). Calpain and PARP are therefore considered to be general therapeutic targets for various neurodegenerative diseases. Accumulation of 8-oxoG in neurons and microglia in the brain should be considered an activator of calpain and PARP in neurodegenerative disorders. Thus, proteins involved in BER and related DNA metabolic events should be considered novel therapeutic targets.

BER is a complex mechanism for repair of various base lesions in DNA, and there are many other factors playing essential roles during BER, such as AP endonucleases, DNA polymerases, Fen1, XRCC1, and DNA ligase and so on (Iyama and Wilson, 2013). DNA polymerase β deficiency in 3xTg-AD mice is reported to exacerbate AD pathology (Sykora et al., 2015), altered stoichiometry of BER proteins, especially FEN1 and DNA polymerase β correlates with increased somatic CAG instability in striatum over cerebellum in HD model mice (Goula et al., 2009), and DNA polymerase β may mediate MPTP-induced neuronal death in PD model (Zhang et al., 2014). These findings indicate that BER system is involved in neurodegenerative process in different manners, and further study is required to delineate the entire roles of BER in neurodegeneration.

References

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

Acevedo-Torres, K., Berrios, L., Rosario, N., Dufault, V., Skatchkov, S., Eaton, M. J., Torres-Ramos, C. A., and Ayala-Torres, S. (2009) Mitochondrial DNA damage is a hallmark of chemically induced and the R6/2 transgenic model of Huntington's

disease. *DNA Repair*, *8*, pp. 126-136.

Al-Tassan, N., Chmiel, N. H., Maynard, J., Fleming, N., Livingston, A. L., Williams, G. T., Hodges, A. K., Davies, D. R., David, S. S., Sampson, J. R.*, et al.* (2002) Inherited variants of MYH associated with somatic G:C-->T:A mutations in colorectal tumors. *Nat. Genet.*, *30*, pp. 227-232.

Alam, Z. I., Jenner, A., Daniel, S. E., Lees, A. J., Cairns, N., Marsden, C. D., Jenner, P., and Halliwell, B. (1997) Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *J. Neurochem.*, *69*, pp. 1196-1203.

Aliev, G., Seyidova, D., Lamb, B. T., Obrenovich, M. E., Siedlak, S. L., Vinters, H. V., Friedland, R. P., LaManna, J. C., Smith, M. A., and Perry, G. (2003) Mitochondria and vascular lesions as a central target for the development of Alzheimer's disease and Alzheimer disease-like pathology in transgenic mice. *Neurol. Res.*, *25*, pp. 665-674.

Arai, T., Fukae, J., Hatano, T., Kubo, S., Ohtsubo, T., Nakabeppu, Y., Mori, H., Mizuno, Y., and Hattori, N. (2006) Up-regulation of hMUTYH, a DNA repair enzyme, in the mitochondria of substantia nigra in Parkinson's disease. *Acta Neuropathol.*, *112*, pp. 139-145.

Ayala-Pena, S. (2013) Role of oxidative DNA damage in mitochondrial dysfunction and Huntington's disease pathogenesis. *Free Radic. Biol. Med.*, *62*, pp. 102-110.

Bo, H., Kang, W., Jiang, N., Wang, X., Zhang, Y., and Ji, L. L. (2014) Exercise-induced neuroprotection of hippocampus in APP/PS1 transgenic mice via upregulation of mitochondrial 8-oxoguanine DNA glycosylase. *Oxid. Med. Cell. Longev.*, *2014*, pp. 834502.

Boiteux, S., and Radicella, J. P. (2000) The human *OGG1* gene: structure, functions, and its implication in the process of carcinogenesis. *Arch. Biochem. Biophys.*, *377*, pp. 1-8.

Bradley-Whitman, M. A., Timmons, M. D., Beckett, T. L., Murphy, M. P., Lynn, B. C., and Lovell, M. A. (2014) Nucleic acid oxidation: an early feature of Alzheimer's disease.

J. Neurochem., *128*, pp. 294-304.

Brouillet, E. (1999) Replicating Huntington's disease phenotype in experimental animals. *Prog. Neurobiol.*, *59*, pp. 427-468.

Browne, S. E., Bowling, A. C., MacGarvey, U., Baik, M. J., Berger, S. C., Muqit, M. M., Bird, E. D., and Beal, M. F. (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann. Neurol.*, *41*, pp. 646-653.

Cadet, J., Douki, T., and Ravanat, J. L. (2010) Oxidatively generated base damage to cellular DNA. *Free Radic. Biol. Med.*, *49*, pp. 9-21.

Cardozo-Pelaez, F., Sanchez-Contreras, M., and Nevin, A. B. (2012) *Ogg1* null mice exhibit age-associated loss of the nigrostriatal pathway and increased sensitivity to MPTP. *Neurochem. Int.*, *61*, pp. 721-730.

Chan, P. H. (1994) Oxygen Radicals in Focal Cerebral Ischemia. *Brain Pathology*, *4*, pp. 59-65.

Chan, P. H. (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. *J .Cereb. Blood Flow Metab.*, *21*, pp. 2-14.

Cobb, C. A., and Cole, M. P. (2015) Oxidative and nitrative stress in neurodegeneration. *Neurobiol. Dis.*, 84, pp. 4-21.

Cooke, M. S., Evans, M. D., Dizdaroglu, M., and Lunec, J. (2003) Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.*, *17*, pp. 1195-1214.

Coppede, F., Mancuso, M., Lo Gerfo, A., Manca, M. L., Petrozzi, L., Migliore, L., Siciliano, G., and Murri, L. (2007) A Ser326Cys polymorphism in the DNA repair gene *hOGG1* is not associated with sporadic Alzheimer's disease. *Neurosci. Lett.*, *414*, pp. 282-285.

Coppede, F., Migheli, F., Ceravolo, R., Bregant, E., Rocchi, A., Petrozzi, L., Unti, E., Lonigro, R., Siciliano, G., and Migliore, L. (2010) The *hOGG1* Ser326Cys polymorphism and Huntington's disease. *Toxicology*, *278*, pp. 199-203.

Coppede, F., and Migliore, L. (2015) DNA damage in neurodegenerative diseases. *Mutat.*

Res., *776*, pp. 84-97.

Dasuri, K., Zhang, L., and Keller, J. N. (2013) Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radic. Biol. Med.*, *62*, pp. 170-185.

Dauer, W., and Przedborski, S. (2003) Parkinson's disease: mechanisms and models. *Neuron*, *39*, pp. 889-909.

de la Monte, S. M., Luong, T., Neely, T. R., Robinson, D., and Wands, J. R. (2000) Mitochondrial DNA damage as a mechanism of cell loss in Alzheimer's disease. *Lab. Invest.*, *80*, pp. 1323-1335.

De Luca, G., Russo, M. T., Degan, P., Tiveron, C., Zijno, A., Meccia, E., Ventura, I., Mattei, E., Nakabeppu, Y., Crescenzi, M.*, et al.* (2008) A role for oxidized DNA precursors in Huntington's disease-like striatal neurodegeneration. *PLoS Genet.*, *4*, pp. e1000266.

Dehmer, T., Lindenau, J., Haid, S., Dichgans, J., and Schulz, J. B. (2000) Deficiency of inducible nitric oxide synthase protects against MPTP toxicity *in vivo*. *J. Neurochem.*, *74*, pp. 2213-2216.

Dherin, C. (1999) Excision of oxidatively damaged DNA bases by the human alpha-hOgg1 protein and the polymorphic α-hOgg1(Ser326Cys) protein which is frequently found in human populations. *Nucl. Acids Res.*, *27*, pp. 4001-4007.

Dizdaroglu, M., and Jaruga, P. (2012) Mechanisms of free radical-induced damage to DNA. *Free Radic. Res.*, *46*, pp. 382-419.

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., Marshall, F. J., Ravina, B. M., Schifitto, G., Siderowf, A.*, et al.* (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, *68*, pp. 384-386.

Duffy, A. M., and Holscher, C. (2013) The incretin analogue D-Ala^{2}GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. *Neuroscience*, *228*, pp. 294-300.

Esteves, A. R., Arduino, D. M., Swerdlow, R. H., Oliveira, C. R., and Cardoso, S. M. (2010) Dysfunctional mitochondria uphold calpain activation: contribution to Parkinson's disease pathology. *Neurobiol. Dis.*, *37*, pp. 723-730.

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

Furuichi, M., Yoshida, M. C., Oda, H., Tajiri, T., Nakabeppu, Y., Tsuzuki, T., and Sekiguchi, M. (1994) Genomic structure and chromosome location of the human *mutT* homologue gene *MTH1* encoding 8-oxo-dGTPase for prevention of A:T to C:G transversion. *Genomics*, *24*, pp. 485-490.

Furuta, A., Iida, T., Nakabeppu, Y., and Iwaki, T. (2001) Expression of hMTH1 in the hippocampi of control and Alzheimer's disease. *Neuroreport*, *12*, pp. 2895-2899.

Gabbita, S. P., Lovell, M. A., and Markesbery, W. R. (1998) Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J. Neurochem.*, *71*, pp. 2034-2040.

Gabbita, S. P., Lovell, M. A., and Markesbery, W. R. (2002) Increased Nuclear DNA Oxidation in the Brain in Alzheimer's Disease. *J. Neurochem.*, *71*, pp. 2034-2040.

Gladding, C. M., Sepers, M. D., Xu, J., Zhang, L. Y., Milnerwood, A. J., Lombroso, P. J., and Raymond, L. A. (2012) Calpain and STriatal-Enriched protein tyrosine phosphatase (STEP) activation contribute to extrasynaptic NMDA receptor localization in a Huntington's disease mouse model. *Hum. Mol. Genet.*, *21*, pp. 3739-3752.

Goula, A. V., Berquist, B. R., Wilson, D. M., 3rd, Wheeler, V. C., Trottier, Y., and Merienne, K. (2009) Stoichiometry of base excision repair proteins correlates with increased somatic CAG instability in striatum over cerebellum in Huntington's disease transgenic mice. *PLoS Genet*, *5*, pp. e1000749.

Hill, J. W., and Evans, M. K. (2006) Dimerization and opposite base-dependent catalytic impairment of polymorphic S326C OGG1 glycosylase. *Nucl. Acids Res.*, *34*, pp.

1620-1632.

Hill, J. W., Hu, J. J., and Evans, M. K. (2008) OGG1 is degraded by calpain following oxidative stress and cisplatin exposure. *DNA Repair*, *7*, pp. 648-654.

Hoang, T., Choi, D. K., Nagai, M., Wu, D. C., Nagata, T., Prou, D., Wilson, G. L., Vila, M., Jackson-Lewis, V., Dawson, V. L.*, et al.* (2009) Neuronal NOS and cyclooxygenase-2 contribute to DNA damage in a mouse model of Parkinson disease. *Free Radic. Biol. Med.*, *47*, pp. 1049-1056.

Hokama, M., Oka, S., Leon, J., Ninomiya, T., Honda, H., Sasaki, K., Iwaki, T., Ohara, T., Sasaki, T., LaFerla, F. M.*, et al.* (2014) Altered expression of diabetes-related genes in Alzheimer's disease brains: the hisayama study. *Cereb. Cortex*, *24*, pp. 2476-2488.

Hwang, B. J., Jin, J., Gunther, R., Madabushi, A., Shi, G., Wilson, G. M., and Lu, A. L. (2015) Association of the Rad9-Rad1-Hus1 checkpoint clamp with MYH DNA glycosylase and DNA. *DNA Repair*, *31*, pp. 80-90.

Iida, T., Furuta, A., Nishioka, K., Nakabeppu, Y., and Iwaki, T. (2002) Expression of 8-oxoguanine DNA glycosylase is reduced and associated with neurofibrillary tangles in Alzheimer's disease brain. *Acta Neuropathol.*, *103*, pp. 20-25.

Iyama, T., and Wilson, D. M., 3rd (2013) DNA repair mechanisms in dividing and non-dividing cells. *DNA Repair*, *12*, pp. 620-636.

Izuo, N., Nojiri, H., Uchiyama, S., Noda, Y., Kawakami, S., Kojima, S., Sasaki, T., Shirasawa, T., and Shimizu, T. (2015) Brain-Specific Superoxide Dismutase 2 Deficiency Causes Perinatal Death with Spongiform Encephalopathy in Mice. *Oxid. Med. Cell. Longev.*, *2015*, pp. 238914.

Jacob, K. D., Noren Hooten, N., Tadokoro, T., Lohani, A., Barnes, J., and Evans, M. K. (2013) Alzheimer's disease-associated polymorphisms in human OGG1 alter catalytic activity and sensitize cells to DNA damage. *Free Radic. Biol. Med.*, *63*, pp. 115-125.

Jaiswal, M., LaRusso, N. F., Nishioka, N., Nakabeppu, Y., and Gores, G. J. (2001) Human

Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide. *Cancer Res.*, *61*, pp. 6388-6393.

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

Jiang, G., Xu, L., Wang, L., Song, S., and Zhu, C. (2008) Association study of human MTH1 Ile45Thr polymorphism with sporadic Parkinson's disease. *Eur. Neurol.*, *59*, pp. 15-17.

Jones, S., Emmerson, P., Maynard, J., Best, J. M., Jordan, S., Williams, G. T., Sampson, J. R., and Cheadle, J. P. (2002) Biallelic germline mutations in *MYH* predispose to multiple colorectal adenoma and somatic G:C-->T:A mutations. *Hum. Mol. Genet.*, *11*, pp. 2961-2967.

Kang, D., Takeshige, K., Sekiguchi, M., and Singh, K. (1999) Introduction. In Mitochondrial DNA mutations in aging, disease and cancer, Singh, K., eds. (Springer-Verlag: Berlin) pp. 1-15.

Kasai, H., and Nishimura, S. (1984) Hydroxylation of Deoxyguanosine at the C-8 Position by Ascorbic-Acid and Other Reducing Agents. *Nucl. Acids Res.*, *12*, pp. 2137-2145.

Kauppinen, T. M., and Swanson, R. A. (2007) The role of poly(ADP-ribose) polymerase-1 in CNS disease. *Neuroscience*, *145*, pp. 1267-1272.

Kimura, Y., Oda, S., Egashira, A., Kakeji, Y., Baba, H., Nakabeppu, Y., and 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*, pp. e57.

Knopperts, A. P., Nielsen, M., Niessen, R. C., Tops, C. M., Jorritsma, B., Varkevisser, J., Wijnen, J., Siezen, C. L., Heine-Broring, R. C., van Kranen, H. J.*, et al.* (2013) Contribution of bi-allelic germline *MUTYH* mutations to early-onset and familial colorectal cancer and to low number of adenomatous polyps: case-series and literature review. *Fam. Cancer*, *12*, pp. 43-50.

Kohno, T., Sakiyama, T., Kunitoh, H., Goto, K., Nishiwaki, Y., Saito, D., Hirose, H., Eguchi,

T., Yanagitani, N., Saito, R., *et al.* (2006) Association of polymorphisms in the MTH1 gene with small cell lung carcinoma risk. *Carcinogenesis*, *27*, pp. 2448-2454.

Kovtun, I. V., Liu, Y., Bjoras, M., Klungland, A., Wilson, S. H., and McMurray, C. T. (2007) OGG1 initiates age-dependent CAG trinucleotide expansion in somatic cells. *Nature*, *447*, pp. 447-452.

Leandro, G. S., Lobo, R. R., Oliveira, D. V., Moriguti, J. C., and Sakamoto-Hojo, E. T. (2013) Lymphocytes of patients with Alzheimer's disease display different DNA damage repair kinetics and expression profiles of DNA repair and stress response genes. *Int. J. Mol. Sci.*, *14*, pp. 12380-12400.

Leon, J., Sakumi, K., Castillo, E., Sheng, Z., Oka, S., and Nakabeppu, Y. (2016) 8-Oxoguanine accumulation in mitochondrial DNA causes mitochondrial dysfunction and impairs neuritogenesis in cultured adult mouse cortical neurons under oxidative conditions. *Sci. Rep.*, *6*, pp. 22086.

Lillenes, M. S., Stoen, M., Gomez-Munoz, M., Torp, R., Gunther, C. C., Nilsson, L. N., and Tonjum, T. (2013) Transient OGG1, APE1, PARP1 and Polβ expression in an Alzheimer's disease mouse model. *Mech. Ageing Dev.*, *134*, pp. 467-477.

Lin, L. H., Cao, S., Yu, L., Cui, J., Hamilton, W. J., and Liu, P. K. (2000) Up-regulation of base excision repair activity for 8-hydroxy-2'-deoxyguanosine in the mouse brain after forebrain ischemia-reperfusion. *J. Neurochem.*, *74*, pp. 1098-1105.

Liu, D., Croteau, D. L., Souza-Pinto, N., Pitta, M., Tian, J., Wu, C., Jiang, H., Mustafa, K., Keijzers, G., Bohr, V. A., *et al.* (2011) Evidence that OGG1 glycosylase protects neurons against oxidative DNA damage and cell death under ischemic conditions. *J. Cereb. Blood Flow Metab.*, *31*, pp. 680-692.

Liu, P., Hsu, C., Dizdaroglu, M., Floyd, R., Kow, Y., Karakaya, A., Rabow, L., and Cui, J. (1996) Damage, repair, and mutagenesis in nuclear genes after mouse forebrain ischemia-reperfusion. *J. Neurosci.*, *16*, pp. 6795-6806.

Lovell, M. A., and Markesbery, W. R. (2007) Oxidative DNA damage in mild cognitive

impairment and late-stage Alzheimer's disease. *Nucl. Acids Res.*, *35*, pp. 7497-7504.

Lovell, M. A., Soman, S., and Bradley, M. A. (2011) Oxidatively modified nucleic acids in preclinical Alzheimer's disease (PCAD) brain. *Mech. Ageing Dev.*, *132*, pp. 443-448.

Luncsford, P. J., Manvilla, B. A., Patterson, D. N., Malik, S. S., Jin, J., Hwang, B. J., Gunther, R., Kalvakolanu, S., Lipinski, L. J., Yuan, W.*, et al.* (2013) Coordination of MYH DNA glycosylase and APE1 endonuclease activities via physical interactions. *DNA Repair*, *12*, pp. 1043-1052.

Lyras, L., Cairns, N. J., Jenner, A., Jenner, P., and Halliwell, B. (1997) An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. *J. Neurochem.*, *68*, pp. 2061-2069.

Lyras, L., Cairns, N. J., Jenner, A., Jenner, P., and Halliwell, B. (2002) An Assessment of Oxidative Damage to Proteins, Lipids, and DNA in Brain from Patients with Alzheimer's Disease. *J. Neurochem.*, *68*, pp. 2061-2069.

MacKenna, M., Dienel, G., Sonnewald, U., HS, W., and A, S. (2012) Energy Metaboilsm of the Brain. In Basic. Neurochemistry, Brady, S., and Siegel, G., eds. (Academic Press: Waltham, MA, USA) pp. 200-231.

Mao, G., Pan, X., Zhu, B. B., Zhang, Y., Yuan, F., Huang, J., Lovell, M. A., Lee, M. P., Markesbery, W. R., Li, G. M.*, et al.* (2007) Identification and characterization of *OGG1* mutations in patients with Alzheimer's disease. *Nucl. Acids Res.*, *35*, pp. 2759-2766.

Martire, S., Mosca, L., and d'Erme, M. (2015) PARP-1 involvement in neurodegeneration: A focus on Alzheimer's and Parkinson's diseases. *Mech. Ageing Dev.*, *146-148*, pp. 53-64.

McBrayer, M., and Nixon, R. A. (2013) Lysosome and calcium dysregulation in Alzheimer's disease: partners in crime. *Biochem. Soc. Trans.*, *41*, pp. 1495-1502.

Mecocci, P., MacGarvey, U., and Beal, M. F. (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.*, *36*, pp. 747-751.

Miller-Pinsler, L., Pinto, D. J., and Wells, P. G. (2015) Oxidative DNA damage in the in utero initiation of postnatal neurodevelopmental deficits by normal fetal and ethanol-enhanced oxidative stress in oxoguanine glycosylase 1 knockout mice. *Free Radic. Biol. Med.*, *78*, pp. 23-29.

Moore, D. J., West, A. B., Dawson, V. L., and Dawson, T. M. (2005) Molecular pathophysiology of Parkinson's disease. *Annu. Rev. Neurosci.*, *28*, pp. 57-87.

Moritz, E., Pauly, K., Bravard, A., Hall, J., Radicella, J. P., and Epe, B. (2014) hOGG1-Cys326 variant cells are hypersensitive to DNA repair inhibition by nitric oxide. *Carcinogenesis*, *35*, pp. 1426-1433.

Morreall, J., Limpose, K., Sheppard, C., Kow, Y. W., Werner, E., and Doetsch, P. W. (2015) Inactivation of a common OGG1 variant by TNF-alpha in mammalian cells. *DNA Repair*, *26*, pp. 15-22.

Nakabeppu, Y. (2014) Cellular Levels of 8-Oxoguanine in either DNA or the Nucleotide Pool Play Pivotal Roles in Carcinogenesis and Survival of Cancer Cells. *Int. J. Mol. Sci.*, *15*, pp. 12543-12557.

Nakabeppu, Y., Oka, S., Sheng, Z., Tsuchimoto, D., and Sakumi, K. (2010) Programmed cell death triggered by nucleotide pool damage and its prevention by MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase. *Mutat. Res.*, *703*, pp. 51-58.

Nakabeppu, Y., Tsuchimoto, D., Yamaguchi, H., and Sakumi, K. (2007) Oxidative damage in nucleic acids and Parkinson's disease. *J. Neurosci. Res.*, *85*, pp. 919-934.

Nishioka, K., Ohtsubo, T., Oda, H., Fujiwara, T., Kang, D., Sugimachi, K., and 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*, pp. 1637-1652.

Nunnari, J., and Suomalainen, A. (2012) Mitochondria: in sickness and in health. *Cell*, *148*, pp. 1145-1159.

Oda, H., Nakabeppu, Y., Furuichi, M., and Sekiguchi, M. (1997) Regulation of expression of

the human *MTH1* gene encoding 8-oxo-dGTPase. Alternative splicing of transcription products. *J. Biol. Chem.*, *272*, pp. 17843-17850.

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

Ohno, M., Sakumi, K., Fukumura, R., Furuichi, M., Iwasaki, Y., Hokama, M., Ikemura, T., Tsuzuki, T., Gondo, Y., and Nakabeppu, Y. (2014) 8-oxoguanine causes spontaneous de novo germline mutations in mice. *Sci. Rep.*, *4*, pp. 4689.

Ohtsubo, T., Nishioka, K., Imaiso, Y., Iwai, S., Shimokawa, H., Oda, H., Fujiwara, T., and 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. *Nucl. Acids Res.*, *28*, pp. 1355-1364.

Oka, S., and Nakabeppu, Y. (2011) DNA glycosylase encoded by MUTYH functions as a molecular switch for programmed cell death under oxidative stress to suppress tumorigenesis. *Cancer Sci.*, *102*, pp. 677-682.

Oka, S., Ohno, M., Tsuchimoto, D., Sakumi, K., Furuichi, M., and Nakabeppu, Y. (2008) Two distinct pathways of cell death triggered by oxidative damage to nuclear and mitochondrial DNAs. *EMBO J.*, *27*, pp. 421-432.

Parker, A., Gu, Y., Mahoney, W., Lee, S. H., Singh, K. K., and Lu, A. L. (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*, pp. 5547-5555.

Polidori, M. C., Mecocci, P., Browne, S. E., Senin, U., and Beal, M. F. (1999) Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex. *Neurosci. Lett.*, *272*, pp. 53-56.

Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W., and Ferri, C. P. (2013) The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers*

Dement., *9*, pp. 63-75 e62.

Pringsheim, T., Wiltshire, K., Day, L., Dykeman, J., Steeves, T., and Jette, N. (2012) The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. *Mov. Disord.*, *27*, pp. 1083-1091.

Puzzo, D., Gulisano, W., Palmeri, A., and Arancio, O. (2015) Rodent models for Alzheimer's disease drug discovery. *Expert Opin. Drug Discov.*, *10*, pp. 703-711.

Ross, C. A., and Tabrizi, S. J. (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol.*, *10*, pp. 83-98.

Saito, K., Elce, J. S., Hamos, J. E., and Nixon, R. A. (1993) Widespread activation of calcium-activated neutral proteinase (calpain) in the brain in Alzheimer disease: a potential molecular basis for neuronal degeneration. *Proc. Natl. Acad. Sci. USA*, *90*, pp. 2628-2632.

Sakai, Y., Oda, H., Yoshimura, D., Furuichi, M., Kang, D., Iwai, S., Hara, T., and 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*, pp. 660-670.

Sakumi, K., Furuichi, M., Tsuzuki, T., Kakuma, T., Kawabata, S., Maki, H., and Sekiguchi, M. (1993) Cloning and expression of cDNA for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *J. Biol. Chem.*, *268*, pp. 23524-23530.

Sanders, L. H., McCoy, J., Hu, X., Mastroberardino, P. G., Dickinson, B. C., Chang, C. J., Chu, C. T., Van Houten, B., and Greenamyre, J. T. (2014) Mitochondrial DNA damage: molecular marker of vulnerable nigral neurons in Parkinson's disease. *Neurobiol. Dis.*, *70*, pp. 214-223.

Shao, C., Xiong, S., Li, G. M., Gu, L., Mao, G., Markesbery, W. R., and Lovell, M. A. (2008) Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. *Free Radic. Biol. Med.*, *45*, pp. 813-819.

Sheng, Z., Oka, S., Tsuchimoto, D., Abolhassani, N., Nomaru, H., Sakumi, K., Yamada, H., and Nakabeppu, Y. (2012) 8-Oxoguanine causes neurodegeneration during MUTYH-mediated DNA base excision repair. *J. Clin. Invest.*, *122*, pp. 4344-4361.

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

Siddiqui, A., Rivera-Sanchez, S., Castro Mdel, R., Acevedo-Torres, K., Rane, A., Torres-Ramos, C. A., Nicholls, D. G., Andersen, J. K., and Ayala-Torres, S. (2012) Mitochondrial DNA damage is associated with reduced mitochondrial bioenergetics in Huntington's disease. *Free Radic. Biol. Med.*, *53*, pp. 1478-1488.

Sieber, O. M., Lipton, L., Crabtree, M., Heinimann, K., Fidalgo, P., Phillips, R. K., Bisgaard, M. L., Orntoft, T. F., Aaltonen, L. A., Hodgson, S. V.*, et al.* (2003) Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N. Engl. J. Med.*, *348*, pp. 791-799.

Simonelli, V., Camerini, S., Mazzei, F., Van Loon, B., Allione, A., D'Errico, M., Barone, F., Minoprio, A., Ricceri, F., Guarrera, S.*, et al.* (2013) Genotype-phenotype analysis of S326C OGG1 polymorphism: a risk factor for oxidative pathologies. *Free Radic. Biol. Med.*, *63*, pp. 401-409.

Slupska, M., Baikalov, C., Luther, W., Chiang, J., Wei, Y., and Miller, J. (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*, pp. 3885-3892.

Slupska, M., Luther, W., Chiang, J., Yang, H., and Miller, J. (1999) Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. *J. Bacteriol.*, *181*, pp. 6210-6213.

Song, X. N., Zhang, L. Q., Liu, D. G., Lin, J., Zheng, J. D., Dai, D. P., Hei, A. L., Hayakawa, H., Sekiguchi, M., and Cai, J. P. (2011) Oxidative damage to RNA and expression patterns of MTH1 in the hippocampi of senescence-accelerated SAMP8 mice and

Alzheimer's disease patients. *Neurochem. Res.*, *36*, pp. 1558-1565.

Sorolla, M. A., Reverter-Branchat, G., Tamarit, J., Ferrer, I., Ros, J., and Cabiscol, E. (2008) Proteomic and oxidative stress analysis in human brain samples of Huntington disease. *Free Radic. Biol. Med.*, *45*, pp. 667-678.

Sosa-Ortiz, A. L., Acosta-Castillo, I., and Prince, M. J. (2012) Epidemiology of dementias and Alzheimer's disease. *Arch. Med. Res.*, *43*, pp. 600-608.

Stuart, J. A., Mayard, S., Hashiguchi, K., Souza-Pinto, N. C., and Bohr, V. A. (2005) Localization of mitochondrial DNA base excision repair to an inner membrane-associated particulate fraction. *Nucl. Acids Res.*, *33*, pp. 3722-3732.

Sultana, R., Perluigi, M., and Allan Butterfield, D. (2013) Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. *Free Radic. Biol. Med.*, *62*, pp. 157-169.

Sykora, P., Misiak, M., Wang, Y., Ghosh, S., Leandro, G. S., Liu, D., Tian, J., Baptiste, B. A., Cong, W. N., Brenerman, B. M.*, et al.* (2015) DNA polymerase beta deficiency leads to neurodegeneration and exacerbates Alzheimer disease phenotypes. *Nucleic Acids Res*, *43*, pp. 943-959.

Takao, M., Aburatani, H., Kobayashi, K., and Yasui, A. (1998) Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage. *Nucl. Acids Res.*, *26*, pp. 2917-2922.

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

Ventura, I., Russo, M. T., De Nuccio, C., De Luca, G., Degan, P., Bernardo, A., Visentin, S., Minghetti, L., and Bignami, M. (2013) hMTH1 expression protects mitochondria from Huntington's disease-like impairment. *Neurobiol. Dis.*, *49*, pp. 148-158.

Wang, J., Markesbery, W. R., and Lovell, M. A. (2006) Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J. Neurochem.*, *96*,

pp. 825-832.

Wang, J., Xiong, S., Xie, C., Markesbery, W. R., and Lovell, M. A. (2005) Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.*, *93*, pp. 953-962.

Wirdefeldt, K., Adami, H.-O., Cole, P., Trichopoulos, D., and Mandel, J. (2011) Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Euro. J. Epidemiol.*, *26*, pp. 1-58.

Xiong, H., Callaghan, D., Wodzinska, J., Xu, J., Premyslova, M., Liu, Q. Y., Connelly, J., and Zhang, W. (2011) Biochemical and behavioral characterization of the double transgenic mouse model (APPswe/PS1dE9) of Alzheimer's disease. *Neurosci. Bull.*, *27*, pp. 221-232.

Yakushiji, H., Maraboeuf, F., Takahashi, M., Deng, Z. S., Kawabata, S., Nakabeppu, Y., and Sekiguchi, M. (1997) Biochemical and physicochemical characterization of normal and variant forms of human MTH1 protein with antimutagenic activity. *Mutat. Res.*, *384*, pp. 181-194.

Yamaguchi, H., Kajitani, K., Dan, Y., Furuichi, M., Ohno, M., Sakumi, K., Kang, D., and 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*, pp. 551-563.

Yamashima, T. (2013) Reconsider Alzheimer's disease by the 'calpain-cathepsin hypothesis'--a perspective review. *Prog. Neurobiol.*, *105*, pp. 1-23.

Yan, M. H., Wang, X., and Zhu, X. (2013) Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease. *Free Radic. Biol. Med.*, *62*, pp. 90-101.

Zhang, J., Perry, G., Smith, M. A., Robertson, D., Olson, S. J., Graham, D. G., and Montine, T. J. (1999) Parkinson's disease is associated with oxidative damage to cytoplasmic DNA and RNA in substantia nigra neurons. *Am. J. Pathol.*, *154*, pp. 1423-1429.

Zhang, Z., Zhang, Z., Wang, H., Zhang, G., Hu, D., Xiong, J., Xiong, N., Wang, T., Cao, X., and Mao, L. (2014) Proliferating cell nuclear antigen binds DNA polymerase-beta

and mediates 1-methyl-4-phenylpyridinium-induced neuronal death. *PLoS One*, *9*, pp. e106669.

Zhou, P. T., Li, B., Ji, J., Wang, M. M., and Gao, C. F. (2015) A systematic review and meta-analysis of the association between OGG1 Ser326Cys polymorphism and cancers. *Med. Oncol.*, *32*, pp. 472.

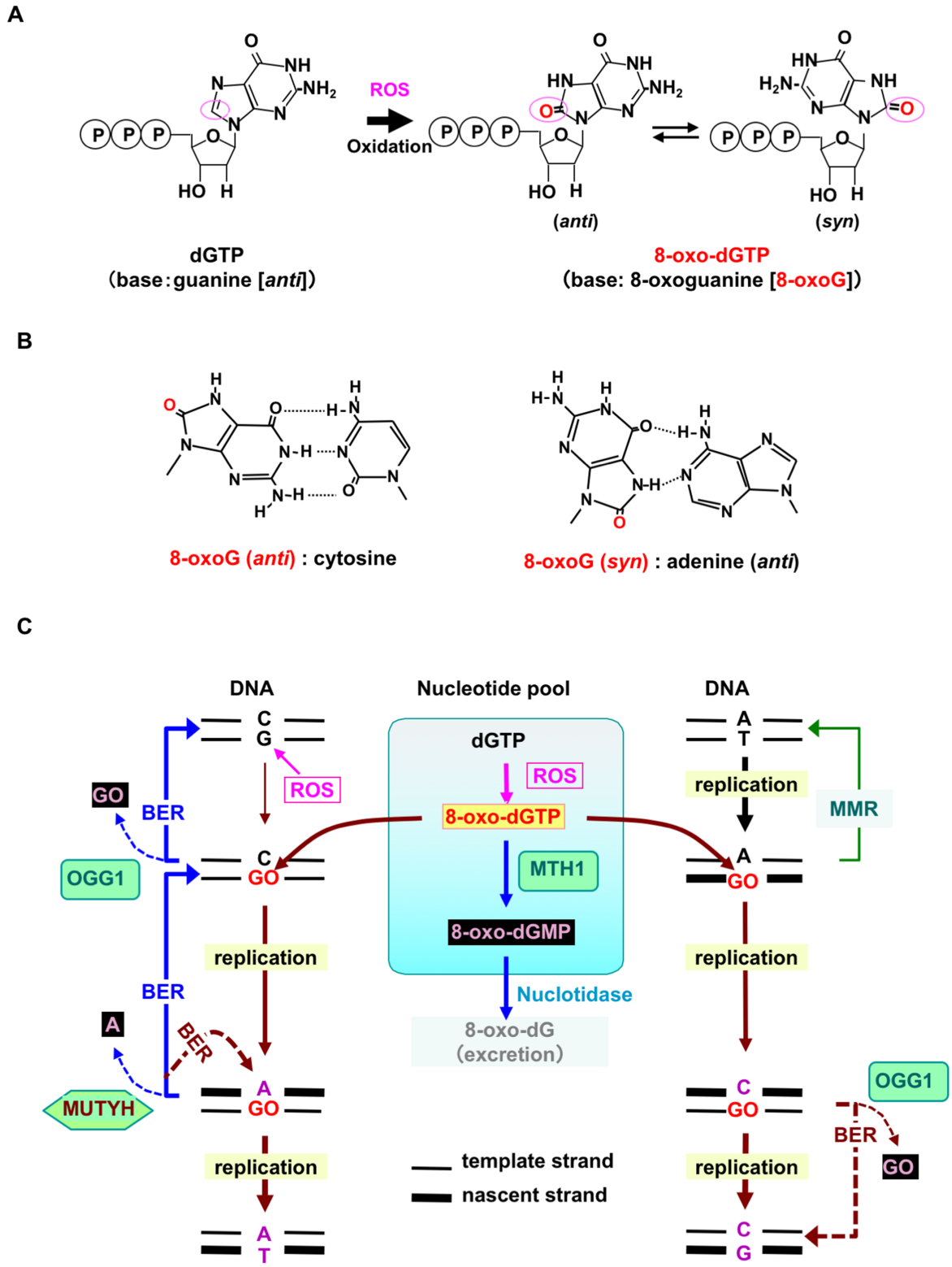

Fig. 1. Oxidation of guanine and defense mechanisms to minimize its accumulation in DNA.

(A) Oxidation of guanine by ROS. ROS preferentially attack free nucleotides (dGTP) compared with guanine bases in DNA, thereby generating 8-oxo-dGTP in the nucleotide pool. dGTP mainly takes the *anti*-configuration; however, 8-oxo-dGTP is in equilibrium between *anti*- and *syn*- configurations.
(B) 8-OxoG (*syn*) forms a base pair with adenine as well as with cytosine in DNA.
(C) Mutagenesis caused by 8-oxoG and mammalian defense systems. 8-oxoG (GO) accumulates in DNA via the incorporation of 8-oxo-dGTP from the nucleotide pool or due to direct oxidation. This outcome increases the occurrence of A:T to C:G or G:C to T:A transversion mutations after two rounds of replication. Dark red line: mutagenic pathway. In the defense systems, MTH1 hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate. 8-oxo-dGMP is further converted to nucleoside, 8-oxodeoxyguanosine (8-oxo-dG), thus preventing its incorporation into DNA. 8-oxoG DNA glycosylase (OGG1) removes 8-oxoG to initiate BER. OGG1 preferentially excises 8-oxoG opposite cytosine. MUTYH excises the adenine inserted opposite 8-oxoG in the template strand. Once cytosine is inserted opposite 8-oxoG during BER initiated by MUTYH, OGG1 can remove the 8-oxoG residue opposite cytosine. However, adenine can be reinserted opposite 8-oxoG during BER (dashed dark red line). In mammals, the mismatch repair (MMR) machinery recognizes 8-oxoG opposite adenine in template DNA and excises the 8-oxoG containing nascent strand (green line). Thus, in the absence of MTH1, OGG1, and MUTYH, only G to T transversion mutations are increased (Ohno et al., 2014). OGG1 may enhance A to C transversions if it excises 8-oxoG opposite a cytosine that had been inserted opposite 8-oxoG paired with the adenine in the template DNA (dashed dark red line). Blue lines: BER pathways.

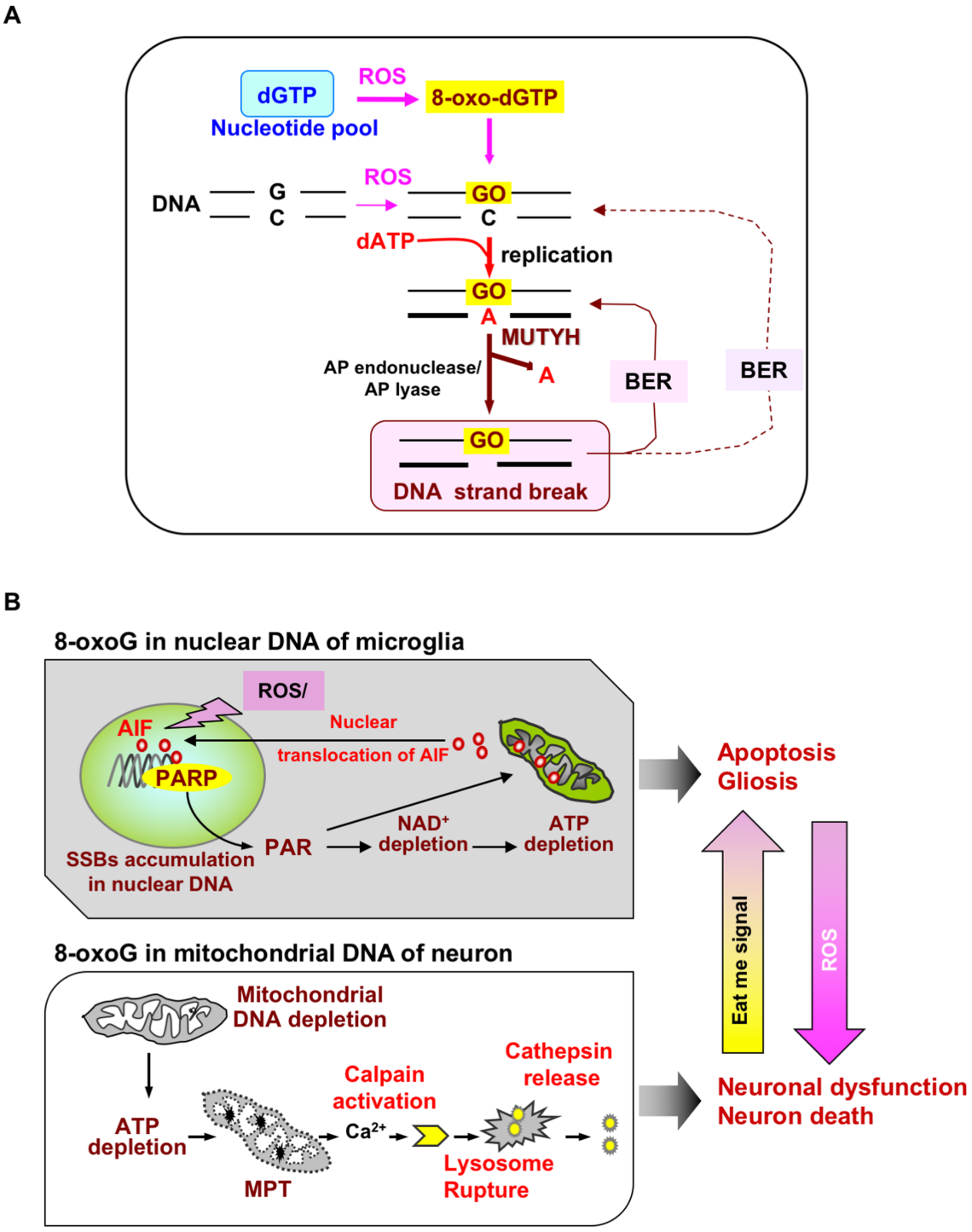

Fig. 2. 8-OxoG causes neurodegeneration during MUTYH-mediated BER.

(A) MUTYH is a molecular switch for programmed cell death under conditions of oxidative stress. Under oxidative conditions, 8-oxoG may be highly accumulated in DNA. During replication, adenine can be inserted opposite 8-oxoG in template DNA, and MUTYH initiates BER by excising the adenine residue. MUTYH-initiated BER might induce a futile

BER cycle (red arrows), because an adenine can be reinserted opposite an 8-oxoG during repair by DNA polymerases, such as POLβ, thus increasing accumulation of SSBs in the nascent strand. 8-OxoG accumulation in nuclear DNA triggers PARP-AIF-dependent apoptosis, while accumulation in mitochondrial DNA causes calpain-dependent cell death, as shown in B.

(B) Two distinct cell death pathways induced by MUTYH-dependent BER cause a complex process of neurodegeneration. When neurons accumulate high levels of 8-oxoG under oxidative conditions, SSBs build-up substantially in mitochondrial DNA in a MUTYH-dependent manner, resulting in calpain activation and neuronal death. “Eat me signals” released from dead or damaged neurons activate microglia, which produce RO/NS. Activated microglia accumulate significant quantities of 8-oxoG in nuclear DNA during their proliferation, and subsequently SSBs in nuclear DNA in a MUTYH-dependent manner, thus activating the PARP-AIF pathway to induce apoptosis. Consequently, microgliosis is further exacerbated resulting in severe neurodegeneration.