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Programmed cell death triggered by nucleotide pool damage and its prevention by MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase

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Abbreviation

8-oxoG: 8-oxoguanine
8-oxo-dGTP: 8-oxo-2’-deoxyguanosine triphosphate
2-OH-A: 2-hydroxyadenine
2-OH-dATP: 2-hydroxy-2’-deoxyadenosine triphosphate
AIF: apoptosis inducing factor
BER: base excision repair
NO: nitric oxide
PARP: poly (ADP-ribose) polymerase
PAR:poly (ADP-ribosy)lation
ROS: reactive oxygen species
SOD: superoxide dismutase
SSBs: single strand breaks

Abstract

Accumulation of oxidized bases such as 8-oxoguanine in either nuclear or mitochondrial DNA triggers various cellular dysfunctions including mutagenesis, and programmed cell death or senescence. Recent studies have revealed that oxidized nucleoside triphosphates such as 8-oxo-dGTP in the nucleotide pool are the main source of oxidized bases accumulating in the DNA of cells under oxidative stress. To counteract such deleterious effects of nucleotide pool damage, mammalian cells possess MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase and related enzymes, thus minimizing the accumulation of oxidized bases in cellular DNA. Depletion or increased expression of the MTH1 protein have revealed its significant roles in avoiding programmed cell death or senescence as well as mutagenesis, and accumulating evidences indicate that MTH1 is involved in suppression of degenerative disorders such as neurodegeneration.

Keywords

8-oxo-dGTP; 2-OH-dATP; nucleotide pool; MTH1; OGG1; MUTYH; programmed cell death

1. Introduction

Cellular components such as lipids, proteins and nucleic acids are at high risk of being oxidized by reactive oxygen species (ROS). ROS are inevitable byproducts of electron transport in the mitochondria or other normal metabolic pathways and are also generated as useful products for various biological processes such as host defense, neurotransmission, vasodilation and signal transduction. Their production is markedly enhanced by various environmental exposures. Such oxidative damage is considered to be a major cause for various types of cellular dysfunction resulting in cell death or mutagenesis, which may in turn cause degenerative disorders and neoplasms [1].

Organisms are equipped with defense mechanisms to minimize the accumulation of ROS. For example, superoxide dismutases convert superoxide to oxygen and hydrogen peroxide and the latter is further detoxified by peroxidases or catalases. Mice lacking the *SOD2* gene encoding mitochondrial superoxide dismutase have severe abnormalities in development and growth, including cardiomyopathy and neurodegeneration [2]. Once excessive ROS accumulates in the cells, these cells can no longer avoid severe oxidative damage. Even in the presence of functional superoxide dismutases, accumulation of oxidized macromolecules in human tissues gradually occurs during normal aging; hence, oxidative damage has been implicated in aging and degenerative disorders and may well be the major cause of these disorders [1].

Among the various types of oxidative damage to cellular macromolecules, damage to nucleic acids is particularly hazardous because of the genetic information present in cellular DNAs (nuclear and mitochondrial), can be altered. Furthermore, oxidized nucleotides can disturb various cellular processes. Such oxidative damage accumulating in cells often results not only in mutagenesis but also in programmed cell death. The former can initiate carcinogenesis in somatic cells, and mutations fixed in germ lines cause genetic polymorphisms or cause hereditary diseases with a malfunction of the gene(s), while the latter often causes degenerative diseases [3-6].

There are two pathways for the accumulation of oxidized bases in cellular DNA or RNA: one is a result of the incorporation of oxidized nucleotides generated in nucleotide pools while the other is a result of the direct oxidation of bases in DNA or RNA [7]. Recent progress in studies of the sanitization of nucleotide pools, as well as DNA repair, has revealed that the impact of oxidation of free nucleotides is

unexpectedly large, in comparison with the direct oxidation of DNA [8]. In this review, we focus on the programmed cell death induced when oxidized purine nucleoside triphosphates are accumulated in the nucleotide pools and how their sanitizing enzyme MTH1 prevents such biological consequence.

2. Oxidation of purine nucleotides and their incorporation into cellular DNA.

Among the nucleobases, guanine is known to be the most susceptible to oxidation and its simple oxidized form, 8-oxoguanine (8-oxoG), is one of the major oxidation products in DNA or nucleotides [9]. *In vitro* exposure of the guanine base to H_2O_2 and ascorbic acid or to Fe_2^+-EDTA generates 8–9 times more 8-oxoG residues in the nucleotide dGTP than in DNA. Interestingly, the C-8 position of dATP is not oxidized in the treatments; instead, the C-2 position of dATP is oxidized, thus yielding 2-hydroxy-2′-deoxyadenosine triphosphate (2-OH-dATP). However, treatment with Fe_2^+-EDTA generates 2-hydroxyadenine (2-OH-A) residues in DNA to as little as 1.5% of the level of 2-OH-A residues that are formed from dATP [10]. Free nucleotides are thus more susceptible to oxidation by ROS than is DNA.

These *in vitro* studies indicated that dGTP is likely to be most susceptible to oxidation by *in vivo* generated ROS, thus generating 8-oxo-dGTP. Although there have been few reports measuring the *in vivo* concentration of 8-oxo-dGTP in the nucleotide pool, it has recently been reported that 8-oxo-dGTP is present at 0.2–2 μM range in the mitochondrial dNTP pools of several rat tissues under normal conditions [11].

It has been established that 8-oxo-dGTP and 2-OH-dATP are frequently misinserted opposite template adenine or guanine, respectively, in DNA by various DNA polymerases for bacterial genomes, and in the nuclear and mitochondrial DNA in mammals, because of their altered base pairing properties [11-18] (Fig. 1A). 8-oxoG pairs with adenine and cytosine at equal efficiency because it prefers the *syn*-form compared with guanine, which takes mostly an *anti*-form and exclusively pairs with cytosine. However, 2-OH-A also can pair with guanine in a *syn*-form in addition to thymine. It has been shown that these oxidized nucleotides indeed increased certain mutations when they were introduced into *E. coli* or mammalian cells [19, 20].

As summarized in Fig. 1B, 8-oxo-dGTP is misinserted opposite template adenine as well as cytosine in DNA, thus causing mainly an A:T to C:G transversion

mutation after two rounds of replication. 2-OH-dATP tends to be misinserted opposite guanine mostly, thus inducing mainly G:C to T:A transversion mutation.

3. MTH1 is a major oxidized purine nucleoside triphosphatase in mammals.

E. coli mutT mutants exhibit the strongest mutator phenotype among all known *E. coli* mutator mutants and the spontaneous occurrence of A:T to C:G transversion mutation increases 1000-fold compared with wild type. Maki and Sekiguchi demonstrated that the MutT protein hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate, thus sanitizing the nucleotide pool [12]. The MutT protein also efficiently hydrolyzes 8-oxo-GTP and *mutT* mutants accumulate 8-oxoG in DNA and mRNA; 8-oxoG in the latter also results in the production of mutant proteins [21]. The *E. coli* Orf135 protein hydrolyzes 2-OH-dATP [22] and its mutants exhibit a 2-fold increase in the spontaneous occurrence of A:T to C:G transversion. The introduction of 2-OH-dATP, but not 8-oxo-dGTP or other nucleotides, into Orf135 mutants, specifically increases the mutation frequency compared with wild type [23]. MutT and Orf135 proteins share the nudix (nucleoside diphosphate linked moiety X) motif corresponding to the 23 residues from Gly37 to Gly59 of *E. coli* MutT, which constitute the phosphohydrolase module for hydrolysis of phosphodiester bonds of the substrates [24, 25].

We have identified a human homolog of the MutT protein and designated it as MTH1 (MutT homolog-1) [26-28]. However, it is now referred to as NUDT1 because it is the first identified protein with the nudix motif protein in eukaryotes. In contrast to MutT, MTH1 efficiently hydrolyzes two forms of oxidized dATP, 2-OH-dATP and 8-oxo-dATP, as well as 8-oxo-dGTP. It also hydrolyzes the corresponding ribonucleotides, 2-OH-ATP, 8-oxo-GTP and 8-oxo-ATP. Among these, MTH1 has the highest affinity to 2-OH-ATP (Km = 4.3 μM), while the highest catalytic efficiency was observed in 2-OH-dATP (kcat/Km = 1.68 $s^{-1}\mu M^{-1}$) [29, 30]. We determined the solution structure of MTH1 by multi-dimensional heteronuclear NMR spectroscopy [31]. The protein adopts a highly similar folding pattern to *E. coli* MutT, despite the low sequence similarity outside the conserved nudix motif [32]. The substrate binding pockets are dissimilar, which might account for the different substrate specificities observed for the two enzymes [33]. Based on the arrangement of the pocket-forming residues, combined with the mutagenesis data, we generated models for the substrate recognition of MTH1 in which Asn-33 and Asp-119 play

pivotal roles in discriminating the oxidized form of the purine, namely 8-oxoG and 2-OH-A, while Trp-117 is important for determining the affinity with purine rings [34, 35]. Among known proteins with the nudix motif, two other mammalian proteins, MTH2 (NUDT15) and NUDT5, were identified with the potential to hydrolyze either 8-oxo-dGTP or 8-oxo-(d)GDP to 8-oxo-(d)GMP, respectively [36-38]. NUDT5 also hydrolyzes 8-oxo-dADP and to a lesser extent 2-OH-dADP [39]. The discovery of NUDT5 with 8-oxo-(d)GDPase activity, further revealed that MTH1 and MutT can both hydrolyze 8-oxo-GDP [38, 40]. MTH1 also recognizes oxidized forms of dATP and ATP as mentioned above. Therefore, we expect that their diphosphate forms can be hydrolyzed by MTH1, suggesting that MTH1 is the most powerful enzyme for the sanitization of nucleotide pools [8] (Fig. 1B). Gene knockdown experiments for MTH1, MTH2 and NUDT5 in cultured human cells revealed that MTH1 deficiency induced an increased occurrence of A:T to C:G transversion mutations when 8-oxo-dGTP was introduced into cells [41].

4. MTH1 deficiency increases susceptibility to cellular dysfunction caused by ROS.

We reported that lung adenomas/carcinomas developed spontaneously in 8-oxoG DNA glycosylase 1 (OGG1)-null mice at about 1.5 years after birth, and that 8-oxoG was highly accumulated in their genomes because of the lack of excision repair of 8-oxoG [42]. In that study, we found that no tumor was formed in the lungs of mice lacking both the OGG1 and MTH1 proteins, despite an increased accumulation of 8-oxoG in these mice. This observation suggests that *Mth1* gene disruption resulted in a suppression of the tumorigenesis caused by an OGG1 deficiency. If cell death is caused by the accumulation of a large amount of oxidized purine nucleoside triphosphates in nucleotide pools with MTH1 deficiency, in addition to the accumulation of 8-oxoG in cellular DNA because of the OGG1 deficiency, then cells with premutagenic lesions might not survive to produce precancerous cells with mutations in either proto-oncogenes or tumor suppressor genes. This might be why carcinogenesis is suppressed in mice lacking both the OGG1 and MTH1 proteins [43].

We have demonstrated that MTH1-null mouse embryo fibroblasts (MEF) are highly susceptible to cell dysfunction and death caused by exposure to H_2O_2, with condensed nuclei and degenerated mitochondria in which electron dense deposits

were seen in place of intact cristae [44]. The cell death observed was not dependent on either poly (ADP-ribose) polymerase or caspases. A continuous accumulation of 8-oxoG, both in the nuclear and mitochondrial DNA, was observed after exposure to H_2O_2. All of the H_2O_2-induced alterations observed in MTH1-null MEFs were effectively suppressed by the expression of wild-type human MTH1 (hMTH1), while they were only partially suppressed by the expression of mutant hMTH1 which possessed either 8-oxo-dGTPase or 2-OH-dATPase activity. MTH1 thus protects the cells from H_2O_2-induced cell dysfunction and death by hydrolyzing oxidized purine nucleotides.

It has been shown that hMTH1 depletion in p53-proficient human cancer-derived or SV40-transformed cell lines promotes H_2O_2-induced apoptosis through a Noxa- and caspase-3/7- mediated signaling pathway [45]. In contrast, hMTH1 depletion in primary human cells results in rapid cellular senescence with an increased accumulation of 8-oxoG in genomic DNA and upregulation of tumor suppressor genes including *p53*, especially under high oxygen tension (20%) [46]. In both cases, nuclear accumulation of γ-H2AX immunoreactivity was observed, suggesting that incorporation of 8-oxoG into nuclear DNA results in double-strand breaks, thus inducing p53-dependent responses. These results indicate that the nucleotide pool is a critical target of intracellular ROS and that oxidized nucleotides, unless continuously eliminated, can rapidly induce programmed cell death or senescence [8].

5. Two distinct pathways of cell death are triggered by 8-oxoG accumulating in nuclear and mitochondrial DNAs.

Under oxidative stress conditions, generation of 8-oxo-dGTP in the nucleotide pool as well as direct oxidation of guanine in DNA results in the increased accumulation of 8-oxoG in nuclear and mitochondrial DNAs [44, 47], thus inducing programmed cell death or senescence (Fig. 2). However, it is not clear which form of DNA is involved—nuclear or mitochondrial—or how such programmed processes are executed. To distinguish the biological effects of 8-oxoG accumulation in nuclear or mitochondrial DNA, we established cells that accumulate 8-oxoG selectively in either type of DNA by expression of a nuclear or mitochondrial form of human OGG1 proteins. These selectively excise 8-oxoG opposite cytosines in DNA in OGG1-null mouse cells [48, 49]. The increased accumulation of 8-oxoG in nuclear DNA caused

poly-(ADP-ribose) polymerase (PARP)-dependent nuclear translocation of apoptosis-inducing factor (AIF). On the other hand, the increased accumulation of 8-oxoG in mitochondrial DNA caused mitochondrial dysfunction followed by Ca^{2+} efflux and activation of calpains. Both types of cell death were accompanied by increased accumulation of single-strand breaks (SSBs) in the respective DNAs. These were suppressed by knockdown of MUTYH that excises adenine inserted opposite 8-oxoG in DNA during replication, thus initiating base excision repair (BER). Recently, it has been shown that DNA polymerase λ efficiently insert cytosine opposite 8-oxoG after adenine excision by MUTYH, thus ensuring the faithful repair of A;8-oxoG mispairs [50]. Under increased accumulation of 8-oxoG in template DNA, however, MUTYH might induce futile BER because an adenine can be reinserted opposite an 8-oxoG during BER, thus causing accumulation of SSBs in the nascent strand [51] (Fig. 2A). Knockdown of MUTYH resulted in escape from both types of cell death, indicating that MUTYH functions as a molecular switch for the two types of programmed cell death when 8-oxoG accumulates in either nuclear or mitochondrial DNA. These results indicate that MUTYH-dependent excision of adenines paired with 8-oxoGs lead to the accumulation of SSBs in each type of DNA [48]. SSBs accumulating in nuclear DNA activate PARP followed by nuclear translocation of AIF, thus executing cell death [52, 53] (Fig. 2B). In contrast, SSBs accumulating in mitochondrial DNA results in their degradation, and in mitochondrial dysfunctions such as ATP depletion and opening the membrane permeability transition pore. These lead to Ca^{2+} efflux from mitochondria causing activation of the Ca^{2+}-dependent proteases, calpains, in the cytoplasm. Activated calpains induce lysosomal rupture and cell death [54, 55] (Fig. 2C).

We recently found that mice lacking MUTYH, OGG1 and MTH1 proteins are highly susceptible to the rapid development of various types of spontaneous tumors (our unpublished data), thus demonstrating that MUTYH-dependent programmed cell death is why mice lacking both OGG1 and MTH1 proteins do not develop the lung tumors observed in mice lacking only the OGG1 protein.

6. Oxidation of the nucleotide pool for mitochondrial DNA causes MUTYH-dependent cell death

We reported that both 8-oxoG accumulation and the expression levels of MTH1 are highly increased in the cardiovascular tissues of a rat model of genetic

hypertension compared with control rats, suggesting that the oxidation of nucleotide pools may play a role in the development of hypertension [56]. Cardiovascular tissues are constitutively exposed to nitric oxide (NO), a vasodilator and neurotransmitter, which produces peroxynitrite in the presence of superoxide [1]. Peroxynitrite itself produces the hydroxyl radical, which is known to vigorously oxidize nucleic acids *in vitro*; however, it has not been clear whether or how NO participates in the oxidation of nucleic acids *in vivo* [57].

We examined whether hMTH1 would prevent cellular dysfunction induced by sodium nitroprusside, a spontaneous NO donor [58]. Exposure caused 8-oxoG accumulation in the DNA of proliferating MTH1-null cells, which underwent mitochondrial degeneration and subsequently died. Quiescent MTH1-null cells also died with the 8-oxoG accumulation but only when it affected mitochondrial and not nuclear DNA. In both proliferative and quiescent conditions, the accumulation of 8-oxoG in DNA and the consequent cell death were effectively prevented by hMTH1 treatment. Knockdown of MUTYH in quiescent MTH1-null cells significantly reduced cell death, suggesting that 8-oxoG incorporated into mitochondrial DNA is a main cause of this form of cell death. To verify this possibility, an artificially modified hMTH1 with a mitochondrial targeting peptide (mTP), namely mTP-EGFP-hMTH1, which localizes exclusively in mitochondria, was expressed in MTH1-null cells [58]. mTP-EGFP-hMTH1 selectively prevented the accumulation of 8-oxoG in mitochondrial, but not nuclear DNA, after exposure of proliferating cells to NO and also efficiently prevented cell death. We thus conclude that exposure of cells to NO causes oxidation of mitochondrial deoxynucleotide pools and that the buildup of oxidized bases in mitochondrial DNA initiates cell death.

It is likely that the accumulation of 8-oxoG in nuclear DNA by the incorporation of 8-oxo-dGTP from the nucleotide pools does not induce acute cell death [58]. The MUTYH protein in mammalian cells functions in a replication-coupled manner by association with proliferating cell nuclear antigen (PCNA), replication protein A (RPA) and MutS homolog 6 (MSH6) in the nucleus [59-61] and the levels of MUTYH in the nucleus increased 3- to 4-fold during progression of the cell cycle and reached maximum levels in S phase compared with levels in early G1 and that MUTYH was localized at the site of DNA replication [62]. Therefore, MUTYH in nuclei selectively recognizes and excises adenine inserted into the nascent strand opposite template 8-oxoG in DNA, but not the template adenine that pairs with 8-oxoG in

nascent strand derived from 8-oxo-dGTP in the nucleotide pool. Thus, 8-oxoG derived from nucleotide pool may not result in accumulation of SSBs through MUTYH-initiated BER. It is likely that mismatch repair might recognize 8-oxoG inserted opposite template adenine in DNA [63] and OGG1 also excises 8-oxoG inserted opposite template cytosine in DNA [64, 65]. However, these processes are not so efficient because 8-oxoG level in nuclear DNA in the absence of MTH1 is still high 24 h after exposure to NO, which might cause delayed cell death through further replication (Fig. 2A,B).

In mitochondria, MUTYH might function independently of replication because mitochondria lack replication coupling factors such as PCNA [43]. It has been shown that the bacterial MutY protein can excise an adenine opposite an 8-oxoG regardless of the origin of the adenine base; the template adenine that pairs with an 8-oxoG in the nascent strand derived from 8-oxo-dGTP in the nucleotide pool (Fig. 2A: gray dotted line), or adenine inserted into the nascent strand opposite template 8-oxoG [66]. Therefore, in mitochondria, MUTYH can excise adenine opposite 8-oxoG regardless of their origin, as does bacterial MutY. We thus suggest that the accumulation of 8-oxoG in mitochondrial DNA in the absence of MTH1 results in excess formation of SSBs in both strands of DNA through MUTYH-initiated BER. This would cause double-strand breaks and thereby induce mitochondrial degeneration followed by cell death (Fig. 2A, C), particularly when cells are exposed to excess NO under conditions of inflammation or excitotoxicity [58, 67].

7. Neuronal accumulation of 8-oxoG causes neurodegeneration, which can be suppressed by MTH1

Oxidatively damaged bases, such as 8-oxoG accumulates in both nuclear and mitochondrial DNAs during aging [44, 68, 69] and such accumulation appears to increase dramatically in patients with various neurodegenerative diseases, such as Parkinson's disease (PD) [70, 71], Alzheimer's disease (AD) [72, 73] or amyotrophic lateral sclerosis (ALS) [74, 75]. We have shown that a significant increase of 8-oxoG in mitochondrial DNA was accompanied by an elevated expression of MTH1 [71], the mitochondrial form of OGG1 (OGG1-2a) [76] and an N-terminally truncated form of MUTYH encoded by an alternatively spliced *MUTYH* mRNA in the substantia nigra neurons of patients with PD [77]. In postmortem tissue specimens from patients with AD, the expression levels of MTH1 in the entorhinal cortex were also elevated, whilst

the levels of MTH1 apparently decreased in the stratum lucidum at CA3, corresponding to mossy fiber synapses, where MTH1 was highly expressed in the control subjects [78]. In contrast, expression level of OGG1-2a was found to decrease in the orbitofrontal gyrus and the entorhinal cortex in patients with AD compared with control subjects [79]. The accumulation of 8-oxoG was increased in most of the large motor neurons in patients with ALS, with a decreased expression of OGG1-2a but not MTH1. It is thus likely that OGG1-2a is indeed unstable under increased oxidative stress, compared with MTH1 [75].

We reported that the levels of 8-oxoG in cellular DNA and RNA increased in the mouse nigrostriatal system during tyrosine hydroxylase (TH)-positive dopamine neuron loss induced by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [80]. In contrast to wild-type mice, MTH1-null mice exhibited a greater accumulation of 8-oxoG in mitochondrial DNA, accompanied by a more significant decrease in TH- and dopamine transporter-positive fibers in the striatum after MPTP administration [80]. We thus demonstrated that MTH1 indeed protects the dopaminergic neurons from oxidative damage in nucleotide pools. This was especially effected by preventing 8-oxoG accumulation in the mitochondrial DNA of striatal nerve terminals of dopaminergic neurons [81], which is likely to cause mitochondrial dysfunction through the MUTYH-initiated BER as shown in Fig.2A,C.

Recently, a transgenic mouse has been established in which the human MTH1 is expressed [82]. Wild-type mice exposed to 3-nitropropionic acid, an inhibitor for mitochondrial succinate dehydrogenase, develop neuropathological and behavioral symptoms that resemble those of Huntington's disease, with an increased 8-oxoG accumulation in medium spiny neurons in striatum. 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 [82]. The findings indicate that oxidized nucleoside triphosphates such as 8-oxo-dGTP accumulating in nucleotide pools in medium spiny neurons have a significant contribution to their degeneration.

Enhanced oxidative stress has been implicated in the excitotoxicity of the central nervous system and 8-oxoG was reported to be accumulated in the rat hippocampus after administration of kainate, an excitotoxin for glutamate receptors [83]. We reported that the 8-oxoG levels in mitochondrial DNA and cellular RNA

increased significantly in the CA3 subregion of the mouse hippocampus 6–12 h after kainate administration but returned to basal levels within a few days [67]. 8-OxoG accumulation in mitochondrial DNA was remarkable in CA3 microglia, whereas that in nuclear DNA or cellular RNA was also detected in the CA3 pyramidal cells and astrocytes. MTH1-null and wild-type mice exhibited a similar degree of CA3 neuron loss after kainate administration; however, the 8-oxoG levels that accumulated in mitochondrial DNA and cellular RNA in the CA3 microglia increased significantly in the MTH1-null mice in comparison with wild-type mice [67]. This demonstrated that MTH1 efficiently suppresses the accumulation of 8-oxoG in both cellular DNA and RNA in the hippocampus—especially in microglia—caused by the excitotoxicity that plays a major role during neurodegeneration [84].

We examined the expression levels of MTH1 and OGG1 in the mouse hippocampus after kainate administration. The *Mth1* mRNA level decreased soon after kainate administration and then quickly recovered beyond the basal level. A continuously raised MTH1 protein level was observed, whereas the *Ogg1* mRNA level remained constant [67]. These results may indicate that oxidative stress in brain induces expression of MTH1 especially in microglia, thus avoiding cellular dysfunction.

8. Future perspectives

Oxidative DNA damage has been considered as one of major threats for organisms, causing mutagenesis and carcinogenesis [5]. Because bases of free nucleotides in the nucleotide pools are more susceptible to oxidation by ROS, compared with those in DNA, oxidized nucleotides generated in the nucleotide pools have greater impact as causes for mutagenesis through their incorporation into DNA. Beyond mutagenesis, the incorporation of oxidized nucleotides into nuclear or mitochondrial DNA from the damaged nucleotide pools triggers programmed processes resulting in cell death or senescence. Such programmed processes are involved in tumor suppression or neurodegeneration in animal models [67, 80, 82]. MTH1, a major sanitizing enzyme for oxidized nucleotide pools plays a crucial role by suppressing their accumulation in cellular DNA. In addition to oxidized purine deoxyribonucleoside triphosphates, MTH1 efficiently hydrolyzes oxidized purine ribonucleoside triphosphates such as 2-OH-ATP, 8-oxo-ATP and, to a lesser extent, 8-oxo-GTP. As a result, cellular dysfunction may also be caused by their

incorporation into RNA. Alternatively, such oxidized purine ribonucleoside triphosphates might interfere with various pathways of signal transduction or metabolisms in which ATP or GTP function as essential mediators of co-factors, thus suggesting that free forms of oxidized purine nucleotides might themselves exert a certain degree of cytotoxicity.

Conflict of interest

There is no conflicting interest.

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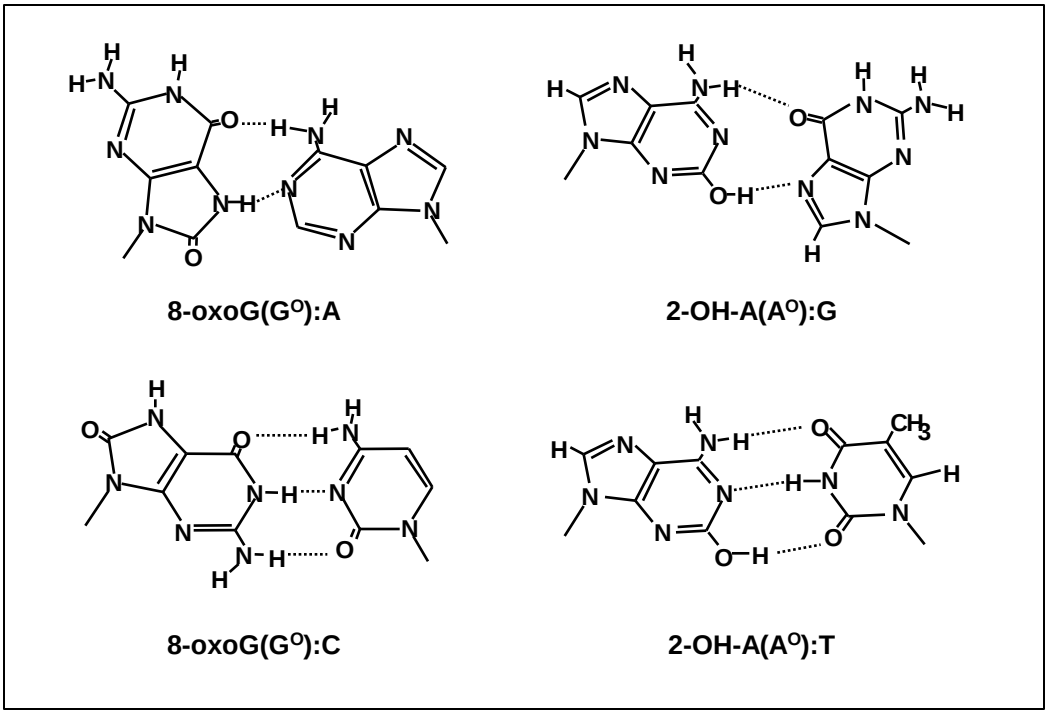
Figure legends

Fig. 1. Altered base pairing and mutagenesis caused by the oxidation of nucleic acids, and defense mechanisms in mammals. A: Altered base pairing of 8-oxoguanine and 2-hydroxyadenine. During DNA replication, 8-oxoG (G^O) and 2-OH-A (A^O) can pair with adenine (A) and guanine (G) as well as with cytosine (C) or thymine (T), respectively. B: Mutagenesis caused by 8-oxoG and 2-OH-A. 8-OxoG accumulates in DNA as a result of the incorporation of 8-oxo-dGTP from nucleotide pools or because of the direct oxidation of guanine in DNA. 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. (Modified from reference 6 with permission).

Fig. 2. MUTYH-dependent programmed cell death triggered by accumulation of 8-oxoguanine in nuclear and mitochondrial DNA. A. Reactive oxygen species (ROS) oxidize dGTP in the nucleotide pool and, to a lesser extent, guanine in DNA. 8-Oxo-dGTP escaping from hydrolysis by MTH1 is utilized by DNA polymerases as a substrate for DNA synthesis, thus increasing the accumulation of 8-oxoG (G^O) in DNA. During the next round of replication, adenine (A) can be inserted opposite 8-oxoG in DNA, MUTYH excises the adenine in the nascent strand and AP endonucleases incise the abasic sites. Cytosine (C) or adenine may be inserted opposite 8-oxoG during repair replication; however, insertion of adenine causes futile cycle of the base excision repair (BER), thus accumulating single strand breaks (SSBs) in the nascent strand when 8-oxoG accumulates to a large extent in the template DNA. B. When 8-oxoG accumulates highly in nuclear DNA, poly (ADP-ribose) polymerase (PARP) binds the SSBs generated by MUTYH-initiated BER, thus increasing poly(ADP-ribosyl)ation (PAR) resulting in nuclear translocation of apoptosis inducing factor (AIF) in mitochondria. AIF executes apoptotic cell death with large chromosomal DNA fragmentation. C. 8-OxoG accumulated highly in mitochondrial DNA causes degradation of mitochondrial DNA through MUTYH-initiated BER, thus causing mitochondrial dysfunction. Mitochondrial membrane permeability transition (MMTP) initiated by ATP depletion causes Ca^{2+} efflux from mitochondria, thus an increased Ca^{2+} in the cytoplasm activates calpains,

which in turn cause lysosomal rupture to execute cell death. (Modified from reference 48 with permission)

A



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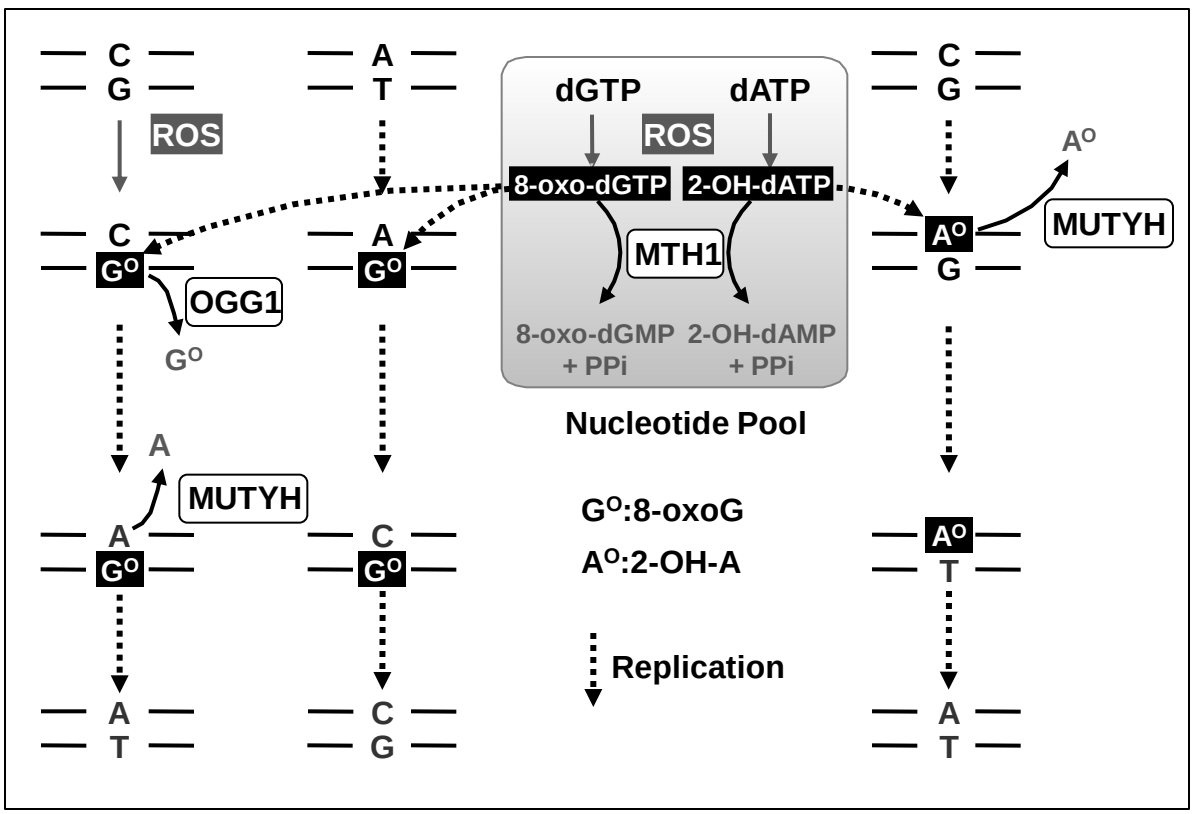
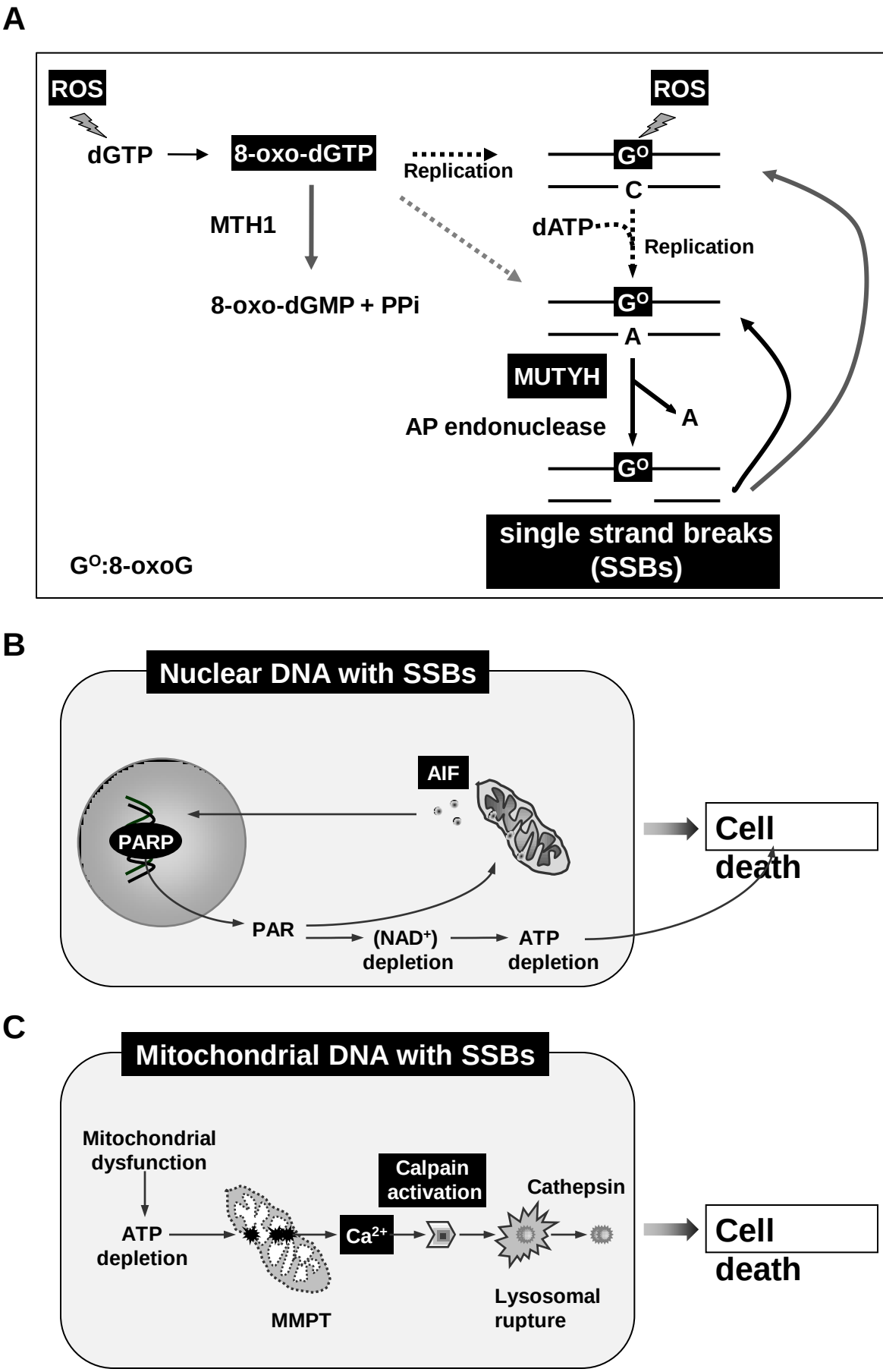


Nakabeppu et al., Figure 1



Nakabeppu et al., Figure 2