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*Review*

# The Defense Mechanisms in Mammalian Cells against Oxidative Damage in Nucleic Acids and their Involvement in the Suppression of Mutagenesis and Cell Death

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To counteract oxidative damage in nucleic acids, mammalian cells are equipped with several defense mechanisms. We herein review that MTH1, MUTYH and OGG1 play important roles in mammalian cells avoiding an accumulation of oxidative DNA damage, both in the nuclear and mitochondrial genomes, thereby suppressing carcinogenesis and cell death. MTH1 efficiently hydrolyzes oxidized purine nucleoside triphosphates, such as 8-oxo-dGTP, 8-oxo-dATP and 2-hydroxy (OH)-dATP, to the monophosphates, thus avoiding the incorporation of such oxidized nucleotides into the nuclear and mitochondrial genomes. OGG1 excises 8-oxoG in DNA as a DNA glycosylase and thus minimizes the accumulation of 8-oxoG in the cellular genomes. MUTYH excises adenine opposite 8-oxoG, and thus suppresses 8-oxoG-induced mutagenesis. MUTYH also possesses a 2-OH-A DNA glycosylase activity for excising 2-OH-A incorporated into the cellular genomes. Increased susceptibilities to spontaneous carcinogenesis of the liver, lung or intestine were observed in MTH1-, OGG1- and MUTYH-null mice, respectively. The increased occurrence of lung tumors in OGG1-null mice was abolished by the concomitant disruption of the *Mth1* gene, indicating that an increased accumulation of 8-oxoG and/or 2-OH-A might cause cell death. Furthermore, these defense mechanisms also likely play an important role in neuroprotection.

*Keywords*: Oxidative damage; DNA repair; Carcinogenesis; Neurodegeneration; Nucleotide pool; Mitochondria

## INTRODUCTION

In eukaryotic cells, more than one genome in a single cell has to be maintained throughout the entire life of the cell, namely with one in the nucleus and the others in the mitochondria. Aerobic respiration in the mitochondria makes it feasible for eukaryotic organisms to produce energy and to maintain life. Electrons leaked from the respiratory chain generate reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals.[1] ROS inevitably oxidize macromolecules in living cells, including lipids, proteins and nucleic acids, thereby leading to various types of cellular dysfunction including cell death and mutagenesis which may cause degenerative disorders and cancer.[2]

8-Oxoguanine (8-oxoG), an oxidized form of guanine base by ROS, is considered to be a major cause for spontaneous mutagenesis and carcinogenesis. Because 8-oxoG can pair with adenine as well as cytosine, with an almost equal efficiency, during DNA replication, its accumulation in DNA increases the occurrence of A:T to C:G or G:C to T:A transversion mutations, respectively.[3–5] There are two distinct pathways which result in the occurrence of 8-oxoG in DNA, namely, the incorporation of an oxidized precursor, 8-oxo-dGTP into DNA during DNA synthesis and the direct oxidation of guanine base in DNA.[6]

Prokaryotes are equipped with several error avoiding mechanisms that minimize the accumulation of 8-oxoG, and in which MutT, MutM and

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TABLE I The defense mechanisms in mammalian cells against oxidative damage in nucleic acids which causes various types of cellular dysfunction

| Enzyme | 8-oxoG DNA glycosylase | 2-OH-A/Adenine DNA glycosylase | Oxidized purine nucleoside triphosphatase |
|---|---|---|---|
| Function | C:GO → C:□ + GO | AO:G → AO + □:G; A:GO → A + □:GO | 2-OH-dATP / 2-OH-ATP<br>8-oxo-dATP (8-oxo-ATP)<br>8-oxo-dGTP (8-oxo-GTP)<br>8-Cl-dGTP → dNMP/NMP + PPi |
| Gene | *OGG1* | *MUTYH* | *MTH1* |
| Localization | Nucleus Mitochondria | Nucleus Mitochondira | Nucleus/Cytoplasm Mitochondria |
| Expression | Brain > Thymus, Testis, Kidney, Spleen, Ovary | Thymus > Brain, Testis, Kidney, Spleen, Ovary | Thymus, Testis, Kidney, Spleen, Ovary > Brain |
| Human disease | PD, SAH (↑) ALS, AD (↓) | PD, Hypoxia (↑) Familial adenomatous polyposis | PD, ALS, BT (↑) AD (↑/↓) |
| KO Mice | Viable lung cancer | Viable intestinal cancers | Viable liver cancer |

GO, 8-oxoguanine; AO, 2-OH-adenine; □, abasic site; PD, Parkinson's disease; AD, Alzheimer's disease; ALA, Amiotrophic lateral scerosis; SAH, Subarachnoid hemorrhage; BT, Brain tumor.

MutY proteins play an important role.[7,8] MutT protein hydrolyses 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate, thus avoiding the incorporation of 8-oxo-dGTP into DNA.[5] The spontaneous occurrence of A:T to C:G transversion mutation in a *mutT*-deficient *Escherichia coli* strain increases from hundreds to a 1000-fold compared to the wild type. MutM protein, originally identified as formamidopyrimidine (Fapy) DNA glycosylase,[9] removes the 8-oxoG paired with cytosine.[10] MutY protein with its DNA glycosylase activity excises adenine incorporated opposite 8-oxoG.[11] The spontaneous occurrence of the G:C to T:A transversion mutation in the *mutM* or *mutY*-deficient *E. coli* strain is 10–50 times higher than that in the wild type strain.

We have previously established that mammalian cells are also equipped with error avoiding mechanisms similar to those found in prokaryotes.[12,13] We have identified and intensively characterized three enzymes, MTH1 as a MutT homolog, OGG1 as functional homologs for MutM, MUTYH or MYH as MutY homolog in mammals, as summarized in Table I.[14] In mammalian cells, the genome in the mitochondria is likely to be more susceptible to ROS-induced oxidative damage as the oxygen metabolism is high. We demonstrated that all three enzymes are located both in the nuclei and mitochondria of mammalian cells.[15–17] We herein describe that three enzymes, namely MTH1, MUTYH and OGG1, play important roles in minimizing the accumulation of oxidative DNA damage in mammalian cells, both in the nuclear and mitochondrial genomes, thereby suppressing carcinogenesis and cell death.[14]

## Oxidized Purine Nucleoside Triphosphatase, MTH1

MTH1 was identified as an enzyme that degrades 8-oxo-dGTP into 8-oxo-dGMP and pyrophosphate.[18] MTH1 efficiently hydrolyzes 2 forms of oxidized dATP, 2-hydroxy (OH)-dATP and 8-oxo-dATP, as well as 8-oxo-dGTP,[19] and MTH1 also efficiently hydrolyzes oxidized ATP, 2-OH-ATP.[20] As a result, MTH1 protein possesses an oxidized purine nucleoside triphosphatase activity.[21,22] Its substrate specificity is different from that of the *E. coli* homolog, MutT which hydrolyzes 8-oxo-dGTP and 8-oxoGTP but not oxidized (d)ATP. The unique substrate specificity of MTH1 is determined by its substrate binding pocket.[23,24] The residues at positions W117 and D119 of MTH1 showed apparently different chemical shift perturbations with 8-oxo-dGDP and 2-OH-dADP in NMR analyses, and the residues form the substrate binding pocket and are not conserved in MutT. W117 is essential for MTH1 to recognize both 8-oxo-dGTP and 2-OH-dATP while D119 is only essential for recognizing

2-OH-dATP. Based on these findings, we constructed two different mutant MTH1 (W117Y, D119A) proteins, the former hydrolyses 2-OH-dATP but not 8-oxo-dGTP while the latter hydrolyses 8-oxo-dGTP but not 2-OH-dATP, respectively.[23]

### Nuclear and Mitochondrial 8-OxoG DNA Glycosylase, OGG1

8-OxoG DNA glycosylase, encoded by the *OGG1* gene, excises the 8-oxoG as a free base from the DNA. The DNA glycosylase activity of OGG1 preferentially excises 8-oxoG opposite cytosine, and OGG1 possesses an AP lyase activity.[16,25]

The human *OGG1* gene located on chromosome 3p25, has 8 major exons. More than seven alternatively spliced forms of *OGG1* mRNAs are produced, which are 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).[16] Types 1a and 2a mRNAs are major *OGG1* transcripts in various human tissues. OGG1-1a has a nuclear localization signal (NLS) at the C-terminal end, and thus is located in the nucleus, while OGG1-2a, which has a unique C-terminal region consisting of two distinct regions: namely the N-terminal sided acidic region (amino acid residues from $Ile^{345}$ to $Asp^{381}$) and the C-terminal sided hydrophobic region (the last 20 residues), located exclusively in the mitochondria. Both OGG1-1a and OGG1-2a carry a relatively poor mitochondrial targeting sequence (MTS), consisting of residues 9–26 at their common N-terminal region, and the MTS is not sufficient to locate the nuclear OGG1-1a with NLS into the mitochondria.[16]

### MUTYH, a Bifunctional DNA Glycosylase for 2-Hydroxyadenine Opposite Guanine and Adenine Opposite 8-Oxoguanine

DNA polymerases may insert adenine into the nascent strand when they encounter 8-oxoG in template strand during DNA replication, thus increasing the occurrence of G:C to T:A transversion.[3] A DNA glycosylase encoded by the *MUTYH* gene excises the adenine inserted opposite 8-oxoG in the template strand.[26,27] MUTYH protein also has an ability to excise 2-OH-A incorporated opposite guanine in template.[17] It has been shown that the adenine base in DNA is barely oxidized, while adenine nucleotides are easily oxidized *in vitro*, thus suggesting that 2-OH-A in DNA is mostly derived through the incorporation of 2-OH-dATP during DNA replication.[28] It is therefore likely that MUTYH has to specifically recognize adenine or 2-OH-A incorporated into the nascent strand during DNA replication. It has been demonstrated that MUTYH has a functional proliferating cell nuclear antigen (PCNA) binding motif,[29] and we have shown that the MUTYH repair activity for adenine incorporated opposite 8-oxoG in plasmid DNA transfected into cultured cells is dependent on the PCNA binding motif.[30] However, we recently found that the PCNA-binding motif in MUTYH is not essential to suppress the increased spontaneous mutation rate observed in MUTYH-null cells.[31] It has been shown that MUTYH interacts with other replication-associated proteins such as RPA or MSH2/MSH6 which also can interact with PCNA, thus suggesting that the interaction of MUTYH and PCNA may be indirectly mediated by these proteins.[32]

The human *MUTYH* gene is located on the short arm of chromosome 1 between p32.1 and p34.3, and consists of 16 exons.[26] In human cells, we previously reported that there are three major *MUTYH* transcripts, namely type α,β,γ with a different 5′ sequence or first exon and each transcript is alternatively spliced, thus multi-forms of human MUTYH (hMUTYH) proteins are present in the nuclei and in the mitochondria.[17,33] hMUTYH protein encoded by type α mRNA possesses a MTS, consisting of the amino terminal 14 residues which are required for its localization in the mitochondria, while those encoded by type β and γ mRNAs lack the MTS, and are localized in the nuclei. As a result, the subcellular localization of hMUTYH in human cells indicates that mitochondrial DNA is an important target for BER initiated by MUTYH as well as OGG1, probably because of their increased oxidative stress. Rodent MUTYH proteins deduced from mouse and rat MUTYH cDNA clones lack an amino-terminal sequence corresponding to the MTS in hMUTYH, and they are localized mostly in the nucleus, and to a lesser extent, in the mitochondria.[17]. We recently found a novel N-terminally truncated form of MUTYH (MUTYHβ) protein in murine cultured cells and mouse tissues, which is encoded by an alternatively spliced *Mutyh* mRNA.[34] It is likely that rats and humans also possess such a N-terminally truncated form of MUTYH, which is highly expressed in the mitochondria in the neurons in rat cerebellum.[35] Since MUTYHβ lacks a minor groove reading motif which is important for the adenine DNA glycosylase activity, MUTYH protein in the mitochondria may have a different biochemical function.[34]

### Increased Susceptibility to Spontaneous Carcinogenesis in Mice Lacking MTH1, OGG1 or MUTYH

MTH1-null embryonic stem cells exhibits a 2-fold increased spontaneous mutation rate,[36] thus confirming that MTH1 indeed plays a role in avoiding errors caused by 8-oxo-dGTP or 2-OH-dATP in the nuclear genome. Furthermore, MTH1-null mice exhibited a several-fold increased incidence of

spontaneous carcinogenesis in the liver at about 1.5 years after birth, in comparison to wild-type mice,[36] probably because of their mutator phenotype. We also recently reported that an overexpression of hMTH1 in mismatch-deficient mouse embryo fibroblasts efficiently suppresses the increased spontaneous mutagenesis, thus indicating that the oxidation of deoxynuceloside triphosphate pool is a significant contributor to spontaneous genetic instability in mismatch repair deficient cells.[37]

We found that lung adenoma/carcinoma spontaneously developed in OGG1-null mice at about 1.5 years after birth, in which 8-oxoG was found to accumulate in their genomes.[38] The mean number of tumors per mouse was 0.71 in the OGG1-null mice, which was five times higher than that observed in the wild-type mice (0.14). Two other groups established OGG1-null mice and they found an increased spontaneous mutagenesis in the reporter genes such as the *lacI* gene, however, they did not observe any increased occurrence of tumorigenesis at about 1 year after birth.[39,40] This discrepancy might be attributed to the shorter period for observation.

G:C to T:A transversion mutations that can be caused by 8-oxoG or 2-OH-A are frequently observed in the p53 gene in human cancer,[41] especially in the lung and liver, and this finding correlates with our observations in OGG1 or MTH1-null mice.

Recently, familial alterations in the human *MUTYH* gene have been reported to be possible causative mutations for certain types of autosomal recessive colorectal adenomatous polyposis,[42,43] thus suggesting that the absence of the MUTYH function in human cells might also result in a mutator phenotype. Among the many genes involved in BER, *MUTYH* is the first candidate gene for a hereditary neoplasm in human beings. We generated MUTYH-null mouse embryonic stem (ES) cell lines, and reported that the spontaneous mutation rate in MUTYH-null cells increased 2-fold in comparison to wild-type cells, thus indicating that the absence of the MUTYH function in mammalian cells results in a moderate mutator phenotype as well as in the absence of the MTH1 or OGG1 function.[31] MUTYH-null mice are now available in our lab, and we found an increased occurrence of intestinal adenoma or adenocarcinoma at about 1.5 years after birth (our unpublished observation). This observation may thus provide definitive evidence supporting the hypothesis that in the human *MUTYH* gene familial alterations are the causative mutations for either autosomal recessive colorectal adenomatous polyposis or multiple colorectal adenomas.

The expression of a mutant mMUTYH protein with an amino acid substitution (G365D) that corresponds to a germ-line mutation (G382D) found in patients with multiple colorectal adenomas could not suppress the elevated spontaneous mutation rate of the MUTYH-null ES cells, thus indicating that the germ-line mutation (G382D) of the human *MUTYH* gene is therefore likely to be responsible for the occurrence of a mutator phenotype in these patients.[31]

## MTH1 Suppresses Cell Death Caused by Oxidative Stress

We established MTH1-null mouse embryo fibroblasts that were highly susceptible to cell dysfunction and death caused by exposure to $H_2O_2$, with morphological features of pyknosis and electron dense deposits accumulated in the mitochondria.[44] The cell death observed was independent of both poly (ADP-ribose) polymerase and caspases. A continuous accumulation of 8-oxo-G both in 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 hMTH1, while they were only partially suppressed by the expression of mutant hMTH1 which possessed either 8-oxo-dGTPase or 2-OH-dATPase activity. Human MTH1 thus protects cells from $H_2O_2$-induced cell dysfunction and death by hydrolyzing oxidized purine nucleotides including 8-oxo-dGTP and 2-OH-dATP, and these alterations may be partly attributed to a mitochondrial dysfunction.

We recently reported that no tumor was found in the lungs of mice lacking both OGG1 and MTH1 proteins, in spite of the increased accumulation of 8-oxoG in the mice.[38] This observation suggests that a *Mth1* gene disruption resulted in the suppression of the tumorigenesis caused by an OGG1-deficiency. Based on the fact that MTH1 suppresses the cell death caused by oxidative stress,[44] we speculate that the simultaneous loss of OGG1 and MTH1 enhances cell death when a large amount of 8-oxo-dGTP in a nucleotide pool or 8-oxoG in cellular genome accumulates. Under such circumstances, damaged cells may not survive to produce progenitors with mutations in protooncogenes or tumor suppressor genes, thus suppressing carcinogenesis in mice lacking both OGG1 and MTH1 proteins.

The increased expression of hMTH1 protein in human cancerous tissues including brain tumors, kidney and lung cancers is generally coincidental with an increased accumulation of 8-oxoG,[45–47] which indicates increased oxidative stress in cancerous tissues. In glioma cases, the levels of MTH1 expression and 8-oxoG accumulation correlated significantly with the malignancy of glioma, thus suggesting that MTH1 may protect cancer cells against increased oxidative stress.[46] As a result, cancer cells exposed to higher oxidative stress

require an increased expression of MTH1 for their survival. It is noteworthy that OGG1 is easily inactivated under oxidative conditions, and we did not observe an increased expression of OGG1 in cancer tissues.[48]

Based on these findings, we propose that compounds which suppress the MTH1 function or expression must be new candidates for chemotherapy of cancer to sensitize cancer cells against certain type of anticancer drugs. MTH1-null mouse embryo fibroblasts expressing hMTH1 can be used as a practical screening system for such compounds.[44]

### Altered Expression of MTH1 and OGG1 in Various Neurodegenerative Diseases

It has been established that oxidative DNA damage, such as 8-oxoG, accumulates both in nuclear and mitochondrial genomes during aging, and such accumulation is likely to be increased dramatically in patients with various neurodegenerative diseases, such as Parkinson's disease (PD),[49,50] Alzheimer's disease (AD)[51] or amyotrophic lateral sclerosis (ALS).[52]

We have shown that a significant increase of 8-oxo-G accumulated in the cytoplasm or mitochondria with a coincidentally elevated expression of hMTH1 in the substantia nigral neurons of PD patients.[49] In postmortem, tissue specimens from patients with AD, the expression levels of hMTH1 in the entorhinal cortex were also elevated, while the levels of hMTH1 apparently decreased in the stratum lucidum at CA3 corresponding to mossy fiber synapses, where hMTH1 is highly expressed in control subjects.[53] Regarding the expression of mitochondrial OGG1, hOGG1-2a was found to decrease in the orbitofrontal gyrus and the entorhinal cortex in AD patients in comparison to that in control cases.[54] 8-OxoG accumulation increased in majority of the large motor neurons in the ALS cases with a decreased expression of hOGG1-2a but not hMTH1.[52]

Our results thus indicate that oxidative damage accumulates in the mitochondria of affected neurons in PD, AD and ALS and an increased expression of MTH1 may therefore, suppress the cell death caused by increased oxidative stress. We have shown that OGG1 itself is vulnerable to oxidative stress,[48] therefore, the repair function of OGG1 is not sufficient to excise 8-oxoG efficiently in these damaged neurons. A dysfunction of MTH1 may therefore, increase the loss of the neurons in these neurodegenerative diseases. Furthermore, we found that the expression of MTH1 was significantly higher in reactive astrtocytes and oligodendrocytes in the brains of patients with cerebrovascular diseases, than in control patients, and that MTH1 expression was found to be inducible in cultured glyoma cells after exposure to $H_2O_2$.[55] Similar levels of OGG1 expression were observed in both control and patient brains, thus indicating that the expression of MTH1 in the brain is inducible by oxidative stress, thus protecting the brain function.

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