*Review Article*

# Significance of error-avoiding mechanisms for oxidative DNA damage in carcinogenesis

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**Reactive oxygen species (ROS) are produced through normal cellular metabolism, and their formation is further enhanced by exposure to ionizing radiation and various chemicals. ROS attack DNA, and the resulting oxidative DNA damage is considered to contribute to aging, carcinogenesis and neurodegeneration. Among various types of oxidative DNA damage, 8-oxo-7,8-dihydroguanine (8-oxoguanine or 8-oxoG) is the most abundant, and plays significant roles in mutagenesis because of its ability to pair with adenine as well as cytosine. Enzymatic activities that may be responsible for preventing 8-oxoG-evoked mutations were identified in mammalian cells. We have focused on the following three enzymes: MTH1, OGG1 and MUTYH. MTH1 is a mammalian ortholog of *Escherichia coli* MutT, which hydrolyzes 8-oxo-dGTP to its monophosphate form in nucleotide pools, thereby preventing incorporation of the mutagenic substrate into DNA. OGG1, a functional counterpart of *E. coli* MutM, has an 8-oxoG DNA glycosylase activity. MUTYH, a mammalian ortholog of *E. coli* MutY, excises an adenine paired with 8-oxoG. These three enzymes are thought to prevent mutagenesis caused by 8-oxoG in mammals. To analyze the functions of mammalian MTH1 (Mth1), OGG1 (Ogg1) and MUTYH (Mutyh) *in vivo*, we established mutant mice for these three enzymes by targeted mutagenesis, and investigated spontaneous tumorigenesis as well as mutagenesis. Here we discuss our recent investigation of mutagenesis and carcinogenesis in these mutant mice. (*Cancer Sci* 2007; 98: 465–470)**

ROS generated in cells oxidize DNA as well as proteins and lipids. Numerous oxidative lesions in DNA, such as base modifications, abasic sites, deoxyribose damage and single- or double-strand breaks, are all formed under endogenous oxidative stress as well as exogenous oxidative stress caused by environmental exposure to mutagens and carcinogens.[1] The endogenous attack of ROS on DNA is most likely a major cause of the low steady-state levels of oxidative lesions that have been detected in DNA from human cells. It has been proposed that the steady-state level of oxidative lesions present in DNA may be an important determinant of longevity.[2] There are more than 100 different types of modified bases identified as endogenous oxidative lesions in mammalian DNA, of which 8-oxoG is the most abundant lesion in DNA.

8-OxoG is formed in nucleotide pools as well as in DNA by ROS, which are generated via many naturally occurring compounds, including cellular metabolic intermediates, as well as by X-ray irradiation (Fig. 1).[3] 8-OxoG is particularly mutagenic because of its propensity to form base pairs with adenine and cytosine.[4] 8-Oxo-G : A mispairs can arise via two pathways: (i) oxidation of dGTP in the nucleotide pools followed by misincorporation of 8-oxo-dGTP opposite adenine during replication; and (ii) oxidation of guanine in DNA followed by misincorporation of adenine.[5,6] Thus, 8-oxoG directly produced in DNA by ROS causes a G : C to T : A transversion, whereas 8-oxo-dGTP incorporated into DNA during DNA replication causes a A : T to C : G transversion as well as a G : C to T : A transversion after two rounds of DNA replication.[7] Studies on mutator mutants of *E. coli* revealed that three enzymes, encoded by the *mutT*, *mutM* and *mutY* genes, play important roles in avoiding 8-oxoG-related mutagenesis. MutT hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate, thus preventing the incorporation of 8-oxo-dGTP into DNA during replication.[5,6] MutM removes 8-oxoG paired with cytosine.[8] MutY is an adenine DNA glycosylase that excises adenine paired with guanine or 8-oxoG.[9,10] The functional cooperation of these three enzymes prevents mutations caused by 8-oxoG in bacteria.[7] In mammalian cells, similar enzymatic activities have been found, suggesting that similar systems are used to avoid 8-oxoG-related mutagenesis in mammals. *MTH1* (*Mth1*) and *MUTYH* (*Mutyh*) have been identified as human (mouse) mutT[11–13] and mutY orthologs,[14,15] respectively. There is no *mutM* ortholog in the human or the mouse genomes. However, an ortholog for the yeast gene encoding 8-oxoG DNA glycosylase, *Ogg1*, a functional counterpart of *Escherichia coli mutM*, has been identified in humans (*OGG1*) and mice (*Ogg1*).[16–18]

To assess the roles of the mammalian enzymes counteracting 8-oxoG-related mutagenesis, we have generated gene-knockout mice for these three enzymes by targeted mutagenesis.

## Mammalian MutT homolog proteins and their genes, *MTH1*

Taking advantage of the high level of 8-oxo-dGTPase activity in Jurkat cells (a human T-cell lymphoma cell line), a homogenous preparation of the enzyme was obtained with four steps of column chromatography and sodium dodecylsulfate–polyacrylamide gel electrophoresis. Based on the partial amino acid sequence determined from the resulting 18-kDa protein, a cDNA for the human enzyme was cloned comprising 156 amino acid residues.[12] The human gene for 8-oxo-dGTPase was named *MTH1* (human *mutT* homolog). *MTH1* cDNA was expressed in *E. coli mutT*$^-$ cells, whereupon the level of spontaneous mutagenesis was examined. The mutation frequency of mutT$^-$ cells expressing human MTH1 protein was significantly lower than that of *mutT*$^-$ cells transfected with empty vector.[12] Additionally, more significant suppression was observed when mouse *Mth1* cDNA was expressed in the *mutT*$^-$ cells.[13]

A certain degree of sequence homology has been noted in the *E. coli* MutT, and human and mouse MTH1 proteins.[12,13,19]

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Abbreviations: 2-OH-A, 2-hydroxyadenine or 1,2-dihydro-2-oxoadenine; 8-oxoG, 8-oxoguanine or 8-oxo-7,8-dihydroguanine; ARCAP, autosomal recessive colorectal adenomatous polyposis; ES, embryonic stem; MMR, mismatch repair; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; UV, ultraviolet.

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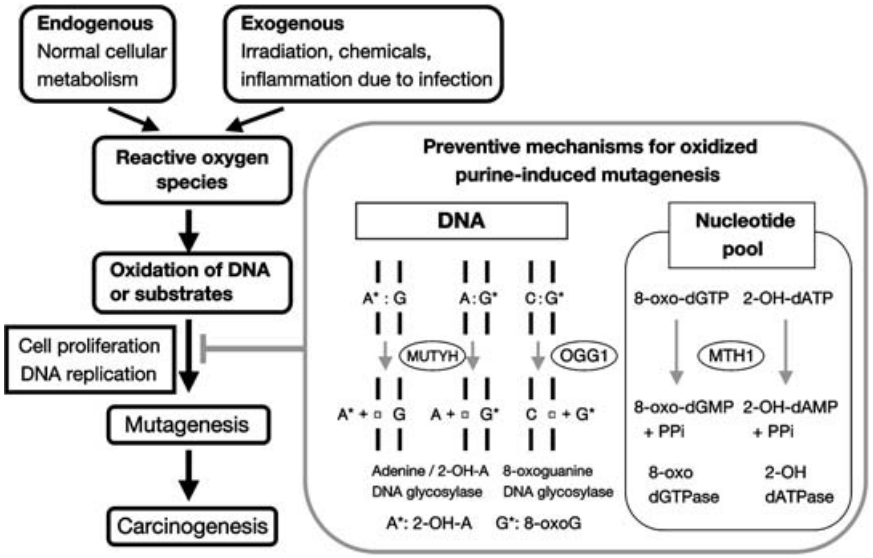


**Fig. 1.** Mutations caused by oxidative damage. Genomes and their precursor nucleotides are constantly exposed to reactive oxygen species, which are generated by cellular metabolism or molecular executors in the host defense, and by environmental exposure to ionizing radiation and chemicals. To counteract this oxidative damage in nucleic acids, mammalian cells are equipped with three distinct enzymes. One is MTH1, a sanitizing enzyme for the nucleotide pool, and the others are DNA glycosylases, which remove mutagenic lesions in DNA. OGG1 excises 8-oxo-7,8-dihydroguanine paired with cytosine in DNA, whereas MUTYH removes adenine paired with 8-oxo-7,8-dihydroguanine and 1,2-dihydro-2-oxoadenine paired with guanine in DNA.

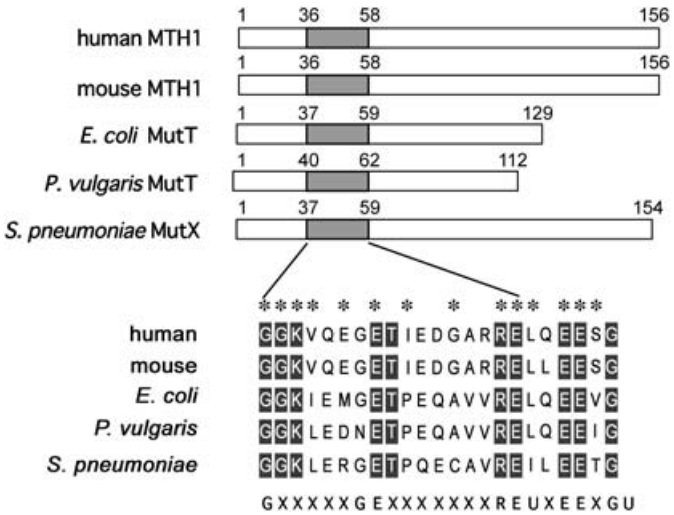


**Fig. 2.** Comparison of structures of MutT family proteins. Comparison of the predicted amino acid sequences of MutT homologs from humans,[12] mouse,[13] *Escherichia coli*,[19] *Proteus vulgaris*[55] and *Streptococcus pneumoniae*[56] are shown. Shaded boxes represent the regions with a high degree of amino acid sequence homology, with their amino acid sequences given below. The amino acid residues conserved through these five species are shown as white bold letters in black boxes. Numbers correspond to the positions of amino acid residues from the N-termini. Asterisks indicate the residues that could not be replaced by any other residue without losing function.[20] Two mammalian MTH1 proteins are each composed of 156 amino acids. This highly conserved sequence motif, consisting of 23 amino acid residues, is now recognized as the Nudix box, shown on the bottom line as boldface letters.[21] Nudix hydrolases, a superfamily of $Mg^{2+}$-requiring enzymes, catalyze the hydrolysis of nucleoside diphosphates linked to other moieties, X, and contain the sequence motif or Nudix box, $GX_5EX_7REUXEEXGU$, where U is a bulky hydrophobic residue and X is any residue.

Comparison of the amino acid sequences of bacterial and mammalian MutT orthologs is shown in Fig. 2. Besides similarities in size, the sequence alignment demonstrates that all carry a highly conserved sequence in nearly the same region (corresponding to amino acids 36–58 for human protein). Ten out of 23 amino acid residues are identical, suggesting that this region may constitute the active site for the enzyme. This view has been supported by site-directed mutagenesis experiments, thereby outlining the significance of this conserved amino acid sequence for the antimutator activity of human MTH1.[20] This highly conserved 23-residue sequence motif is now recognized as the phosphohydrolase module[20] or Nudix box, $GX_5EX_7REUXEEXGU$, where U is a bulky hydrophobic residue and X is any residue.[21] Nudix hydrolases, a superfamily of $Mg^{2+}$-requiring enzymes found in more than 250 species including viruses, bacteria and eukaryotes, catalyze the hydrolysis of nucleoside diphosphates linked to other moieties, X. In spite of the similarity, MTH1 has broader substrate specificity than MutT. While MutT hydrolyzes 8-oxo-dGTP and 8-oxo-GTP, human MTH1 efficiently degrades two forms of oxidized dATP, 2-OH-dATP and 8-OH-dATP, in addition to 8-oxo-dGTP and 8-oxo-GTP.[22]

The human *MTH1* gene spans approximately 10 kb on chromosome 7p22 and is found to contain five major exons.[23] There are two alternative exon 1 and three alternatively spliced exon 2 segments (exons 2a, 2b and 2c), and accordingly seven different *MTH1* transcripts were proven to be produced.[24] There is a SNP in the *MTH1* gene resulting in replacement of the amino acid valine 83 (Val83) with methionine (Met83). This substitution has been found to increase structural and functional thermolability.[25] Molecular epidemiological studies have revealed that this mutation with another tightly linked SNP is a risk factor for hepatocellular carcinoma and small-cell lung carcinoma.[26,27] Furthermore, the allele frequencies of Met83 in patients with gastric cancer were higher than those observed in healthy volunteers.[28] Whether or not the region of chromosome 7p22 is related to a certain type of inherited disease or to familial cancer is not fully evident, but it is possible that a defect in this region might lead to an increased incidence of cancer because a defect in sanitization of nucleotide pools increases spontaneous mutations.

Western blot analysis of various mouse tissues for the MTH1 protein revealed that all organs except the small intestine contain substantial amounts of the protein, and the highest expression was seen in the liver.[13] Among the various tissues and cell lines examined, the highest level of 8-oxo-dGTPase activity was found in ES cells, which have an intense proliferative capacity and thus a high oxygen consumption. The higher oxygen consumption may correlate with the higher level of oxidative stress, and the level of expression of the *MTH1* (*Mth1*) gene might be regulated to respond to the higher oxidative stress.


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**Table 1. Spontaneous tumorigenesis in wild-type and *Mth1*-deficient mice**

| Organ | Males ($n$ = 88) | | Females ($n$ = 95) | |
|---|---|---|---|---|
| | –/– | +/+ | –/– | +/+ |
| Lung | | | | |
| Carcinoma, adenoma | 2 | 1 | 3 | 1 |
| No. mice with tumors | 5 | 2 | 5 | 0 |
| Subtotal | 7 (17) | 3 (7) | 8 (16) | 1 (2) |
| Liver | | | | |
| Carcinoma, adenoma | 10 | 2 | 0 | 0 |
| No. mice with tumors | 6 | 4 | 2 | 0 |
| Subtotal | 16 (38) | 6 (13) | 2 (4) | 0 (0) |
| Stomach | | | | |
| Carcinoma, adenoma | 2 | 1 | 0 | 0 |
| No. mice with tumors | 4 | 1 | 2 | 0 |
| Subtotal | 6 (14) | 2 (4) | 2 (4) | 0 (0) |
| Total no. mice examined | 42 | 46 | 51 | 44 |

## Tumorigenesis, carcinogenesis and mutagenesis in mice lacking MTH1

To analyze the function of MTH1 *in vivo*, we established a mouse line carrying a mutant *Mth1* allele created by targeted gene disruption.[29] *Mth1*$^{-/-}$ mutant mice were found to have a physically normal appearance, but had no 8-oxo-dGTPase activity in liver extracts, thus being MTH1-null. Notably, approximately two-fold higher mutation rate was observed in two independently isolated *Mth1*$^{-/-}$ ES cells compared with *Mth1*$^{+/+}$ cells when mutation rates toward 6-thioguanine resistance were determined by the fluctuation test. Because the mutagenic substrate 8-oxo-dGTP can be sanitized from nucleotide pools by MTH1 protein,[6] we examined the susceptibility of MTH1-null mice to spontaneous tumorigenesis. Groups of wild-type or MTH1-null males and females (50 per group) were kept under specific pathogen-free conditions for 1.5 years, and then all of the animals were subjected to necropsy. No distinct difference in the survival rates of these wild-type and MTH1-null males and females was observed. Systematic pathological examination revealed a statistically significant increase in the incidence of tumors in MTH1-null mice; more tumors were formed in the three internal organs (lungs, liver and stomach) of MTH1-null mice than in those of wild-type mice (Table 1). The elevated incidence of tumor formation in the liver of MTH1-null mice was well correlated with the highest level of MTH1 protein in this organ of the wild-type mice.[13] Because there are different expression profiles for antioxidant enzymes, such as superoxide dismutases and catalases, in each organ, the susceptibility to tumorigenesis may differ among organs, probably reflecting the metabolic balance between antioxidants and oxidative stress, which may determine the levels of oxidative lesions in the nucleotide pools as well as in DNA.

The *in vivo* study of mutations has been facilitated by the use of transgenic mice, where mutational responses can be measured in virtually any tissue as a function of age, sex and external stresses. To examine *in vivo* mutation events due to MTH1 deficiency, the *rpsL* gene of *E. coli* was introduced into MTH1-null mice as a reporter gene, as the *rpsL* gene is highly sensitive to base substitutions as well as frameshifts, deletions and insertions, making the transgene an ideal choice for recovery of spontaneous and induced mutations. Spleen DNA samples from three 24-week-old wild-type and MTH1-null animals, hemizygous for the *rpsL* transgene, were analyzed by the forward mutation detection assay in order to investigate the frequency and specificity of mutations in the transgene *in vivo*.[30] The spontaneous mutation frequency observed in spleen samples from MTH1-null mice showed no apparent difference compared with the wild-type mice (less than two-fold). However, the site distribution of the mutations that occurred in the *rpsL* gene were slightly different between the spleen samples from the two groups of mice. Unlike MutT-deficient *E. coli*,[7,19] an increase in the frequency of A : T to C : G transversions was not evident in MTH1-null mice. Nevertheless, the frequency of 1-bp frameshift mutations at the mononucleotide runs was 5.7-fold higher in spleens of MTH1-null mice than in those of wild-type mice. Because the elevated incidence of single-base frameshifts at mononucleotide runs is a hallmark of a defect in the MSH2-dependent MMR system, this weak site-specific mutator effect of MTH1-deficiency may be attributed to a partial sequestration of the MMR function that may act to correct mispairs with the oxidized nucleotides. Consistent with this hypothesis, a significant increase in the frequency of G : C to T : A transversions was observed in MTH1/MSH2 double mutants compared with either mutant alone.

There are several possible explanations for this relatively low spontaneous mutation rate in the absence of cellular MTH1 protein. Figure 3 shows oxidative damage-induced mutagenesis and its avoidance mechanisms in mammals. First, OGG1, an 8-oxoG DNA glycosylase, efficiently removes 8-oxoG incorporated opposite cytosine in DNA. Secondly, MUTYH can excise adenine misincorporated opposite 8-oxoG or 2-OH-A mispaired with guanine in the template strand during DNA replication. Such removal of relevant adenine or 2-OH-A would result in possible reversion to a correct cytosine, thus preventing G : C to T : A transversion in the absence of MTH1 protein. Thirdly, in addition to the above two enzymes, mismatch repair may also participate in the avoidance of oxidized purine-related mutagenesis in mammalian cells, as deficiency of MMR in mouse ES cells leads to an accumulation of 8-oxo-G in the genome.[31] Interestingly, it is suggested that OGG1-initiated base excision repair and MMR represent a major mechanism in avoiding 8-oxoG-related mutagenesis in *Saccharomyces cerevisiae*, which lacks MutT- and MutY-related proteins.[32] Furthermore, overexpression of human MTH1 in MSH2-deficient cells efficiently decreased the elevated level of mutations, including frameshifts and G : C to T : A transversions.[33] Recently, other mammalian counterparts of MutT have been identified. MTH2 (NUDT15) degrades 8-oxo-dGTP to 8-oxo-dGMP, as does MTH1.[34] NUDT5 has little activity toward 8-oxo-dGTP but efficiently hydrolyzes 8-oxo-dGDP to the related nucleoside monophosphate.[35] Mice deficient in these nudix proteins should provide new insights into the significance of nucleotide pool sanitization under normal or oxidative stress conditions.

## Tumorigenesis, carcinogenesis and mutagenesis in mice lacking OGG1

OGG1 (8-oxoG DNA glycosylase) preferentially excises 8-oxoG opposite cytosine, and possesses an apurinic/apyrimidic lyase activity. The human *OGG1* gene is located on chromosome 3p25, a region showing frequent loss of heterozygosity in lung and kidney tumors.[36] In addition, some OGG1 polymorphisms have been reported to positively correlate with a variety of cancers,[37] suggesting a role for OGG1 in tumor suppression.

We have generated OGG1-null mice and analyzed spontaneous mutagenesis and carcinogenesis. We found that adenoma and carcinoma developed in the lungs of OGG1-null mice at the age of 1.5 years, where a five-fold increased level of 8-oxoG was found to accumulate in their nuclear genomes in comparison to wild-type mice.[38] The mean number of tumors per mouse was 0.71 in OGG1-nulls, compared with 0.14 in wild-type mice. Spontaneous mutagenesis in OGG1-nulls at 4–5 weeks was analyzed using the *rpsL* transgenic mice. The observed total mutation frequency was $1.00 \times 10^{-5}$ in both wild-type and OGG1-null

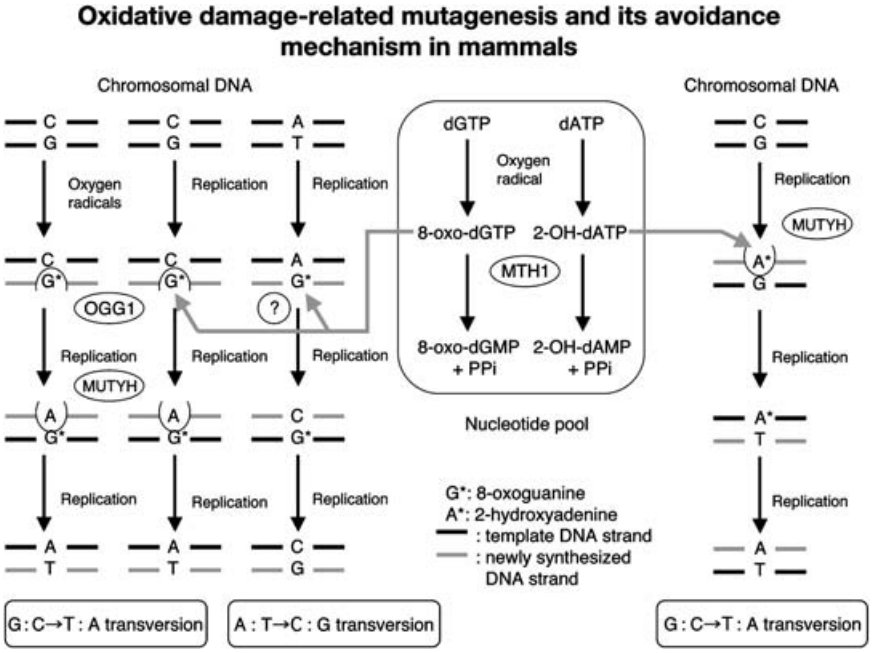


**Fig. 3.** Oxidaive damage-induced mutagenesis and avoidance mechanisms in mammals. Among the various types of oxidative damage in DNA, the oxidized forms of guanine and adenine, 8-oxoguanine and 1,2-dihydro-2-oxoadenine, can form relatively stable base pairs with adenine or guanine, respectively, in DNA. During DNA replication, they are thought to induce spontaneous mutagenesis, such as A : T to C : G and G : C to T : A transversions. The direct oxidation of DNA by reactive oxygen species has been reported to generate a substantial amount of 8-oxo-7,8-dihydroguanine but little 1,2-dihydro-2-oxoadenine. In contrast, 1,2-dihydro-2-oxoadenine is generated exclusively by the oxidation of dATP in the nucleotide pool. Studies on mutator mutants have revealed that *Escherichia coli* has several error-avoiding mechanisms that minimize the deleterious effects of 8-oxo-7,8-dihydroguanine, and in which MutT, MutM and MutY proteins play important roles. MutT protein hydrolyzes 8-oxo-dGTP to 8-oxo-dGMT and pyrophosphate, thus avoiding the occurrence of A : T to C : G transversion mutations during DNA replication. MutM and MutY proteins are DNA glycosylases, the former excises 8-oxoG paired with cytosine whereas the latter removes adenine paired with 8-oxo-7,8-dihydroguanine. Mammalian cells are also equipped with elaborate error-preventing mechanisms similar to those found in prokaryotes; MTH1 as a MutT homolog, OGG1 as a functional homolog for MutM, and MUTYH (MYH) as a MutY homolog. Recent studies showed that MTH1 effectively hydrolyzes 2-OH-dATP as well as 8-oxo-dGTP, and MUTYH has the ability to excise 1,2-dihydro-2-oxoadenine inserted opposite guanine in the template strand as well as the ability to remove adenine incorporated opposite 8-oxo-7,8-dihydroguanine in the template. As a result of the cooperative action of MTH1/OGG1/MUTYH and other repair pathways, mammalian cells effectively protect the occurrence of spontaneous mutations such as A : T to C : G and G : C to T : A transversions, which are caused by 8-oxo-7,8-dihydroguanine and 1,2-dihydro-2-oxoadenine.

mice. Analysis of the mutation spectrum, however, revealed that the frequency of G : C to T : A transversions increased five-fold in OGG1-nulls compared with wild-type mice.[39]

Two other groups also established OGG1-null mice and they found an increased spontaneous mutation frequency in mice carrying the *lacI* or *gpt* gene as the reporter gene. However, they did not observe any increased incidence of tumor at about 1 year after birth.[40,41] One group recently reported that significant increases in spontaneous lung tumorigenesis were observed when the *OGG1* mutation was combined with a MUTYH- or MSH2-deficient condition, and that an increased occurrence of G : C to T : A transversions in the *K-ras* gene was also observed in the tumors. Thus, OGG1 may suppress spontaneous lung tumorigenesis in mammal.[42]

Production of ROS is enhanced by exposure to sunlight, especially UVB. We thus investigated skin tumorigenesis in OGG1-null mice with UVB irradiation.[43] UVB irradiation significantly enhanced the accumulation of 8-oxoG in nuclei of epidermal cells within 12 h of irradiation. The level of 8-oxoG in OGG1-null mice 24 h after UVB irradiation remained high compared to wild-type and heterozygous mice. After chronic UVB irradiation at a dose of 2.5 kJ/m$^2$ three times a week for 40 weeks, we found that OGG1-null mice showed an increased susceptibility to UVB-induced skin tumorigenesis. The mean number of tumors was 3.71 in OGG1-null mice, 1.71 in wild-type mice and 2.28 in heterozygous mutant mice. The OGG1-null mice developed more malignant tumors (squamous cell carcinomas 73.1%, sarcomas 15.4%) than did wild-type mice (squamous cell carcinomas 41.7%, sarcomas 8.3%). These results clearly demonstrate that OGG1 plays an important role in preventing the development of skin cancers caused by oxidative DNA damage induced by sunlight.

## Tumorigenesis, carcinogenesis and mutagenesis associated with MUTYH deficiency

In addition to 8-oxoG, recent studies suggested that 2-hydoxyadenine, an oxidized form of adenine, may be involved in mutagenesis because of its miscoding property. MUTYH efficiently excises 2-OH-A mispaired with guanine in DNA, and is thus considered to play an important role in preventing G : C to T : A transversions.[44] The human *MUTYH* gene, consisting of 16 exons, resides on the short arm of chromosome 1 between p32.1 and p34.3.[14,44] Recently, several germline mutations in the *MUTYH* gene have been linked to certain types of ARCAP.[45,46] Although these patients have no germline mutation in the *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 in the context of GAA sequences. Accordingly, these observations strongly suggest that a MUTYH deficiency causes somatic mutations in the *APC* gene, thus resulting in multiple colorectal tumors. The expression of a mutant form of mouse MUTYH protein bearing G365D amino acid substitution, corresponding to a germline MUTYH (G382D) found frequently in patients with ARCAP, could not suppress the elevated spontaneous


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mutation rate of MUTYH-deficient ES cells.[15] Biochemical analyses revealed that MUTYH (G365D) retained the DNA glycosylase activity for excising adenine opposite 8-oxoG but compromised the activity excising 2-OH-A opposite guanine (only 1.5% level of the wild-type activity).[47] This finding suggests that the reduced repair capacity of MUTYH (G382D) for excising 2-OH-A opposite guanine could result in an increased occurrence of somatic G : C to T : A transversions in the *APC* gene.

Xie et al. reported that no significant difference in tumor incidence occurred between MUTYH-null and wild-type mice within a 12-month period, albeit with a relatively small number of animals. They also showed that mice lacking both MUTYH and OGG1, both of which are considered to synergistically prevent 8-oxoG-related mutagenesis, are predisposed to tumors, predominantly lung and ovarian tumors and lymphomas, and to a lesser extent gastrointestinal-tract tumors.[42] To assess the contribution of MUTYH in suppression of tumorigenesis caused by oxidative lesions, 8-oxoG and 2-OH-A in living animals, we also generated MUTYH-null ES cells and mice. MUTYH-null ES cell lines showed that the spontaneous mutation rate of the *Hprt* gene was increased two-fold in comparison to wild-type cells. This indicated that the absence of MUTYH function in mammalian cells results in a moderate mutator phenotype similar to that observed in the absence of MTH1 function.[15] Examination for tumor development using a large cohort of wild-type and MUTYH-null mice around the age of 18 months revealed an increased occurrence of tumors in various internal organs of MUTYH-null mice compared to wild-type mice (Tsuzuki T, Nakatsu Y and Naka beppu Y, unpublished data). Particularly, MUTYH-null mice appeared to have a higher susceptibility to intestinal adenoma and adenocarcinoma, suggesting a linkage between oxidative damage and spontaneous tumorigenesis in the intestine.

## Perspective

Tumor development is a multistep process that requires the accumulation of mutations in genes regulating cell growth, differentiation and apoptosis. DNA lesions introduced by ROS are a major source of spontaneous mutation in organisms living under aerobic conditions.[48] Thus, compromise of the cellular system involved in mechanisms for the avoidance of oxidative DNA damage-related mutagenesis could lead to an increase in spontaneous mutagenesis and tumorigenesis in mammals. The biochemical and genetic data discussed in this review indicate that MTH1, OGG1 and MUTYH play roles in the avoidance mechanisms of oxidative DNA damage-related mutagenesis, and thus suppress spontaneous tumorigenesis caused by oxidative stress in mammals.

It has been shown that in certain human tumors, amounts of 2-OH-A are increased several-fold compared to findings in normal non-cancerous tissues.[49] 2-OH-A is poorly produced by direct oxidation of DNA but 2-OH-dATP is produced efficiently by ROS treatment of dATP. Thus a major source of 2-OH-A in DNA would be 2-OH-dATP generated in the nucleotide pools. Studies on the mutagenic potential of damaged nucleotides revealed that 2-OH-dATP is more mutagenic than 8-oxo-dGTP *in vivo*.[50] Because MTH1 efficiently hydrolyzes 2-OH-dATP and 8-OH-dATP as well as 8-oxo-dGTP, MTH1 appears to eliminate the oxidized purine nucleoside triphosphates from the DNA precursor pool.[22] The broad substrate specificity of MTH1 suggests its versatile roles in multiple sanitization pathways. Furthermore, recent studies have indicated that MTH1 protects cells from oxidative stress-induced mitochondrial dysfunction and cell death.[51] These observations further suggest that a loss or decrease in MTH1 activity may be deeply involved in mutagenesis and carcinogenesis, aging and other diseases.

The presence of premutagenic lesions or mutations in the genome is unlikely to be sufficient to cause tumor formation. It is of interest that no increased intestinal tumor formation was observed in OGG1-null mice, whereas the mutation frequency in the small intestine of OGG1-null mice increased as in MUTYH-null mice. These findings suggest that MUTYH and OGG1 play different roles in preventing tumorigenesis in the intestine of mammals, even though these enzymes act synergistically to prevent the mutagenesis caused by 8-oxoG in DNA. When 8-oxo-dGMP is incorporated opposite adenine in a template strand during DNA replication, OGG1 cannot excise 8-oxoG. In such the case, MUTYH would not excise adenine paired with 8-oxoG, because the removal of adenine in the template strand results in fixation of premutagenic lesions to mutations. It should be noted that MUTYH interacts with MSH6,[52] which associates with MSH2 to form a DNA lesion recognition complex that is essential to provoke apoptotic cell death caused by $O^6$-methylguanine.[53,54] These observations give rise to the possibility that MUTYH, together with MMR protein, might recognize the mispairs containing oxidative lesions and activate apoptotic signaling pathways. Possible involvement of MUTYH in apoptosis induced by certain types of oxidative DNA lesions may explain the functional difference between MUTYH and OGG1 in preventing intestinal tumorigenesis in mammals.

More definitive studies designed to evaluate the role of these oxidation-induced DNA damage defense systems in the intact animal will benefit from the double- or triple-mutant mice as resources such as OGG1-deficient mice and MUTYH-deficient mice together with several other DNA repair-deficient mouse lines (e.g. MSH2-deficient mice) are available.

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## References

1 Gajewski E, Rao G, Nackerdien Z *et al*. Modification of DNA bases in mammalian chromatin by radiation-generated free radicals. *Biochemistry* 1990; **29**: 7876–82.
2 Ames BN, Gold LS. Endogenous mutagens and the causes of aging and cancer. *Mutat Res* 1991; **250**: 3–16.
3 Kasai H, Tanooka H, Nishimura S. Formation of 8-hydroxyguanine residues in DNA by X-irradiation. *Gann* 1984; **75**: 1037–9.
4 Grollman AP, Moriya M. Mutagenesis by 8-oxoguanine: an enemy within. *Trends Genet* 1993; **9**: 246–9.
5 Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxoG. *Nature* 1991; **349**: 431–4.
6 Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature* 1992; **355**: 273–5.
7 Tajiri T, Maki H, Sekiguchi M. Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in *Escherichia coli*. *Mutat Res* 1995; **336**: 257–67.
8 Michaels ML, Pham L, Cruz C, Miller JH. MutM, a protein that prevents G: C→T : A transversions, is formamidopyrimidine-DNA glycosylase. *Nucleic Acids Res* 1991; **19**: 3629–32.
9 Au KG, Clark S, Miller JH, Modrich P. *Escherichia coli mutY* gene encodes an adenine glycosylase active on G : A mispairs. *Proc Natl Acad Sci USA* 1989; **86**: 8877–81.
10 Michaels ML, Tchou J, Grollman AP, Miller JH. A repair system for 8-oxo-7,8-dihydrodeoxyguanine. *Biochemistry* 1992; **31**: 10 964–8.
11 Mo JY, Maki H, Sekiguchi M. Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proc Natl Acad Sci USA* 1992; **89**: 11 021–5.
12 Sakumi K, Furuichi M, Tsuzuki T *et al*. Cloning and expression of cDNA

for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *J Biol Chem* 1993; **268**: 23 524–30.
13 Kakuma K, Nishida J, Tsuzuki T *et al*. Mouse MTH1 protein with 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphatase activity that prevents transversion mutation. *J Biol Chem* 1995; **270**: 25 942–8.
14 Slupska MM, Baikalov C, Luther WM *et al*. Cloning and sequencing a human homolog (hMYH) of *Escherichia coli mutY* gene whose function is required for the repair of oxidative DNA damage. *J Bacteriol* 1996; **178**: 3885–92.
15 Hirano S, Tominaga Y, Ichinoe A *et al*. Mutator phenotype of MUTYH-null mouse embryonic stem cells. *J Biol Chem* 2003; **278**: 38 121–4.
16 Rosenquist TA, Zharkov DO, Grollman AP. Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. *Proc Natl Acad Sci USA* 1997; **94**: 7429–34.
17 Arai K, Morishita K, Shinmura K *et al*. Cloning of a human homolog of the yeast *OGG1* gene that is involved in the repair of oxidative DNA damage. *Oncogene* 1997; **14**: 2857–61.
18 Aburatani H, Hippo Y, Ishida T *et al*. Cloning and characterization of mammalian 8-hydroxyguanine-specific DNA glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. *Cancer Res* 1997; **57**: 2151–6.
19 Akiyama M, Horiuchi T, Sekiguchi M. Molecular cloning and nucleotide sequence of the *mutT* mutator of *Escherichia coli* that causes A : T to C : G transversion. *Mol Gen Genet* 1987; **206**: 9–16.
20 Cai JP, Kawate H, Ihara K *et al*. Significance of the conserved amino acid sequence for human MTH1 protein with antimutator activity. *Nucleic Acids Res* 1997; **25**: 1170–6.
21 Bessman MJ, Frick DN, O'Handley SF. The MutT proteins or 'Nudix' hydrolases, a family of versatile, widely distributed, 'housecleaning' enzymes. *J Biol Chem* 1996; **271**: 25 059–62.
22 Fujikawa K, Kamiya H, Yakushiji H *et al*. The oxidized forms of dATP are substrates for the human MutT homologue, the hMTH1 protein. *J Biol Chem* 1999; **274**: 18 201–5.
23 Furuichi M, Yoshida MC, Oda H *et al*. 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* 1994; **24**: 485–90.
24 Oda H, Nakabeppu Y, Furuichi M *et al*. Regulation of expression of the human *MTH1* gene encoding 8-oxo-dGTPase. Alternative splicing of transcription products. *J Biol Chem* 1997; **272**: 17 843–50.
25 Yakushiji H, Maraboeuf F, Takahashi M *et al*. Biochemical and physicochemical characterization of normal and variant forms of human MTH1 protein with antimutagenic activity. *Mutat Res* 1997; **384**: 181–94.
26 Oda H, Taketomi A, Maruyama R *et al*. Multi-forms of human MTH1 polypeptides produced by alternative translation initiation and single nucleotide polymorphism. *Nucleic Acids Res* 1999; **27**: 4335–43.
27 Kohno T, Sakiyama T, Kunitoh H *et al*. Association of polymorphisms in the *MTH1* gene with small cell lung carcinoma risk. *Carcinogenesis* 2006; **27**: 2448–54.
28 Kimura Y, Oda S, Egashira A *et al*. A variant form of hMTH1, a human homologue of the *E. coli* mutT gene, correlate with somatic mutation in the *p53* tumour suppressor gene in gastric cancer patients. *J Medical Genet* 2004; **41**: e57.
29 Tsuzuki T, Egashira A, Igarashi H *et al*. Spontaneous tumorigenesis in mice defective in the *MTH1* gene encoding 8-oxo-dGTPase. *Proc Natl Acad Sci USA* 2001; **98**: 11 456–61.
30 Egashira A, Yamauchi K, Yoshiyama K *et al*. Mutational specificity of mice defective in the *MTH1* and/or *MSH2* genes. *DNA Repair* 2002; **1**: 881–93.
31 DeWeese TL, Shipman JM, Larrier NA *et al*. Mouse embryonic stem cells carrying one or two defective alleles respond abnormally to oxidative stress inflicted by low-level radiation. *Proc Natl Acad Sci USA* 1998; **95**: 11 915–20.
32 Ni TT, Marsischky GT, Kolodner RD. MSH2 and MSH6 are required for removal of adenine misincorporated opposite 8-oxo-guanine in *S. cerevisiae*. *Mol Cell* 1999; **4**: 439–44.
33 Russo M, Blasi M, Chiera F *et al*. The oxidized deoxynucleoside triphosphate pool is a significant contributor to genetic instability in mismatch repair-deficient cells. *Mol Cell Biol* 2004; **24**: 465–74.
34 Cai JP, Ishibashi T, Takagi Y *et al*. Mouse MTH2 protein which prevents mutations caused by 8-oxoguanine nucleotides. *Biochem Biophys Res Commun* 2003; **305**: 1073–7.
35 Ishibashi T, Hayakawa H, Sekiguchi MA. Novel mechanism for preventing mutations caused by oxidation of guanine nucleoside. *EMBO Reports* 2003; **4**: 479–83.
36 Chevillard S, Radicella JP, Levalois C *et al*. Mutations in *OGG1*, a gene involved in the repair of oxidative DNA damage, are found in human lung and kidney tumours. *Oncogene* 1998; **16**: 3083–6.
37 Hung RJ, Hall J, Brennan P, Boffetta P. Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. *Am J Epidemiol* 2005; **162**: 925–42.
38 Sakumi K, Tominaga Y, Furuichi M *et al*. Ogg1 knockout-associated lung tumorigenesis and its suppression by Mth1 gene disruption. *Cancer Res* 2003; **63**: 902–5.
39 Isogawa A. Functional cooperation of Ogg1 and Mutyh in preventing G : C → T : A transversions in mice. *Fukuoka Igaku Zasshi* 2004; **95**: 17–30.
40 Klungland A, Rosewell I, Hollenbach S *et al*. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci USA* 1999; **96**: 13 300–5.
41 Minowa O, Arai T, Hirano M *et al*. Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. *Proc Natl Acad Sci USA* 2000; **97**: 4156–61.
42 Xie Y, Yang H, Cunanan C *et al*. Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-*ras* oncogene in lung tumors. *Cancer Res* 2004; **64**: 3096–102.
43 Kunisada M, Sakumi K, Tominaga Y *et al*. 8-Oxoguanine formation induced by chronic UVB exposure makes Ogg1 knockout mice susceptible to skin carcinogenesis. *Cancer Res* 2005; **65**: 6006–10.
44 Ohtsubo T, Nishioka K, Imaiso Y *et al*. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. *Nucleic Acids Res* 2000; **28**: 1355–64.
45 Al-Tassan N, Chmiel NH, Maynard J *et al*. Inherited variants of MYH associated with somatic G : C to T : A mutations in colorectal tumors. *Nat Genet* 2002; **30**: 227–32.
46 Sieber AM, Lipton L, Crabtree M *et al*. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med* 2003; **348**: 791–9.
47 Ushijima Y, Tominaga Y, Miura T *et al*. A functional analysis of the DNA glycosylase activity of mouse MUTYH protein excising 2-hydroxyadenine opposite guanine in DNA. *Nucleic Acids Res* 2005; **33**: 672–82.
48 Sakai A, Nakanishi M, Yoshiyama K, Maki H. Impact of reactive oxygen species on spontaneous mutagenesis in *Escherichia coli*. *Genes Cells* 2006; **11**: 767–78.
49 Toyokuni S, Mori T, Dizdaroglu M. DNA base modifications in renal chromatin of Wistar rats treated with renal carcinogen ferric nitrilotriacetate. *Int J Cancer* 1994; **57**: 123–8.
50 Inoue M, Kamiya H, Fujikawa K *et al*. Induction of chromosomal gene mutations in *Escherichia coli* by direct incorporation of oxidatively damaged nucleotides. New evaluation method for mutagenesis by damaged DNA precursors *in vivo*. *J Biol Chem* 1998; **273**: 11 069–74.
51 Yoshimura D, Sakumi K, Ohno M *et al*. An oxidized purine nucleoside triphosphatase, MTH1, suppresses cell death caused by oxidative stress. *J Biol Chem* 2003; **278**: 37 965–73.
52 Gu Y, Parker A, Wilson TM, Bai H, Chang DY, Lu AL. Human MutY homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human MutS homolog 2/human MutS homolog 6. *J Biol Chem* 2002; **277**: 11 135–42.
53 Kawate H, Sakumi K, Tsuzuki T *et al*. Separation of killing and tumorigenic effects of an alkylating agent in mice defective in two of the DNA repair genes. *Proc Natl Acad Sci USA* 1998; **95**: 5116–20.
54 Hidaka M, Takagi Y, Takano TY, Sekiguchi M. PCNA-MutSα-mediated binding of MutLα to replicative DNA with mismatched bases to induce apoptosis in human cells. *Nucleic Acids Res* 2005; **33**: 5703–12.
55 Kamath AV, Yanofsky C. Sequence and characterization of *mutT* from *Proteus vulgaris*. *Gene* 1993; **134**: 99–102.
56 Bullions LC, Mejean V, Claverys JP *et al*. Purification of the MutX protein of *Streptococcus pneumoniae*, a homologue of *Escherichia coli* MutT. *J Biol Chem* 1994; **269**: 12 339–44.


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