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Review Article
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Role of Base Excision Repair Enzyme MUTYH in the Repair of 8-Hydroxyguanine and MUTYH-Associated Polyposis (MAP)

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Abstract

8-Hydroxyguanine (8OHG) is an oxidized form of guanine, and the formation of 8OHG in DNA causes a G:C to T:A transversion mutation, since 8OHG can pair with adenine as well as cytosine. The base excision repair gene *MUTYH* encodes a DNA glycosylase for adenine mispaired with 8OHG and is thus involved in the prevention of mutations caused by 8OHG. Biallelic mutations of the *MUTYH* gene are responsible for MUTYH-associated polyposis (MAP), which is a hereditary disease and is characterized by a predisposition to multiple colorectal adenomas and carcinomas. This article reviews the repair function of MUTYH towards 8OHG, the functional characterization of MUTYH variants, the characteristics of MAP tumors, and the management of MAP patients.

Keywords: APC; Base excision repair; Colorectal cancer; DNA glycosylase; 8-hydroxyguanine; MUTYH; MUTYH-associated polyposis

Abbreviations: AFAP; Attenuated Familial Adenomatous Polyposis; BER; Base Excision Repair; CRC; Colorectal Cancer; CI; Confidence Interval; E. coli; Escherichia coli; FAP Familial Adenomatous Polyposis; HhH; Helix-hairpin-Helix; 8OHG; 8-Hydroxyguanine; LOVD; Leiden Open Variation Database; LOH; Loss of Heterozygosity; MSI; Microsatellite Instability; MTS; Mitochondrial Targeting Signal; MEF; Mouse Embryonic Fibroblast; MAP; MUTYH-Associated Polyposis; OR; Odds Ratio; ORF; Open Reading Frame; PCR; Polymerase Chain Reaction; 9-1-1; Rad9-Rad1-Hus1; SIR; Standardized Incidence Ratio

Introduction

8-Hydroxyguanine (8OHG) is an oxidized form of guanine [1]. The formation of 8OHG in DNA and the failure to remove the modified lesion before replication causes a G:C to T:A mutation, since 8OHG can pair with adenine as well as cytosine [2-5]. OGG1, MUTYH (formerly MYH/hMYH), and MTH1 constitute the 8OHG repair pathway in human cells (Figure 1) [6]. Both OGG1 and MUTYH are DNA glycosylases that initiate base excision repair (BER) for 8OHG; OGG1 removes 8OHG from 8OHG mispaired with cytosine (C) [7-9], and MUTYH removes adenine (A) from A:8OHG mispairs [10-14]. MTH1 hydrolyses 8-hydroxy-dGTP in a nucleotide pool to a monophosphate form so that 8-hydroxy-dGTP cannot be incorporated into DNA [15]. The importance of this system for avoiding mutations caused by 8OHG is shown by a direct association between a germline *MUTYH* abnormality and the occurrence of colorectal polyposis and cancer. Nowadays, the multiple colorectal adenomas and carcinomas caused by biallelic inactivating germline mutations in the *MUTYH* gene have been termed MUTYH-associated polyposis (MAP; OMIM #608456) [16,17]. MAP is distinct from other hereditary syndromes featuring colorectal polyposis and carcinomas, such as familial adenomatous polyposis (FAP; OMIM #175100) and attenuated familial adenomatous polyposis (AFAP; OMIM #175100), both of which are caused by a germline mutation in the *APC* gene [18,19]. However, the MAP phenotype partly resembles that of AFAP [18,20]. Here, we review the research findings regarding the functional role of MUTYH, the functional characterization of MUTYH variants, the characteristics of MAP tumors, and the management of MAP patients.

Functional Role of MUTYH

A human homolog of the *Escherichia coli* (*E. coli*) *mutY* gene was cloned and named *hMYH* in 1996 [21]. Later, the name was changed to the *MUTYH* gene. The *MUTYH* gene encodes a DNA glycosylase and is one of the members that participates in BER, a kind of DNA repair system. Based on its DNA glycosylase activity, the following functional assays have been performed for MUTYH: a DNA glycosylase assay, a mutation assay, a binding assay, measurement of the 8OHG residue, and a survival assay.

The MUTYH protein has several functional domains: a helix-hairpin-helix (HhH) motif, a pseudo HhH motif, an iron-sulfur cluster loop motif, a NUDIX hydrolase domain, an intracellular localization

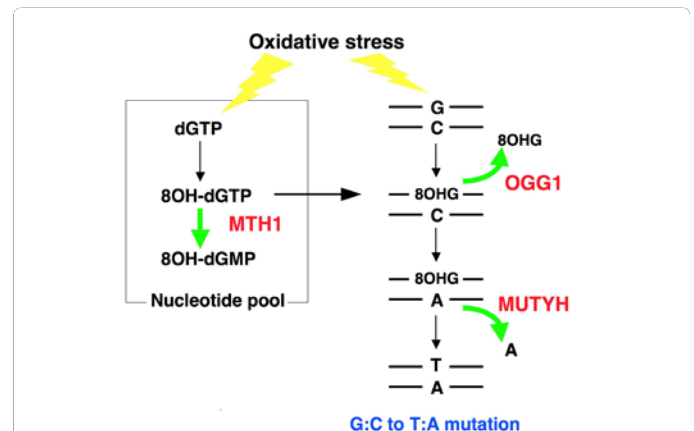

Figure 1: Pathways for the removal of 8-hydroxyguanine (8OHG) in human cells. Oxidative stress causes the formation of 8OHG in DNA and 8-hydroxy-dGTP (8OH-dGTP) in the nucleotide pool. 8OHG paired with cytosine (C) is removed by OGG1 and adenine (A) paired with 8OHG is removed by MUTYH. If the 8OHG:A is not repaired, A pairs with thymine (T) in the next round of replication, thus leading to a G:C to T:A transversion mutation. 8OH-dGTP in a nucleotide pool is hydrolyzed to a monophosphate form by MTH1 so that the 8OH-dGTP can not be misincorporated into DNA.

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sequence, and binding sites for RPA, MSH6, APE1, the Rad9-Rad1-Hus1 (9-1-1) complex, and PCNA [22]. The accumulated results of previous biochemical analyses have suggested that the HhH motif is very important for DNA glycosylase activity. However, other motifs are also indispensable for MUTYH to properly function in cells. After the recognition and catalysis of damaged bases by DNA glycosylase, there are two pathways for completing BER: a single nucleotide insertion pathway (short-patch repair), and a long-patch repair pathway that involves the resynthesis of 2-10 nucleotides [23,24]. The short-patch repair uses POLB for the resynthesis step and requires APE1, XRCC1, PARP1, and either LIG1 or LIG3, while the long-patch repair is PCNA-dependent and involves APE1, RFC, PCNA, RPA, PARP1, FEN1, POLD/POLE, and LIG1. As mentioned above, MUTYH binds to RPA, APE1, and PCNA, suggesting that MUTYH repair is involved in the long-patch BER pathway [25]. However, Dantzer et al. [26] suggested that A:8OHG is repaired in a more complicated fashion in mammalian cells. Hashimoto et al. [27] stated that MUTYH-initiated short-patch BER is futile, and this BER must proceed to long-patch repair, even if it is initiated as a short-patch repair. Later, van Loon and Hübscher [28] observed the specific recruitment of MUTYH, POLL, PCNA, FEN1, and LIG1/LIG3 from human cell extracts to A:8OHG DNA, but not to undamaged DNA, and reconstituted the full pathway for the faithful repair of A:8OHG mismatches in a manner that involved the MUTYH, POLL, FEN1, and LIG1, suggesting the role of POLL in the catalysis of accurate long-patch BER of 8OHG initiated by MUTYH. According to their recent research, MUTYH appeared to promote the stability of POLL by binding it to chromatin [29].

In addition to the binding of MUTYH with PCNA *in vitro*, MUTYH expression is increased in S-phase and remains elevated through mitosis and is associated with replication foci and PCNA *in vivo*, suggesting the role of MUTYH in replication-coupled repair [30,31]. During normal DNA replication, MUTYH coordinates with PCNA; however, in the event of DNA damage, MUTYH is thought to recruit the 9-1-1 checkpoint complex, which in turn enhances the DNA glycosylase activity of MUTYH [32-34]. The interdomain connector between the catalytic domain and the 8OHG recognition domain of MUTYH is a critical element that maintains interactions with the 9-1-1 complex [33]. Luncsford et al. [35] performed a crystal structural analysis for a fragment of MUTYH for the first time and revealed that the interdomain connector adopts a stabilized conformation projecting away from the catalytic domain to form a docking scaffold for the 9-1-1 complex.

Although the *Saccharomyces cerevisiae* MSH2/MSH6 heterodimer (MutSα) binds to A:8OHG mismatches and is involved in their repair [36], MUTYH, not MutSα, is the major protein in human cell extracts recognizing A:G and A:8OHG mismatches by UV cross-linking [37]. Interestingly, MUTYH is physically associated with human MutSα via MSH6, and the DNA glycosylase activity of MUTYH towards A:8OHG mismatches is enhanced by human MutSα suggesting that MUTYH and mismatch repair proteins cooperate to reduce replication errors caused by oxidatively damaged bases [38].

Some reports suggest that MUTYH is phosphorylated *in vivo* [35,39,40]. Kundu et al. [40] showed that MUTYH is phosphorylated at serine 524, which is within the PCNA-binding region, and Ser524 phosphorylation is involved in A:8OHG mismatch recognition by the characterization of phosphomimetic (Ser524Asp) and phosphoablating (Ser524Ala) mutants. Thus, MUTYH activity is likely to be modulated *in vivo* by post-translational modifications.

The excess accumulation of 8OHG in nuclear and mitochondrial DNAs under oxidative stress leads to cell death, and MUTYH is involved in cell death via the induction of single-strand breaks [41]. The involvement of MUTYH in cell death is compatible with other paper's results that synthetic sickness/lethality by the inhibition of specific polymerase in mismatch repair deficient cells was rescued by MUTYH silencing [42].

Cellular exposure to $CoCl_2$ triggers transcriptional changes that mimic the hypoxic response, and one mechanism underlying these changes is the increased generation of reactive oxygen species. Wang et al. [43] found that MUTYH expression is increased in rat neuronal PC12 cells exposed to hypoxia-mimicking concentration of $CoCl_2$. They suggested that the increase in MUTYH expression is associated with cellular DNA damage. Recently, the *MUTYH* gene was shown to be transcriptionally regulated by p73, a p53 family member protein, under DNA-damaged conditions, suggesting a role of p73 in the regulation of DNA damage repair [44].

DNA glycosylase assay

The recognition mechanism for damaged DNA-specific glycosylases have been characterized as "base-flipping" and involves the outward rotation of nucleotides from the DNA double-strand helix [45]. This allows the damaged base to be assessed by fitting into base-specific pockets on the DNA glycosylases. The DNA glycosylase encoded by the *MUTYH* gene recognizes and excises an incorporated adenine opposite 8OHG and an adenine opposite guanine. This activity was reported based on a DNA glycosylase assay performed a few years after the first cloning report of the *MUTYH* gene [10-14]. In addition to A:8OHG and A:G substrates, it was subsequently found that a 2-hydroxyadenine opposite guanine was recognized and catalyzed by MUTYH [46]. The concentrations of both salt and Mg^{2+} have been reported as factors influencing the DNA glycosylase activity of MUTYH [11,12].

Two major MUTYH proteins, i.e., type 1 and type 2, are expressed in human cells as a result of the presence of the alternative splicing of mRNA transcripts and multiple transcription initiation sites [11,14,47]. Type 1 is composed of 535 amino acids and is localized in the mitochondria because of a mitochondrial targeting signal (MTS) in its N-terminal. Type 2 lacks the N-terminal 14 amino acids of type 1, which contain the MTS, and as a result type 2 is localized in the nucleus [11,14,47]. Both types have sufficient DNA glycosylase activity, but the activity of the type 2 protein is greater than that of the type 1 protein under certain conditions [12].

A mutation to asparagine at Asp222 that corresponds to the *E. coli mutY* active site residue Asp138 led to the complete loss of DNA glycosylase activity, suggesting that Asp222 is an active site of MUTYH [48]. The loss of DNA glycosylase activity through the amino acid change has also been shown in other papers [49,50].

Mutation assay

The presence of 8OHG in DNA causes a G:C to T:A transversion mutation, since 8OHG directs the incorporation of cytosine and adenine nucleotides opposite the lesion [2-5]. MUTYH has the ability to suppress the G:C to T:A transversion mutation via its ability to remove adenine mispaired with 8OHG, which has been shown under several experimental conditions. Some research groups have reported that MUTYH suppresses the spontaneous mutation frequency in an *E. coli mutM mutY* mutant or an *E. coli mutY* mutant, as shown using a rifampicin resistance assay [10-12,51,52]. This assay enables the

observation of spontaneous mutations at the rifampicin binding site of *E. coli* RNA polymerases [52]. The accumulation of mutations in an RNA polymerase will render rifampicin less effective as a block to transcription, allowing the propagation of cells even in the presence of the drug. The mutation frequency can then be related to the number of rifampicin resistant colonies relative to the control plates [52]. Using this assay, a high frequency of spontaneous mutations in an *E. coli* defective for *mutY* and complementation of the *E. coli* mutants by MUTYH overproducing plasmids were observed [10-12,51,52]. Hirano et al. [53] showed that the mutation rate was 2-fold increased in *MUTYH*-null mouse embryonic stem cells by a fluctuation assay and that the mutation rate was suppressed in the cells by the expression of exogenous mouse MUTYH. A shuttle vector containing a single 8OHG was utilized for the mutation assay by the other groups [54-56]. In their experiments, an 8OHG:C mispair was introduced at the specific site of the *supF* gene and the shuttle plasmid containing the 8OHG:C mispair was transfected into mammalian cells (Figure 2). The replicated plasmids were then introduced into an indicator *E. coli* and the mutation frequency was calculated. The introduction of 8OHG leads to the elevation of the mutation frequency, especially a G:C to T:A mutation. So far, the role of MUTYH in preventing this mutation induction has been demonstrated in the H1299 lung cancer cell line, the 293T embryonic kidney cell line, and the AGS gastric cancer cell line [54-56].

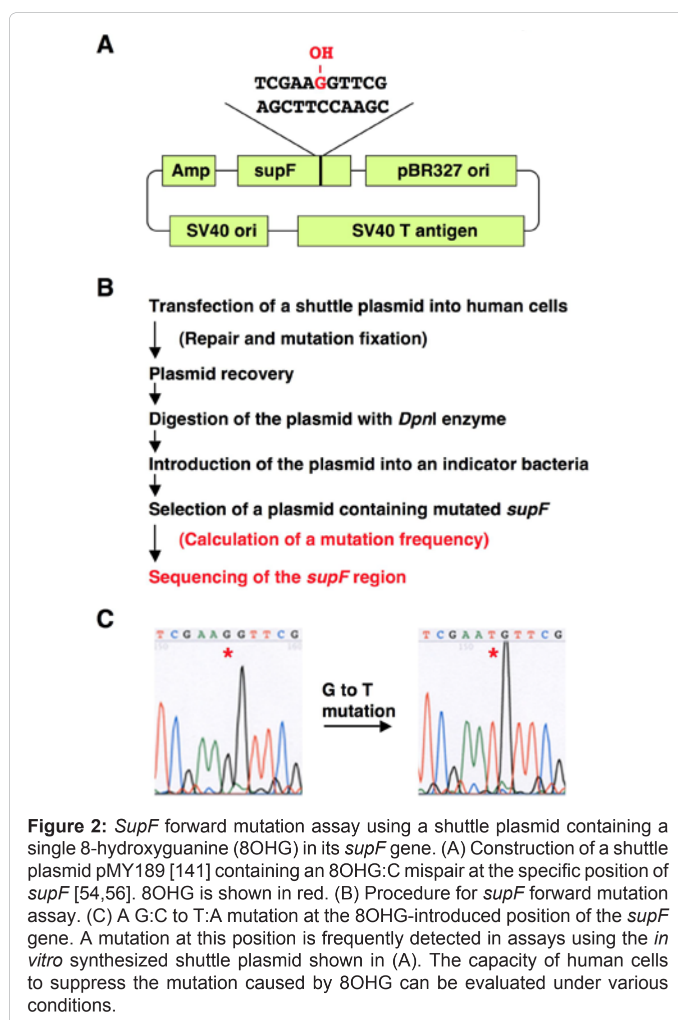

Figure 2: *SupF* forward mutation assay using a shuttle plasmid containing a single 8-hydroxyguanine (8OHG) in its *supF* gene. (A) Construction of a shuttle plasmid pMY189 [141] containing an 8OHG:C mispair at the specific position of *supF* [54,56]. 8OHG is shown in red. (B) Procedure for *supF* forward mutation assay. (C) A G:C to T:A mutation at the 8OHG-introduced position of the *supF* gene. A mutation at this position is frequently detected in assays using the *in vitro* synthesized shuttle plasmid shown in (A). The capacity of human cells to suppress the mutation caused by 8OHG can be evaluated under various conditions.

Other assays

The binding activity of MUTYH towards an A:8OHG mispair-containing oligonucleotide is shown [11,49,57]. An electrophoresis mobility shift assay and a surface plasmon resonance assay are used for the measurement of binding activity. The measurement of 8OHG residues in DNA has also been attempted to assess the MUTYH activity. The amounts of 8OHG residue in DNA at a steady-state level are significantly higher in mouse embryonic fibroblasts (MEFs) derived from *MUTYH* null mice than in wild-type MEFs [58]. The introduction of human MUTYH into the $MUTYH^{-/-}$ MEFs complemented the 8OHG content in DNA, meaning that MUTYH has the ability to regulate the amount of 8OHG residue in DNA.

MUTYH Gene Targeting in Mice

Three groups generated *MUTYH*-null mice. Xie et al. [59] reported no significant difference in the tumor incidence in *MUTYH*-null mice, as compared with the control littermates. They also showed that deficiencies in both *MUTYH* and *Ogg1* genes predispose these mice to develop tumors, predominantly lung and ovarian types, as well as lymphomas and, to a lesser extent, gastrointestinal tract tumors. Sakamoto et al. [60] reported the development of more spontaneous tumors, including intestinal tumors, in *MUTYH*-null mice than in wild-type mice. They also showed that the occurrence of small intestinal tumors dramatically increased in *MUTYH*-null mice treated with $KBrO_3$, a known inducer of oxidative stress in DNA. Sieber et al. [61] reported that $APC^{Min/+}/MUTYH^{-/-}$ mice developed significantly more adenomas in the small intestine and mammary tumors than did $APC^{Min/+}/MUTYH^{+/+}$ or $APC^{Min/+}/MUTYH^{+/-}$ mice. The results of all the above papers suggest that MUTYH has a role in preventing tumorigenesis.

MUTYH Germline Mutations

The association of the *MUTYH* gene mutations with multiple colorectal adenomas and carcinomas was first demonstrated in 2002 by Al-Tassan et al. [16]. They showed that 11 tumors from three affected siblings in a family contained 18 somatic inactivating *APC* mutations and that 15 (83%) of these mutations were G:C to T:A transversions; the proportion was significantly higher than the proportion of G:C to T:A mutations in previously reported somatic *APC* mutations from sporadic colorectal adenomas and carcinomas. They also showed that the siblings were compound heterozygotes for the missense *MUTYH* mutations p.Tyr165Cys and p.Gly382Asp. After their findings, an autosomal recessive disorder characterized by multiple colorectal adenomas and carcinomas arising from biallelic germline *MUTYH* mutations was named MAP, and many reports of the MAP patients have since been accumulated [20].

Reference MUTYH sequence

The mRNA transcript variant α3 (NM_001048171.1) encoding type 1 protein (535 amino acids) has been used as a reference MUTYH sequence. Recently, however, in accordance with the nomenclature rules of the Human Genome Variation Society, the transcript variant α5 (NM_001128425.1), which encodes the longest isoform (549 amino acids), was selected for use as a reference. The latter variant was also chosen in the Leiden Open Variation Database (LOVD) for the *MUTYH* gene (http://chromium.liacs.nl/LOVD2/colon_cancer/home.php?select_db=MUTYH) [62]. By changing the reference sequence, the annotation of the above mentioned p.Tyr165Cys and p.Gly382Asp became p.Tyr179Cys and p.Gly396Asp, respectively. In this review

article, we used the new reference sequence. For the genomic reference sequence, NG_008189.1 was used.

MUTYH germline variants

The first description of *MUTYH* germline variants detected through a screening of a population was reported in a scientific paper published in 2001 [63], and the first functional characterization of MUTYH germline variant proteins was reported in 2000 [12]. After the finding in 2002 that the *MUTYH* gene is responsible for MAP [16], mutational screening for *MUTYH* was performed worldwide and the number of detected variants has since been increasing. So far, 299 unique DNA variants have been reported in the LOVD database, and a significant proportion of these variants has been reportedly found in MAP patients [20,62-89]. Since the DNA variants not influencing the enzymatic activity of MUTYH are not disease-causing variants, all the variants should be tested to determine whether their enzymatic activities are reduced or not. A DNA glycosylase assay, a binding assay, complementation assays in *E. coli mutY* mutants and mouse *MUTYH*-null ES cells, a survival assay, and a splicing assay have been used to evaluate MUTYH variants. When assessing the activities of recombinant MUTYH variant proteins, because of the difficulty in preparing the recombinant human MUTYH proteins, a counterpart amino acid in *E. coli* and mouse homologues of human MUTYH is often substituted into a variant form and the obtained recombinant products have been utilized for functional tests in some papers. Even when human MUTYH variant proteins are analyzed, some papers use type 1 mitochondrial form proteins while other papers use type 2 nuclear form proteins, meaning that there is no standard method at present. In another system, MUTYH variants are evaluated in cell lines derived from patients with a MAP phenotype [90]. To date, p.Arg97X, p.Tyr104X, p.Tyr179Cys, p.Arg182His, p.Arg185Trp, p.Arg241Trp, p.Arg245His, p.Arg245Leu, p.Val246Phe, p.Met283Val, p.Gly286Glu, p.Pro295Leu, p.Gln338Arg, p.Gln338X, c.1145delC, p.Leu388Pro, p.Gln391X, p.Gly396Asp, p.Pro405Leu, p.Glu480del, and p.Glu480X have been shown to be functionally defective [48-50,52,57,58,90-94]. p.His448Asp can excise adenine from an A:8OHG mispair, but not from an A:G mispair [94]. A partially reduced activity was detected for p.Trp152_Met153insIleTrp and p.Ile223Val [49,50,57,58]. Regarding p.Gly396Asp, its repair activity was only partially reduced in some papers [49,57]. The repair activity of p.Arg274Gln was shown to be severely defective in one paper [94], but to be only partially defective in another paper [49]. The repair activities of p.Val22Met, p.Val75Glu, p.Arg309Cys, p.Ala373Val, and p.Ser515Phe have been shown to be retained [49,50,93,94]. Regarding p.Gln338His, which is a common polymorphic form and is detected worldwide, there was no apparent difference in a DNA glycosylase assay and complementation assay in *E. coli* in one paper [12], but a partial reduction in DNA glycosylase activity was detected in another paper [49]. Since the evaluation system, experimental conditions, and definitions of a partial reduction in repair activity differed among the papers, these circumstances have caused some confusion regarding our understanding of the repair activities of some MUTYH variants.

Type 2 nuclear form MUTYH protein is synthesized from both β- and γ-type transcripts, and the IVS1+5G/C variant in the β-type transcript was identified by Yamaguchi et al. [95]. The variant corresponds to c.37-1960G>C in the α-type transcript. The authors found that the variation causes alternative splicing, and the presence of the upstream open reading frame (ORF) on the 5'-side of the native ORF in the β-type transcript from the IVS1+5C allele reduces the translation efficiency of the transcript into the nuclear form protein. Therefore, the IVS1+5C allele may be associated with a reduction in the nuclear form of MUTYH protein in human cells.

The AluYb8 variant, which is an AluYb8 insertion variation, in intron 15 of the *MUTYH* gene is common in the Chinese population [96]. Very interestingly, the allele containing this Alu sequence has been demonstrated to be associated with an increased level of 8OHG in the leukocytic DNA of the carriers [96,97]. The AluYb8 variant is also associated with an increase in the plasma concentration of interleukin 1 of the carrier, suggesting a link between the variant and the inflammation [96]. Future investigations are warranted to know the effect of this common MUTYH variant on various pathological conditions.

Individuals with both c.53C>T and c.74G>A, which are associated with the amino acid substitutions of p.Pro18Leu and p.Gly25Asp, respectively, in the same allele have been reported [63,98], and this missense changes are located near to the functional N-terminal MTS sequence. Wild-type type 1 protein containing MTS is known to be localized in the mitochondria [11,14,47], whereas Chen et al. [99] reported that the MUTYH protein with both p.Pro18Leu and p.Gly25Asp is localized in both the nucleus and mitochondria, suggesting that the *MUTYH* haplotype variation causes the functional differences.

Among the uncharacterized variant MUTYH proteins, there exist truncated proteins caused by nonsense mutations and frameshift mutations. These proteins are estimated to be defective if they lack large parts of MUTYH. However, to evaluate with accuracy whether they are disease-causing alleles or not, they should be functionally evaluated. For a splice-site variation, a splicing assay should be done. At present, the main method for evaluating MUTYH activity is a DNA glycosylase assay for an A:8OHG substrate. In addition to this, the utilization of other methods would increase the accuracy of the evaluation of the repair activity of MUTYH variant proteins.

MUTYH mutation screening

To search for *MUTYH* mutations at a non-specific site, polymerase chain reaction (PCR) amplification of the *MUTYH* gene locus and subsequent direct sequencing of the product are a common method for detecting mutations. A high-resolution melting analysis and PCR-single-strand conformation polymorphism analysis may also be utilized [100]. When searching for specific mutations, several methods such as a TaqMan PCR assay [88], and a tetra-primer amplification refractory mutation system PCR assay exist [101]. Since large deletions destroying the *MUTYH* gene in MAP patients have very recently been reported by two groups [102,103], a combination of the fine evaluation of the allele number and a mutation search for the *MUTYH* gene locus is better for the screening of germline *MUTYH* abnormalities in candidate MAP patients.

To date, there are no generally acknowledged screening criteria for *MUTYH* genetic testing [104]. However, the testing in patients with multiple colorectal polyps and early-onset CRC has been suggested [69,72,105,106].

Characteristics of MAP

Most biallelic *MUTYH* carriers have between 10 and a few hundred colorectal polyps [20]. Thus, there does not seem to be a phenotypic overlap with severe FAP (>1,000 adenomatous polyps). Nielsen et al. [107] analyzed the data of 257 MAP patients from three research groups and stated that the mean age at the presentation of MAP in symptomatic patients is 45 years (range 12-68 years). They also

described that 58% of the MAP patients developed CRC, and the mean age at the time of the diagnosis of CRC was 48 years (range 21-70 years). Regarding the CRC risk, Theodoratou et al. [108] evaluated 20,565 patients with CRC and 15,524 controls for the p.Tyr179Cys and p.Gly396Asp *MUTYH* mutations and showed that the biallelic *MUTYH* mutation status conferred a 28-fold increase in the CRC risk [95% confidence interval (CI) 6.95-115]. Lubbe et al. [104] analyzed a population-based series of 9,268 patients with CRC and 5,064 controls for the p.Tyr179Cys and p.Gly396Asp *MUTYH* mutations and found almost the same result for the CRC risk in biallelic *MUTYH* mutation carriers. They also found that the estimated penetrances at 50 and 60 years of age were 19.5% (95% CI 11.7-31.4) and 42.9% (95% CI 30.5-57.9), respectively, suggesting that the biallelic *MUTYH* mutations are highly penetrant. However, there are some differences in penetrance between their results and the results by Farrington et al. [105].

Characteristics of CRC in MAP patients

CRC in MAP patient is frequently localized in the proximal colon [104,107]. MAP tumors show a high frequency of somatic G:C to T:A mutations in the *APC* and *KRAS* genes [16,64,107,109,110]. The G to T mutation at the GAA sequence of the *APC* gene is well known [16]. Among the *KRAS* mutations, most of them are c.34G>T associated with an amino acid substitution from glycine to cysteine at codon 12 [80,107,109,110]. Microsatellite instability (MSI) is known to be a characteristic of a part of CRCs, and the status of MSI in MAP CRCs has been examined in many studies. Although there are some MAP CRCs with an MSI phenotype [111,112], the majority of MAP CRCs are microsatellite stable [104,107,113,114]. As another characteristic, a high frequency of tumor infiltrating lymphocytes in MAP CRC has been detected in some papers [107,109,114]. The frequent loss of expression of human leukocyte antigen class I was also found in one paper [115].

A high proportion of MAP CRCs are near diploid [109]. A single nucleotide polymorphism microarray analysis for 26 MAP CRCs showed that the CRCs mainly contained the chromosomal regions of copy-neutral loss of heterozygosity (LOH) (71%) in addition to their near-diploid pattern (52%) [116]. In the paper, copy-neutral LOH was suggested to be an important mechanism in the tumorigenesis of MAP. In another whole genome analysis (array comparative genomic hybridization) for 25 colorectal samples derived from 5 MAP patients, a high frequency of aneuploid change in MAP polyps was pointed out [117]. Frequent losses at chromosomes 1p, 17, 19, and 22 and gains affecting chromosomes 7 and 13 have also been detected in MAP adenomas. The different technical platforms and sample preparations may have caused the difference between the above two results.

In a European study cohort, better survival for patients with MAP CRC than for matched control patients with CRC has been shown [118]. The underlying mechanism is at present unclear; however, the authors have discussed some possible biases (selection bias, lead-time bias, and so on) and immune response differences in the paper.

According to the recent research by Nieuwenhuis et al. [119], the CRC risk in MAP patients is not associated with the number of colorectal polyps. The research group also found that about 10% of the patients presenting with polyposis or CRC had developed a primary or metachronous CRC within 5 years of follow-up. These are important findings for the understanding of adequate patient management.

Extracolonic lesions

Vogt et al. [120] evaluated the extracolonic lesions in a cohort of 276 MAP patients from a European multicenter study (Germany, UK, and the Netherlands). Duodenal polyposis occurred in 17% of the MAP patients and the standardized incidence ratio (SIR) of duodenal cancer was 129 (95% CI 16-466), whereas the lifetime risk was 4%. They observed a significant increase in the incidence of ovarian (SIR 5.7, 95% CI 1.2-16.7), bladder (SIR 7.2, 95% CI 2.0-18.4), and skin cancers (SIR 2.8, 95% CI 1.5-4.8) and a trend for increased risk of breast cancer among cases. Therefore, MAP patients are likely susceptible to some types of cancers, in addition to having CRC as their chief symptom.

Clinical management of MAP patients

In the guidelines for the clinical management of MAP [121], the suggested surveillance protocol for MAP patients was similar to that for patients with AFAP. Thus, performing a colonoscopy every 2 years beginning at 18 - 20 years of age is recommended. An upper gastrointestinal endoscopy is also advised beginning at between 25 and 30 years of age. The recommended intervals between screenings depend on the disease severity determined according to the Spigelman classification [122,123]. Surgical treatment of colonic polyposis is also described in the guidelines [121] as follows. If the number of adenomas is small, these polyps can be removed endoscopically in some patients. When surgery is required, a total colectomy with ileorectal anastomosis would be sufficient in most cases to eliminate the cancer risk.

Monoallelic *MUTYH* Variants

Since MUTYH protein has the ability to repair damaged DNA and to avoid the generation of mutation, researchers have considered the possibility that monoallelic variation carriers may have a higher risk of disease and many case-control studies investigating whether *MUTYH* variants are statistically associated with disease onset have been actually performed in various populations.

Evaluation of CRC risk

Many papers have investigated the association of the monoallelic *MUTYH* mutant with the CRC risk; however, in most of them, an independent statistical significance was not obtained for the CRC risk [118]. Three meta-analyses of people from mainly European and North American countries have been recently published [104,108,124]. Lubbe et al. [104] performed a meta-analysis for a total of 18,160 patients and 12,822 controls and found the odds ratio (OR) for all the carriers of monoallelic p.Tyr179Cys and p.Gly396Asp mutations to be not significantly different from unity (OR 1.14, 95% CI 0.96-1.36, P=0.12). In a pooled meta-analysis by Theodoratou et al. [108], the OR for carriers of a monoallelic *MUTYH* mutant was calculated to be 1.16 (95% CI 1.00-1.34). Win et al. [124] described that the association between a monoallelic *MUTYH* mutation carrier for any variant and the CRC was estimated to have a pooled OR of 1.15 (95% CI 0.98-1.36). Thus, even if the risk for CRC is increased in monoallelic *MUTYH* mutation carriers, the level is likely to be modest.

Some papers have investigated the association of MUTYH variants not showing severe reduction of its repair activity and the CRC risk. Regarding the p.Gln338His polymorphism, in a small study composed of 68 Japanese CRC patients and 121 controls, patients with His338-containing alleles were not at an increased risk of CRC [125]. In an association study of 1,785 CRC cases and 1,722 controls from Sweden, an increased risk was associated with the homozygous variant of p.Gln338His in rectal cancer (OR 1.52, 95% CI 1.06-2.17, P=0.02), but not in colon cancer [126]. In another association study of 685 CRC cases and 778 controls from Japan, *MUTYH* variants of c.36+11C>T, c.504+35G>A, c.934-2A>G, and c.1014G>C (p.Gln338His) were examined, and a haplotype containing c.36+11T, c.504+35G, c.934-

2A, and c.1014C was demonstrated to be associated with an increased risk of CRC (OR 1.43, 95% CI 1.005-2.029, *P*=0.04) [127]. Moreover, a statistically significant association was demonstrated between the c.36+11T and an increased CRC risk (OR 1.43, 95% CI 1.012-2.030, *P*=0.04) in that paper. Regarding the p.Pro18Leu and p.Gly25Asp variants, in an association study of 138 CRC cases and 343 controls from China, a haplotype variant allele containing p.Pro18Leu and p.Gly25Asp was shown to be a risk factor of CRC [99].

Evaluation of risk for cancers other than CRC

Some *MUTYH* variants have been investigated for the association with the risk of cancers other than CRC in various populations. In a small study of 101 gastric cancer cases and 129 controls from China, a haplotype variant allele containing p.Pro18Leu and p.Gly25Asp was shown to be a risk factor for gastric cancer [98]. In a study of 148 gastric cancer cases and 292 controls from Japan, Tao et al. [128] examined the distribution of c.934-2A>G but did not detect any association between the variant and the gastric cancer risk. Figueroa et al. [129] examined one intronic *MUTYH* variant and the risk of bladder cancer, but no associations were detected. In a study of 545 breast cancer patients, 762 gastric cancer patients, and controls from China, a *MUTYH* allele containing an AluYb8 insertion was shown to be a risk factor for early-onset breast and gastric cancer [130]. In a study of 108 Japanese lung cancer patients and 121 controls, the lung cancer risk of individuals homozygous for a His338 allele of p.Gln338His compared with individuals homozygous for a Gln338 allele was statistically significant [131]. However, the contribution of p.Gln338His to an increased risk of lung cancer was not observed in an association study of 276 lung cancer cases and 103 controls from the United Kingdom [132] and in another study of 581 non-small cell lung cancer cases and 603 controls from China [133]. No associations between the *MUTYH* variants of p.Val22Met, p.Gln338His, and p.Ser515Phe and the risk of endometrial cancer were obtained in an Australian population [134].

Evaluation of risk for diseases other than cancer

In a study of 565 cases of type 2 diabetes mellitus and 565 controls from China, a *MUTYH* allele containing an AluYb8 insertion was shown to be a risk factor for the disease [97].

MUTYH Abnormality in Non-MAP Tumors

Compared with the examination of *MUTYH* germline mutations, only a limited number of papers examining the status of somatic *MUTYH* mutation in sporadic CRC have been published. Halford et al. [66] reported no somatic mutations of the *MUTYH* gene in 75 sporadic CRC samples collected in the United Kingdom. Vasovcak et al. [135] also reported no somatic *MUTYH* mutations in 103 sporadic CRC samples from Czech patients. Gushima et al. [136] examined 21 ulcerative colitis-associated neoplasia collected in Japan and reported no somatic *MUTYH* mutations. On the other hand, Bougatef et al. [137] found two somatic *MUTYH* mutations in 48 Tunisian sporadic CRC. Considering the results of the above four reports, it seems that somatic *MUTYH* mutations are rare in human sporadic colorectal neoplasia.

Several papers have documented the *MUTYH* abnormality in sporadic gastric cancers. Kim et al. [138] detected two biallelic somatic *MUTYH* mutations (p.Pro405Ser and p.Gln414Arg) from 95 gastric cancers collected in Korea, although they did not examine the level of adenine DNA glycosylase activity for the mutant proteins. Goto *et al.* [139] examined 23 gastric cancer patients exhibiting both 8OHG accumulation and low inflammatory cell infiltration in the stomach for germline *MUTYH* mutations and found no pathogenic mutations. Regarding MUTYH expression in gastric cancer, the mRNA and protein expression levels were reportedly reduced in gastric cancer specimens collected in Japan and, interestingly, the reduced protein expression of MUTYH in the gastric cancer specimens was an independent predictor of a poor survival outcome in an analysis of 353 sporadic gastric cancers [56]. Also in the paper, the MUTYH expression level in gastric cancer cells was shown to define the capacity to repair oxidatively damaged DNA, the capacity to regulate mutation frequency, and the capacity to regulate cellular proliferation. Kobayashi et al. [140] examined 30 Japanese gastric cancer patients and two aberrant transcripts were found more frequently in cancer specimens (67%) than in normal mucosa (10%). The results of the above papers imply that a MUTYH abnormality is involved in a subset of gastric cancers.

In one study, *MUTYH* mutational screening was performed for 66 primary sclerosing cholangitis patients with or without cholangiocarcinoma [94]. Two heterozygous mutations showing a (partial) functional reduction (p.Arg274Gln and p.His448Asp) were found, while the others were polymorphisms. Since MUTYH has the ability to regulate the mutation rate in human cells, MUTYH is considered to have the potential to be associated with various diseases. Therefore, the investigation of MUTYH abnormalities in various pathological settings would be interesting.

Conclusions

The functional role of MUTYH, the evaluation of *MUTYH* variants, the characteristics of MAP tumors, and the management of MAP patients have been reviewed. MUTYH is a DNA glycosylase initiating the excision repair of A in A:8OHG mispairs in DNA. Specific MUTYH mutants are defective in the BER, and such impairments can be detected by a DNA glycosylase assay, mutation assay, and binding assay. MAP tumors show a high frequency of somatic G:C to T:A transversions in the *APC* and *KRAS* genes. MAP CRC is frequently localized in the proximal colon and is microsatellite stable, and the mean age at the diagnosis of CRC is 48 years. An increased incidence of some types of extracolonic carcinomas has been shown in MAP patients. A suggested surveillance protocol for MAP patients is similar to that for patients with AFAP: a colonoscopy performed every 2 years beginning at an age of 18 - 20 years. The past 15 years since the first report of *MUTYH* cloning have contributed enormously to our understanding of *MUTYH*, and biallelic *MUTYH* mutations are now, without a doubt, associated with a susceptibility to colorectal polyposis and carcinomas in humans.

Since the diagnosis of MAP depends on the presence of clinical phenotype characteristics for MAP and the level of repair activity of the MUTYH variants encoded in the two *MUTYH* alleles of the patient, even when gene variations are found in a candidate patient by *MUTYH* mutation screening, information on the level of repair activity of the MUTYH variants is indispensable for making a proper diagnosis of MAP. However, at present, only a small number of *MUTYH* variations have been analyzed for their repair ability. Thus, further effort is needed to evaluate uncharacterized MUTYH variations.

The first crystal structural analysis of MUTYH has been recently done, and the central part of the MUTYH protein containing a catalytic domain and an interdomain connector was analyzed. Further crystal structural analysis of MUTYH, especially the full form of MUTYH covalently complexed with an A:8OHG mispair-containing DNA, may contribute to establishing the correlations between the MUTYH structure and repair function and between MUTYH mutations and impaired repair.

Recently, much progress has been made in technology for whole genome sequencing analyses and "-omics" analyses. The future investigation of MAP tumors using such technology may enrich our knowledge of the molecular and biological characteristics of MAP tumors.

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