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

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 misincorporated 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 polypsis and cancer. Nowadays, the multiple colorectal adenomas and carcinomas caused by biallelic inactivating 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), Lynch syndrome (OMIM #123200), hereditary nonpolyposis colorectal cancer (HNPCC; OMIM #604500), and attenuated familial adenomatous polyposis (AFAP; OMIM #192100), all 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 loop motif, a NUDIX hydrolase domain, an intracellular localization motif, and a survival assay.

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). 8OH-dGTP is a nucleotide pool in a monophosphate form that cannot be misincorporated into DNA. The 8OH-dGTP is removed by OGG1 and MUTYH. If the 8OHG-A is not repaired, A pairs with thymine (T) in the next round of replication, leading to a G:C to T:A transversion mutation. 8OH-dGTP is hydrolyzed to a monophosphate form by MTH1 so that the 8OHG cannot be misincorporated into DNA.
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 the 9-1-1 checkpoint complex, which in turn enhances the DNA glycosylase activity of MUTYH [32-34]. 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 (MutSa) binds to A:8OHG mismatches and is involved in their repair [36], MUTYH, not MutSa, 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 MutSa via MSH6, and the DNA glycosylase activity of MUTYH towards A:8OHG mismatches is enhanced by human MutSa 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 (Ser524 Ala) 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 CoCl2, 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 CoCl2. 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 Mg2+ 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 MTSs, 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 residue [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 *mutT* 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 leads to the elevation of the mutation frequency, especially a G:C to T:A mutation. The introduction of 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 KBrO₃, a known inducer of oxidative stress in DNA. Sieber et al. [61] reported that *APCΔmin/+* MUTYH-/- mice developed significantly more adenomas in the small intestine and mammmary tumors than did *APCΔmin/+* MUTYH-/- or *APCΔmin/+* 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 a3 (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 a5 (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 a significant proportion of these variants has been reportedly found, 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 a significant proportion of these variants has been reportedly found, 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 a significant proportion of these variants has been reportedly found, 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].

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 asymptomatic 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 about 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 showing severe reduction of its repair activity and the CRC risk. Thus, even if the risk for CRC is increased in monoallelic mutation carriers, the level is likely to be modest.

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 not be 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-
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 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 GC 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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