Review Article

DNA repair dysfunction in gastrointestinal tract cancers

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The DNA repair system surveys the genome, which is always suffering from exposure to both exogenous as well as endogenous mutagens, to maintain the genetic information. The fact that the basis of this DNA repair system is highly conserved, from prokaryote to mammalian cells, suggests the importance of precise genome maintenance mechanisms for organisms. In the past 15 years, considerable progress has been made in understanding how repair processes interact and how disruptions of these mechanisms lead to the accumulation of mutations and carcinogenesis. In 1993, two groups reported that DNA mismatch repair could be associated with hereditary non-polyposis colorectal cancer, indicating a connection between faulty DNA repair function and cancer. More recently, an inherited disorder of DNA glycosylase, which removes mutagenic oxidized base from DNA, has been reported in individuals with a predisposition to multiple colorectal adenomas and carcinomas. This is the first report that directly indicates the role of the repair of oxidative DNA in human inherited cancer. Studies from gene knockout mice have elucidated the principal role of these repair systems in the process of carcinogenesis. Moreover, clinical samples derived from cancer patients have shown the direct involvement. This review focuses on the function of DNA mismatch repair and oxidative DNA/nucleotide repair among various DNA repair systems in cells, both of which are essentially involved in the carcinogenesis of gastrointestinal tract cancer. (*Cancer Sci* 2008; 99: 451–458)

Considering the huge spectrum of damage that the genome can suffer from either spontaneously or from exposure to genotoxic environmental agents, it is quite reasonable that cells possess a multitude of mechanisms to confront these events. For example, mutagenic nucleotide substrate and endogenous DNA lesions generated during normal cellular metabolism as well as errors made during replication of undamaged template DNA could be sources for spontaneous mutation. Initially, the molecular mechanisms of spontaneous mutagenesis were elucidated by genetic analyses of *Escherichia coli* mutator strains, and further research has also demonstrated that the basic mechanisms are well conserved evolutionally among various organisms. However, the most definitive difference between a prokaryote and a mammalian is the response to lethal DNA damage. The prokaryote can tolerate lethal damage by adaptation due to the SOS response; in contrast, the mammal might remove highly damaged cells using a specific self-avoiding system called apoptosis.

Recently, considerable progress has been made in understanding how the repair process interacts and how disruptions of these mechanisms lead to the accumulation of mutations and carcinogenesis. In particular, important processes have been elucidated from syndromes that predispose some patients to develop cancer and from inductive methods using gene-targeting approaches, which thus make it possible to investigate those functions *in vivo* in mice. *hMSH2* is the human homolog of bacterial *MutS*, which is responsible for the correction of base/base mismatch as well as insertion/deletion misalignment with other proteins. In 1993, Fishel *et al*. reported that mutations of *hMSH2* could be identified in the familial lines of patients with HNPCC.[1] At the same time, Leach *et al*. cloned the *MutS* homolog using linkage analysis and detected germline mutation of this gene in HNPCC kindreds.[2] These are reports that proved the connection of the disorder of DNA repair function and cancer, particularly in colorectal cancer. Although, oxidative DNA damage has been implicated in cancer etiology, there were no reported human inherited disorders attributed to the repair of oxidative DNA damage by 2002. Al-Tassan *et al*. reported that bi-allelic germline mutations in *MUTYH*, which removes mutagenic oxidized bases from DNA, are present in individuals with a predisposition to multiple colorectal adenomas and carcinomas.[3] This is the first observation to directly elucidate the role of the repair of oxidative DNA in human inherited cancer.

To date, numerous reports from investigations of the role of DNA repair in counteracting the carcinogenesis process have been published. This review article focuses on the MMR and repair against oxidative DNA/nucleotide damage to clarify their correlations to human gastrointestinal cancers and elucidate their roles in avoiding mutation accumulation and carcinogenesis using gene knockout mice *in vivo*.

DNA mismatch repair

In mammalian cells, the MMR system is involved in the correction of errors that arise during DNA replication, DNA damage surveillance, and the prevention of recombination between non-identical sequences.[4] MMR was initially identified for correcting errors made during replication of undamaged template DNA; however, it has been demonstrated that it is involved in the repair or removal of oxidative DNA damage[5] as well as DNA modified by chemicals such as alkylating agent, cisplatin, and 5-fluorouracil.[6] MLH1 and MSH2 are the MMR proteins that are most frequently implicated in an MMR deficiency.[7] MMR genes (*MSH2*, *MSH3*, *MSH6*, *MLH1*, *MLH3*, *PMS1*, *PMS2*) are mutated or inactivated by hypermethylation of the promoter, as a somatic, epigenetic phenomenon.[8]

Cells with a defective MMR system generate mutations at a rate up to 100-fold higher than the rate observed in normal cells, both in mammalian cells and tissues of mice *in vivo*.[9–11] Loeb

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Abbreviations: 8-oxoG, 8-oxo-7,8-dihydroguanine; APC, adenomatous polyposis coli; CRC, colorectal cancer; FAP, familial adenomatous polyposis; HNPCC, hereditary non-polyposis colorectal cancer; LOH, loss of heterozygosity; MAP, MUTYH-associated polyposis; MMR, mismatch repair; MSI, microsatellite instability; OR, odds ratio; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; SCC, squamous cell carcinoma.

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Table 1. Phenotype of mismatch repair-deficient mice

Targeted gene	Increase in mutation frequency†	Cancer susceptibility	Sites of cancer	Reference no.
Msh2	7–160	Cancer prone	lymphoma GI tract Skin	(14–16)
Msh3	2	Low‡	GI tract	(21)
Msh6	2–13	Cancer prone	Lymphoma GI tract	(19)
Mlh1	7–100	Cancer prone	GI tract Lymphoma	(20,22)
Mlh3	–	Cancer prone	GI tract Lymphoma	(17)
Pms1	–	No		(20)
Pms2	5–100	Cancer prone	Lymphoma Sarcoma	(20)

†Data of mutation frequencies were obtained from published studies.(9–11) ‡Overall tumor incidence is not different from wild type, but these mice developed gastrointestinal tract cancer.(21) GI, gastrointestinal.

proposed that this increased mutation frequency (i.e. mutator phenotype) may facilitate the occurrence of mutations in other genes that govern genetic stability, and regulate the cell cycle and apoptosis, therefore causing carcinogenesis.(12) Changes in the length of nucleotide repeats at microsatellite loci, that is, MSI, were observed in cancer cells, and regarded as an important phenotype that reflects the mutator phenotype of cancer cells with MMR deficiencies.(13)

The MMR repair gene knockout mouse was first reported in 1995, 2 years after the *MSH2* gene was cloned. *Msh2*$^{-/-}$ mice were viable but susceptible to malignancies, particularly lymphomas, at an early age.(14,15) Reitmair *et al*. reported that intestinal carcinomas occurred at an older age while the majority of mice developed lymphoma at an early age.(16) Thereafter, other genes involved in MMR were disrupted by a gene-targeting technique and phenotypes were reported.(17–22) As summarized in Table 1, among seven genes involved in mismatch recognition in mammalian cells, all but the *Pms1* gene knockout mice showed the predisposition of cancer development at a more rapid or slower duration from birth.(14–22) In general, *Msh2*$^{-/-}$, *Mlh1*$^{-/-}$ and *Pms2*$^{-/-}$ mice developed lymphoma at an early stage and *Msh2*$^{-/-}$, *Mlh1*$^{-/-}$ mice also tended to generate intestinal carcinomas at a relatively later stage. In contrast, *Msh3*$^{-/-}$, *Msh6*$^{-/-}$ and *Mlh3*$^{-/-}$ mice developed lymphoma and gastrointestinal cancer later than *Msh2*$^{-/-}$ and *Mlh1*$^{-/-}$ developed lymphomas.

Tissue specimens derived from MMR showed increased mutation frequency. The effect of this elevation differs from the target sequence of monitor genes, for example, in the presence or absence of mononucleotide repeat. In general, *Msh2*$^{-/-}$, *Mlh1*$^{-/-}$ and *Pms2*$^{-/-}$ mice show a high frequency of mutation, whereas *Msh3*$^{-/-}$ and *Msh6*$^{-/-}$ mice showed relatively low levels of increased mutation frequencies.(9–11) Considering the mismatch processing, the loss of MSH2 or MLH1 function results in a complete depletion of the MutSα/MutLα (and β) and MutSβ/MutLα (and β) hetero-complex, therefore inactivating the repair of both deletion/insertion misalignment and base/base mismatch. Cells mutated in either one of these two genes therefore show strong mutator phenotypes. In contrast, the mutation of MSH3 or MSH6 results in the depletion of MutSβ/MutLα (and β) or MutSα/MutLα (and β) hetero-complex, respectively. MutSβ/MutLα (and β) recognizes not only deletion/insertion misalignment but also partially recognizes base/base mismatch, and similarly MutSα/MutLα (and β) recognizes not only base/base mismatch but partially recognizes deletion/insertion misalignment. These overlapping recognition activities suppress the mutator effects. Interestingly, mice that showed a high frequency of spontaneous mutation in their tissue developed tumors in an early stage; in contrast, mice with a mild mutator phenotype developed tumors at a late stage. These observations strongly support the idea of the 'mutator phenotype in cancer' proposed by Loeb.(12) However, it is important to note that these observations apply in the case of 'care taker' genes. There was a report that the 'gate keeper' gene, such as *p53*-deficient mice, didn't show elevated mutation frequency.(23)

An MSI analysis is indeed an efficient approach for detecting defective MMR, because MMR include proteins from several gene products and MMR genes have no marked hot spots for mutation in their long coding region. However, the reported frequency for MSI-positive tumors in each malignancy differs widely in the literature.(24) As these discrepancies may relate to problems in the methods used,(13) the authors have used fluorescence-labeled primers and an auto-sequencer for laser scanning to detect precisely the alteration of microsatellite loci.(13) As summarized in Table 2a, the frequencies of MSI in esophageal, stomach, and colorectal cancer analyzed using fluorescent primers and an auto-sequencer were 8%, 24%, and 34.7%, respectively.(24–26) Using this precise fluorescence system, Oda *et al*. pointed out the qualitative difference in the form of alterations at microsatellite loci.(27) In brief, one alteration was drastic and was frequently observed in more than two analyzed loci, whereas the other was subtle and often only observed in one locus. Therefore, the former and latter alterations might be closely connected to MSI-H and MSI-L, respectively. The relationship between MSI and defective MMR may be more complicated than has been suspected, which is fully described in the review article.(24) Interestingly, an extremely high frequency of MSI (all alterations were subtle and might be categorized as MSH-L) was observed in tumors from MSH2-deficient mice (A. Egashira, T. Tsuzuki, Y. Maehara, unpublished data, 2007), whereas the reported frequency of MSI in tumors from MMR deficient animals is not always high.(28)

Because mutations of the MMR genes were identified in the families of patients with HNPCC,(1,2,29) which are among the most common hereditary human cancers, many studies have reported both the hereditary and sporadic cancers. Germline mutations in one of four major HNPCC-associated MMR genes, *MSH2*, *MLH1*, *MSH6* and *PMS2*, are detected in up to 70–80% of such families. Among all mutations reported as predisposing MMR gene mutations, mutations of *MSH2* or *MLH1* account for more than 80%, whereas *MSH6* and *PMS2* mutations account for less than 15% and *MLH3* mutations account for only a small percentage.(8) The mutation of the *PMS1* gene is questionable, because a re-examination of the originally reported HNPCC-like family with a *PMS1* mutation showed this family to have an additional *MSH2* mutation that co-segregated with colon cancer in the family.(30) In addition to the relatively lower frequencies of *MSH6*, *PMS2* and *MLH1* mutations, mutations in these genes often occurred in the families with atypical HNPCC who showed late onset disease. These characteristics are in line with the phenotype observed in analogous gene-disrupted mice. Pms1-knockout mice do not show cancer susceptibility; in contrast to Msh6-, Pms2- and Mlh3-knockout mice, which show a mild elevation of the mutation frequency and later develop tumors in older age.(17,19,20)

DNA mismatch repair dysfunction in gastrointestinal tract cancers

Table 2b shows the results from several studies analyzing the rate of impaired MMR genes and MSI in sporadic esophageal, gastric and colorectal cancers. Esophageal cancer is the sixth most common cause of cancer death in the world. There are two

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Table 2. Mismatch repair dysfunction in sporadic gastrointestinal tract cancer

(a) Frequencies of MSI observed in gastric, stomach, and colorectal cancer

	MSI†	Reference no.	Frequencies cited in the literature‡
(i) Esophagus	8% (MSI-L 8%) (MSI-H 0%)	(26)	5–25%
(ii) Stomach	24% (MSI-L 13%) (MSI-H 11%)	(24)	13–44%
(iii) Colon	34.7% (MSI-L 23.1%) (MSI-H 11.6%)	(25)	15–50%

†MSI is analyzed using fluorescent primers and an auto sequencer.(13) ‡Frequencies of MSI are reviewed in the article.(24) MSI, microsatellite instability.

(b) Involvement of MMR genes in esophageal, gastric, and colorectal cancer

MMR gene	Proportion	Method used for analysis	Reference no. of alteration
(i) Esophagus			
MSH2, MLH1, MSH6 MSH3, PMS2	14%	*In vitro* MMR assay′	(31)
MLH1	33%	MSP	(32)
MSH2, MLH1	28.7%	IHC	(33)
(ii) Stomach			
MLH1	31%	MSP, IHC, RT-PCR	(36)
MLH1	32%	MSP, IHC, WB	(37)
MSH2, MLH1	0%	IHC	(40)
MSH2, MLH1	32–36%	IHC	(39)
(iii) Colon			
MSH2, MLH1 (among MSI)	7%	Mutation or LOH	(43)
MLH1 (among MSI-H,MSS)	25–64%	IHC (64%), MSP (42%) Mutation (26%)	(46)
MSH2 (among MSI)	<1%	Mutation	(44)
MSH2, MLH1	12%	IHC	(45)
MSH6 (among MSI-L)	7–17%	IHC (17%) Mutation (7%)	(47)
MSH2, MLH1, MSH6 PMS2	0–12%	IHC	(48)

IHC, immunohistochemistry; LOH, loss of heterozygosity; MMR, mismatch repair; MSI, microsatellite instability; MSP, methylation-specific PCR; RT-PCR, reverse transcription-polymerase chain reaction; WB, western blotting.

major histological types of esophageal cancer: SCC and adenocarcinoma. Although the latter type has been increasing, particularly in the USA and Europe, the former histological type is predominant worldwide. Although esophageal cancer is not a tumor associated with the HNPCC spectrum, there are several reports analyzing MSI and/or MMR function. Araki *et al*. reported that the frequency of MSI was 8% and 4%, respectively, in patients with esophageal SCC in Japan and China.(26) One represented a relatively high frequency of MSI in esophageal carcinoma.(24) Those authors excluded the suspicious MSI/LOH, which could not be distinguished from LOH by the method used. The suspicious MSI/LOH should not be included when considering the high frequencies of LOH observed in esophageal cancer. Uchida *et al*. reported that no mutation or aberrant expression was found in the MMR genes in esophageal SCC cell lines, but the MMR activity was somehow reduced in 3 of 22 cell lines analyzed.(31) Recently, Guo *et al*. reported MLH1 hypermethylation in esophageal SCC with MSI.(32) However, neither the frequency of MSI, particularly MSI-H, nor the deficient expression of MLH1 protein is necessarily high.(33) Furthermore, the role of MMR in carcinogenesis of the esophagus might be low, and these studies are supported by few reports in which MMR-deficient mice develop esophageal cancer.

Gastric cancer is the second most common extra-colonic malignancy in HNPCC.(34) A large cohort study in Germany showed that either a *MSH2* or *MLH1* germline mutation carrier would develop gastric cancer at the rate of 5.2% and 4.3%, respectively.(35) These reports clearly show a connection between germline mutation of the *MMR* gene and gastric cancer. However, the role of MMR in avoiding sporadic gastric carcinoma is different from that of hereditary disease. The frequencies of MSI in sporadic gastric cancer varies in the literature, from approximately 13–44%.(24) Using precise fluorescent analysis, the frequencies for MSI-H and MSI-L were determined to be 11% and 13%, respectively.(24) Sporadic mutations of MMR genes have not been comprehensively explored in gastric cancers, whereas inactivation of MLH1 due to hypermethylation of a promoter lesion are frequently observed in the gastric cancers with MSH-H.(36,37) These alterations of MLH1 are also detected in the adenomas of the stomach.(38) The expression of MMR protein was investigated in several reports, but the frequency also differs in the literature.(39,40)

HNPCC family members have a lifetime risk of 60–80% for developing CRC,(35) whereas HNPCC might account for 2–5% of all colorectal cancers.(41,42) Among all mutations reported in HNPCC, mutations of *MSH2* or *MLH1* account for more than

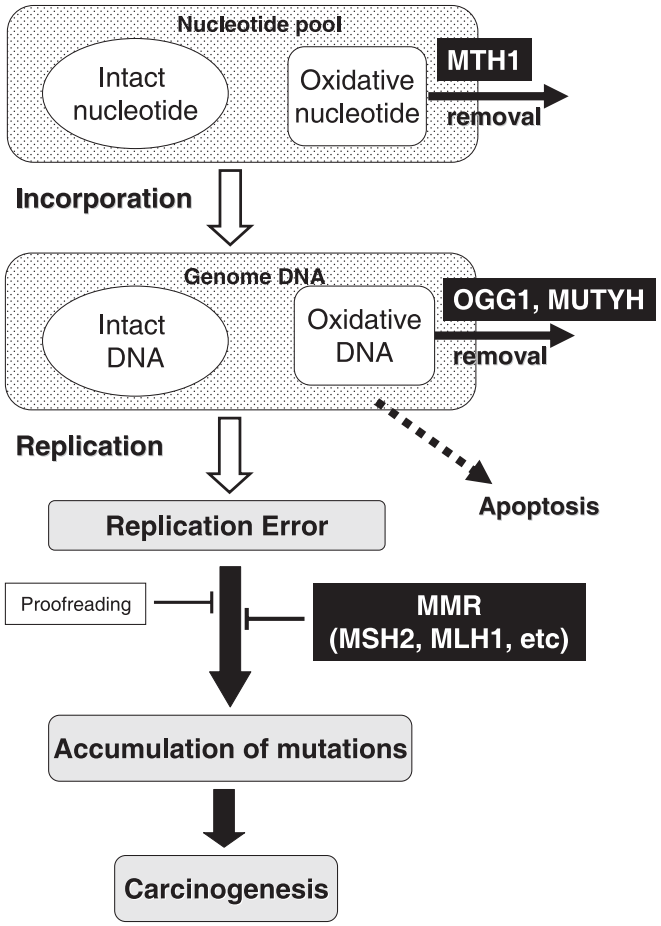

Fig. 1. Proposed process of carcinogenesis due to the accumulation of mutations. There is a considerable amount of oxidative DNA/nucleotides in the genome DNA or nucleotide pool. MTH1 catalyzes 8-oxodGTP to 8-oxodGMP, thus avoiding the incorporation of this mutagenic nucleotide into the genome. Oxidative DNA can be generated in the genome DNA or oxidative nucleotides can be incorporated into genome DNA from the nucleotide pool. OGG1 eliminate 8-oxo-7,8-dihydroguanine (8-oxoG) paired with cytosine, whereas MUTYH efficiently removes adenine paired with 8-oxoG in the genome DNA. In contrast, replicative DNA polymerase causes the mis-incorporation of nucleotides at a low frequency during replication using non-damaged nucleotides. Mismatch repair (MMR) is responsible for correcting these replication errors, which the proofreading activity of the DNA polymerase itself is unable to correct. If the capacity of these repair systems is impaired, then mutations may thus accumulate in an accelerated manner, thereby facilitating the process of carcinogenesis.

80%.[8] Most tumors observed in HNPCC families showed MSI-H.[7] Therefore, CRC from HNPCC families appears to be characterized by a high frequency of MSI-H and MMR gene mutation, particularly the *MSH2* and *MLH1* genes. However, the situation is somewhat different with sporadic CRC. Although the percentages of MSI range from 15 to 50% in the literature,[24] Ikeda *et al*. reported the frequency of MSI-L and MSI-H observed in CRC as 23% and 12%, respectively, using the precise fluorescent system.[25] Table 2b shows that the overall percentage of MMR gene alteration is not high.[43–48] In contrast to the HNPCC families in which tumors with germline mutation of *MSH2* or *MLH1* are closely connected with MSI-H, those who develop sporadic CRC with MSI-H harbor a few somatic *MSH2* or *MLH1* mutations. Sporadic CRC with MSI-H frequently shows inactivation of MLH1 due to hypermethylation of a promoter region, which is also observed in the gastric cancer with MSH-H. Jass *et al*. emphasized that there might be differences in the setting of tumors in HNPCC families and sporadic CRC with MSI-H.[49] In addition, the relationship between MSI, in particular MSI-H, and defective MMR may be highly complicated.[24]

Repair against oxidative DNA/nucleotide damage and the pathogenesis of its deficiency in mouse models

Cells are continuously attacked by reactive species. Oxygen radicals produced endogenously in the course of normal cellular metabolism, as well as in response to various environmental mutagens and ionizing radiation, can damage DNA and its precursors. Among a large variety of DNA lesions caused by oxygen radicals, an oxidized form of guanine, 8-oxoG, is thought to be a key lesion due to its abundance and its possible role in carcinogenesis and aging.[50] 8-OxoG nucleotides formed in DNA as well as 8-oxodGTP formed in the nucleotide pool induce mutations. 8-OxoG has a propensity to form base pairs with adenine as well as cytosine, therefore 8-OxoG in DNA causes a G:C to T:A transversion, if not repaired, while 8-oxo-dGTP incorporated into DNA during DNA replication causes both G:C to T:A and A:T to C:G transversions.[51] From *E. coli* to higher eukaryotes, organisms are equipped with elaborate mechanisms to counteract the mutagenesis caused by the oxidized nucleotides in both DNA and nucleotide pools. Three enzymes, MTH1, OGG1, and MUTYH, have been shown to play important roles in counteracting the accumulation of 8-oxoG in cellular genomes of human and rodent cells (Fig. 1).[52] MTH1 hydrolyzes 8-oxo-dGTP and 2-OH-dATP to their monophosphate forms and pyrophosphates, thereby preventing the incorporation of 8-oxo-dGTP and 2-OH-dATP into DNA during replication.[53] OGG1, an 8-oxoG DNA glycosylase, excises 8-oxoG opposite cytosine in DNA, which minimizes the formation of a pre-mutagenic base pair, A-8-oxoG. MUTYH, a mammalian homolog of *E. coli* MutY, is a DNA glycosylase that has been shown to excise 2-hydroxyadenine incorporated opposite guanine and adenine incorporated opposite 8-oxoG.[54] As a result, MUTYH is considered to play a crucial role in preventing G:C to T:A transversion in mammals.[52]

To analyze the function of these proteins *in vivo*, mutant mice were generated with each of these genes by targeted disruption (Table 1). Klungland *et al*. reported that Ogg1-deficient mice accumulated 8-oxoG in DNA.[55] Minowa *et al*. reported that Ogg1-deficient mice showed increased mutation frequency.[56] However, neither group observed the development of malignancies in these mice. However, Sakumi *et al*. reported that spontaneous lung adenoma/carcinomas developed in Ogg1-deficient mice 1.5 years after birth.[57] Tsuzuki *et al*. reported that Mth1-deficient mice developed malignancies in some organs, proving the direct connection between oxidative DNA damage and carcinogenesis in a mouse model.[58] Interestingly, the overall mutation frequency showed no apparent increase (less than twofold) in *Mth1*$^{-/-}$ mice, while MutT-deficient *E. coli* shows a 1000-fold increased mutation frequency.[10] Nevertheless, the frequency of 1-base pairs frameshift mutations at the mononucleotide runs in the reporter gene was 5.7-fold higher in the 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 could be attributed to the involvement of 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, It is interesting to note that mismatch repair may participate in the avoidance of 8-oxoG-related mutagenesis in mammalian cells, as a deficiency of MMR in Msh2-null mouse embryonic cells leads to an accumulation of 8-oxoG in the genome.[59] Xie *et al*. reported that no significant differences in

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Table 3. Phenotype of oxidative DNA/nucleotide repair-deficient mice

Targeted gene	Increased MF	Cancer susceptibility	Sites of cancer	Reference no.
Mth1	≤2	Cancer prone	Liver Lung Stomach	(10,58)
Ogg1	3	No		(55)
		Cancer prone	Lung	(57)
Mutyh	2†	No		(60)
		Cancer prone	Small intestine Spleen (Angiosarcoma)	(61)

†Obtained from embryonic stem cells.(82) MF, mutation frequency.

Table 4. Association between oxidative DNA/nucleotide repair dysfunction and sporadic gastrointestinal tract cancer

Gene	Mutation/polymorphism	Reference no.
(a) Esophagus		
MTH1	–	
OGG1	Ser326Cys	(75)
MUTYH	–	
(b) Stomach		
MTH1	Val83Met	(78)
OGG1	Arg154His	(71)
	Ser326Cys	(76)
MUTYH	Pro391Ser, Gln400Arg	(70)
(c) Colon		
MTH1	no correlation	(79)
OGG1	Arg154His	(73)
	Ser326Cys	(77)
MUTYH	Tyr165Cys, Gly382Asp	(67,68)

tumor incidence occur between Mutyh-null and wild-type mice within a 12-month period.(60) However, our examination of tumor development using a large cohort of wild-type and Mutyh-null mice at the age of approximately 18 months revealed an increased occurrence of tumors in various internal organs of Mutyh-null mice compared with wild-type mice.(61) The elevation of mutation frequencies observed in these mice was lower than those observed in *E. coli*, which demonstrate a 10–1000-fold higher frequency of mutation in comparison to wild type.(51) This difference might be due to the involvement of multiple anti-mutagenic pathways including other repair systems, such as a mismatch recognition mechanism, for the mutagenesis caused by oxidative DNA and nucleotide damage.(10) Xie *et al*. reported that *Ogg1*$^{-/-}$/*Mutyh*$^{-/-}$ mice are predisposed to lung adenoma/carcinoma, lymphoma and gastrointestinal adenoma/carcinoma, whereas the single mutant did not show predisposition for tumors.(60) Furthermore, it is of interest that the MMR system seems to be involved in the avoidance of mutagenesis caused by oxidative DNA damage.(5,10) Ogg1-, Mth1- or Mutyh-deficient mice develop tumors more frequently than wild-type mice at a later stage.(57,58,61) These observations might be correlated with the findings that the elevated mutation frequencies observed in these mice were not extremely high, in contrast to the Msh2-deficient mice that showed a strongly elevated mutation frequency and highly developed tumors at an earlier stage.(9,14,15) It should be mentioned that in *Saccharomyces cerevisiae* in which the orthologue of *MutY* does not exist, MMR and error-free translesion DNA synthesis could prevent the mutagenic effect of 8-oxoG in cooperation with Ogg1.(62,63) It is also of great interest that DNA polymerase η seems to efficiently promote the error-free incorporation of cytosine opposite 8-oxoG, whereas high-fidelity replicative DNA polymerase δ frequently mis-incorporate adenine opposite 8-oxoG, even in mammalian cells.(63) Furthermore, the fact that the accuracy of polymerase η was enhanced in the addition of PCNA and/or RPA, which is part of the replication machinery, thus suggesting how complicated it is to maintain the fidelity during replication containing 8-oxoG residues.(64)

Dysfunction of oxidative DNA/nucleotide damage repair in gastrointestinal tract cancer

Al-Tassan *et al*. reported a unique somatic mutation pattern of the *APC* gene and *MUTYH* hypomorphic mutation.(3) They analyzed a family that is affected with multiple colorectal adenomas and carcinomas but lacks the germline *APC* gene that is associated with FAP. They showed a high incidence of somatic G:C to T:A transversion mutations in the *APC* gene that is uncommon in tumors from classic FAP individuals and also demonstrated siblings with tumors that were compound heterozygous for *MUTYH* gene mutations (Tyr165Cys and Gly382Asp). Thereafter, the attenuated phenotype of inherited polyposis is proposed as MAP, which is an autosomal recessive disease with germline mutations in the *MUTYH* gene.(65,66) Carriers with a bi-allelic germline mutation of the *MUTYH* gene had an increased risk of colorectal cancer. Furthermore, carriers with a mono-allelic mutation also seem to have an increased risk of colorectal cancer;(67,68) however, further study with a large cohort is necessary to verify these observations. There are few reports that investigate the role of somatic *MUTYH* mutation in carcinogenesis (Table 4). Meanwhile, Halford *et al*. found no somatic mutations of *MUTYH* in any of 75 unselected CRC and CRC cell lines.(69) However, Kim *et al*. demonstrated that 2 of 95 sporadic gastric cancers had bi-allelic disruption of the *MUTYH* gene with somatic mutation of one allele and LOH of the remaining allele.(70)

Other reports have demonstrated a correlation between *OGG1* gene polymorphism and cancer susceptibility (Table 4). The Arg154His mutation was initially observed in a gastric cancer cell line(71) and this mutation was shown to alter the activity of this enzyme.(72) Kim *et al*. analyzed 625 CRC (including 29 FAP, 19 HNPCC, and 86 suspected HNPCC) and 527 normal control cases for *OGG1* Arg154His. They showed that Arg154His was a rare polymorphism associated with sporadic CRC ($P = 0.053$).(73) The OGG1 protein encoded by the Ser326 allele exhibited substantially higher activity than the Cys326 variants in an *in vitro E. coli* complementation activity assay.(74) Xing *et al*. investigated the association between Ser326Cys polymorphism and esophageal SCC among 201 normal controls and 196 patients with esophageal cancer in China. They found that individuals homozygous for the Cys/Cys genotype had a significantly increased risk of developing esophageal SCC, with an OR adjusted for age, sex, and smoking of 1.9.(75) Takezaki *et al*. investigated the association of the *OGG1* gene mutation with stomach cancer risk using 101 stomach cancer patients and 198 controls. In that investigation, the *OGG1* Ser326Cys polymorphism did not alter the overall ORs for stomach cancers; however, subgroup analyses revealed increased ORs with a frequent drinking habit in Cys/Cys carriers.(76) Kim *et al*. investigated 125 colon cancer patients and 247 controls. There was no significant difference in Ser326Cys genotype distribution between the patients and controls. Subgroup analysis revealed increased ORs with smoking or with frequent consumption of meat in Cys/Cys carriers, although the statistical significance of the former factor is marginal.(77)

For MTH1, a polymorphism of Val83Met has been found and studies have reported that Met83-MTH1 expressed in *E. coli* is

more thermolabile than Val83-MTH1, with both its secondary structure and 8-oxodGTPase activity.[52] Kimura *et al*. demonstrated that this polymorphic variation of MTH1 in gastric cancer patients occurs significantly more frequently than in healthy individuals. Furthermore, the *p53* mutation correlated with the variant form of MTH1. The frequency of the *p53* mutation was significantly higher in tumors harboring at least one Met83 allele than in those without the Met83 allele ($P = 0.034$).[78]

It is critical to carefully interpret the results of an association between polymorphism and cancer susceptibility. Recently, Schafmayer *et al*. investigated the variation of several repair genes in sporadic colorectal cancer and reported no association with cancer susceptibility, even in cases with the previously indicated risk variant.[79] This result could be affected by race or cohort scale, or even by the statistical methods used. Because the etiology of esophageal cancer is deeply connected with exposure to both tobacco and alcohol consumption, environmental factors as well as genetic factors are thus considered to play an important role in the process of carcinogenesis.[80]

Mutator phenotype in DNA repair-deficient cells and mice

An elevation of the mutation frequency could play a role in carcinogenesis in MMR-deficient cells and it might occur when there is a defect of some repair function against oxidative DNA/nucleotide damage. It is important to determine whether the proto-oncogene or the tumor suppressor gene could be mutated in DNA repair-deficient cells. As Al-Tassan *et al*. reported, germline *MUTYH* mutations seem to be correlated with *APC* tumor suppressor gene mutations in colonic adenoma and adenocarcinoma.[3] Because *Mutyh*-deficient mice were susceptible to intestinal tumor development, as observed in MAP patients, current experiments of analyzing mutations in the tumor-associated genes, such as the *Apc* gene, by amplifying genomic DNA derived from the intestinal tumors, found in *Mutyh*-deficient mice, would provide significant insights into the involvement of Mutyh function in oxidation-induced carcinogenesis. In addition, Xie *et al*. reported the tumors in $Ogg1^{-/-}/Mutyh^{-/-}$ mice to have mutations in the *K-ras* oncogene.[60] Kimura *et al*. showed a correlation between *MTH1* hypomorphic polymorphism and *p53* mutation in gastric cancer.[78]

Insertion/deletion mutations are observed mostly in the mononucleotide run within the cording region of several genes, such as *TGFβRII*, *BAX*, *MSH3*, etc. There might be base substitutions in the proto-oncogene or tumor suppressor gene. Oda *et al*. demonstrated that a *p53* gene mutation is strongly associated with a certain subtype of MSI,[27] in accordance with several reports that noted the connection between MSI-L and mutation of the proto-oncogene or tumor suppressor gene.[43] The authors observed several germline base substitutions within the cording region of proto-oncogenes and tumor suppressor genes in MSH2-deficient mouse tumors (A. Egashira, T. Tsuzuki, Y. Maehara, unpublished data, 2007). Although the possibility that the mutations observed merely resulted from phenotypical advantage could not be ruled out, these observations might support the idea of the mutator phenotype, which has been proposed by Loeb.

Perspectives

The detection of defective DNA repair in tumor tissue property may provide important information that can be used to guide the clinical management of patients. For instance, MMR-deficient cells are tolerant to DNA methylating agents, anti-metabolites, and intra-strand cross-linking agents,[4] which are commonly used for cancer treatment. There have been several reports that have demonstrated a correlation between MSI status and the effect of adjuvant chemotherapy. Furthermore, oxidative DNA/nucleotide damages could be generated by some therapeutic agents. DNA repair properties not only play an important role in avoiding carcinogenesis in gastrointestinal cancers, but may also control the effects of some drugs even if the tumor was completely removed macroscopically.[81] Understanding the DNA repair capacity of an individual may enable the personalized choice of effective therapeutic agents, most of which function by producing specific types of DNA damage in cancer cells. For this purpose, it is efficient to use animal models that can mimic the phenotype of the cancer by administering some drugs that specially eliminate or reduce specific repair functions. To elucidate the mechanism of DNA repair in carcinogenesis, the results from mice could be applied to humans, and these approaches enable us to achieve a comprehensive understanding of these complicated mechanisms discussed above.

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