Gastroenterology and Hepatology From Bed to Bench.

REVIEW ARTICLE

MUTYH the base excision repair gene family member associated with polyposis colorectal cancer

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ABSTRACT

Colorectal cancer is classified in to three forms: sporadic (70-75%), familial (20-25%) and hereditary (5-10%). hereditary colorectal cancer syndromes classified into two different subtypes: polyposis and non-polyposis. Familial adenomatous polyposis (FAP; OMIM #175100) is the most common polyposis syndrome, account for <1% of colorectal cancer incidence and is characterized by germline mutations in the adenomatous polyposis coli (APC, 5q21- q22; OMIM #175100). FAP is a dominant cancer predisposing syndrome, which 20-25% cases are *de novo*. There is also another polyposis syndrome; *MUTYH* associated polyposis (MAP, OMIM 608456), which is caused by mutation in *human Mut Y homologue MUTYH (MUTYH*; OMIM 604933) and it is associated with multiple (15-100) colonic adenomas. In this paper we discuss *MUTYH* mechanism as an important member of Base Excision Repair (BER) family and its important role in polyposis condition.

Keywords: Colorectal cancer, MAP, *MUTYH*, Base excision repair (BER).

(Please cite as: **Kashfi SMH, Golmohammadi M, Behboudi F, Nazemalhosseini-Mojarad E, Zali MR. *MUTYH* the Base Excision repair (BER) gene family member associated with colorectal cancer polyposis. Gastroenterol Hepatol Bed Bench 2013;6(Suppl.1):S1-S10).**

Introduction

It is estimated that 20,000 DNA damages occur in each cell per day (1). Gastrointestinal tract is a main target for oxidizing elements, which are highly mutagenic (2). Therefore, colorectal cancer is considered as a main cancer, which arises from exposure to this kind of agents. Beside mismatch repair (MMR) and nucleotide excision repair (NER) pathways, which are the fundamental repair pathways that interact with the mismatch pairs and aberrant nucleotide occurs in the replication process. Respectively, the base excision repair (BER) pathway is one of the main and primary DNA repair mechanisms that is involved in correcting the base mutations arised from oxidative, alkylation, deamination and depurination/ depyrimidination damages (3). *MUTYH* is a DNA glycosylase and it belongs to BER family. The *MUTYH* protein is involved in the repair of post-replicative mispairs within DNA replication (4,5).

MUTYH function and interactions

A human homologue of *Escherichia coli* (*E. coli*) *mutY* gene was first cloned in 1996 (6), while

Received: 13 May 2013 *Accepted*: 18 July 2013
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identification of the functional activity of the *MUTYH* gene goes back to 2000 (7). This gene is called *MUTYH* and is often known as *hMYH* or *MYH*. Although this is not a correct name, because *MYH* is the gene symbol for the myosin heavy-chain gene. *MUTYH* is located on the short arm of chromosome 1(1p34.1) and spans 11.2 kb. This gene is a DNA glycosylase, which is involved in the repair of post-replicative mispairs and plays a critical role in base excision repair (BER) pathway (4,5) .The oxidized form of guanine is 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxoG) which is considered as a frequent and stable element (8). In replication process 8-oxoG can pair with adenine as well as cytosine. The modified guanine (8-oxoG) is replicated in each round and the failure to remove the oxidized nucleotides before replication results in G:C to T:A transversion mutation (9,10).

MUTYH mediates to remove A from A:8-oxoG mispairs (7, 11) and OGG1 the other member of BER pathway detects and then removes 8-oxoG opposite cytosine (8-oxoG:C) (12-13). Thus the cooperation of OGG1 and *MUTYH* together prevents G:C to T:A transversion mutation due to oxidative damages within replication process.

When an aberrant base is incised and then removed, it produces a gap called apurinic/a pyrimidinic (AP) sites which are mutagenic and should be corrected quickly (14). Completion of the repair process requires involvement of many additional proteins. More than 8 specific proteins detect specific DNA mutations, which produce a basic or apurinic/ apyrimidinic (AP) sites (15).

Based on proteins involved in the process of BER activity, there are two main mechanisms to repair AP site created by DNA damage: short and long patch repair pathway (16).

Short-patch repair pathway involves making the association between *POLB*, *APE1*, *XRCC1*, *PARP1*, and either *LIG1* or *LIG3* genes. These related genes are activated when a single nucleotide insertion occurs and an AP endonuclease (also known as *APE1* or *APEX*) incises the incorrect matched DNA at the AP site resulting in the formation of a 3-hydroxyl end (3′OH) and a 5′ basic sugar phosphate end (5′dRP) (17). Since *MUTYH* has no AP-lyase activity, *APE1* detects a basic site and then proceed the excision process. At the end, repair procedure of an aberrant nucleotide is accomplished by DNA ligase III (16).

A long-patch repair pathway requires *PCNA, APE1, RFC, RPA, PARP1, FEN1, POLD/POLE* and *LIG1* for BER activity and they get involved when 2-10 nucleotides mispaired in a DNA strand genes. In long patch repair pathway cleavage process accomplished by AP endonuclease (*APEX1*), and repair process is completed by proliferating cell nuclear antigen (*PCNA*), which has different types of functions including DNA repair as well as cell cycle and DNA replication (3,18).

Among repair genes, *MUTYH* is the main protein that detects peculiar A:G and A:8-oxoG mispairs on DNA helix (19).

The *MUTYH* protein structure consists of many functional domains such as the N-terminal domain on the 5' side and the C-terminal domain on the 3' side. The N-terminal domain contains the catalytic region and includes a helix-hairpin-helix (HhH), pseudo HhH and an iron-sulfur cluster loop motif, which are also common regions in other BER glycosylases; the C-terminal domain on the 3' side of the *MUTYH* protein structure reported to have a role in recognition of 8-oxoG and shares homology with *MTH1* (member of the BER family) (20-22).

Association between *MUTYH* and Replication Protein A (*RPA*), Proliferating Cell Nuclear Antigen (*PCNA*), p73, p53 and APE1 has also reported in several studies (18, 23). Many papers suggested that in the damage condition, *PCNA* increases *MUTYH* activity (24-25). Association of *MUTYH* gene and MMR genes such as *MSH6*,

MSH2 and *MLH1* has also been discussed (26-28). Although most of the *APC* mutations produce truncated proteins, most pathogenic *MUTYH* variants are missense and splice site mutations and only a minority of variants is truncating mutations (29). The distribution of *MUTYH* mutations in MAP patients shows ethnic differences. Some variants are more common in other populations including: E480X in Indian (30), Y104X in Pakistani (31), c.1437_1439delGGA in Italian (32), c.1228_1229insGG in Portuguese (33), Q498H in German (34), and G25D and P18L in Chinese populations (35). Also, Y179C and G396D (previously known as Y165C and G382D) are two most common *MUTYH* mutations (80%) in Caucasian populations (30). Since only a few variants of *MUTYH* gene analyzed for their repair activity so far, it is recommended that other variants of *MUTYH* need to be examined for their involvement in pathogenesis of MAP (36).

Frequency of large deletions in *MUTYH* gene seems to be low and only two studies revealed the presence of large deletions in *MUTYH* gene so far (5,37). Loss of heterozygosity (LOH) of 1p is frequently happening in CRC tumors with chromosomal instability (CIN) (38). Since LOH is a common event in CRC tumors with CIN; LOH in MAP tumors display a distinct pattern of loss of heterozygosity with loss of parts of chromosomes without copy number alterations termed copy-neutral loss of heterozygosity, which is not a frequent event in CRC tumors with CIN (39-40). Croitoru et al showed that LOH detected in 20% of biallelic and 47% of monoallelic *MUTYH* mutation carriers (41). As demonstrated in several studies, microsatellite stable (MSS) is a dominant pattern of MSI in MAP tumors (42,43).

MUTYH association polyposis characterizations

Mutation in *MUTYH* gene causes a predisposing condition to CRC termed *MUTYH* association polyposis (MAP) (2, 30). Al Tassan et al first reported MAP while they were evaluating 'family N'. In this family three of seven siblings had a phenotype resemble with AFAP without aberrant mutation in *APC* gene, instead they observed that 11 tumors from three affected siblings had 18 somatic *APC* mutations which 15 mutations were G:C to T:A transversion mutations. This finding highlighted the possibility of deficiency in repair process of 8-oxoG mutations. They also reported that all three affecting siblings had biallelic mutation in *MUTYH* gene since it wasn't detected in rest of the four siblings (2). Mean age of diagnosis of MAP patients is 48 and patients have between 10 and 100 colorectal polyps. The penetrance of this syndrome is 20–80% between 50 and 80 years (42-44). The phenotype of MAP patients resembles with Attenuated Familial Adenomatous polyposis coli AFAP (AFAP; OMIM #175100) individuals (30, 44).

Diagnosis of MAP patients with cases present overlapping features or AFAP patients is difficult. Since they share some similarities such as number of polyps, proximal location of polyps and early onset of CRC (42-45). In MAP patient's polyps are frequently small and mostly located left side of the colon (42). Proximal location of polyps is the key point to distinguish cases with moderate adenomatous polyps from those of sporadic (42,46). In comparison with the general population CRC risk in MAP patients, associated with 28- to 93-fold (42, 47). Histopathologically adenomas (tubular or tubulo-villous) are detected in the entire colon are considered as predominant lesions in AFAP/FAP and also in MAP patients. Since serrated polyps: hyperplastic polyps, sessile serrated polyps (also referred to as sessile serrated adenomas) and traditional serrated adenomas are not present in affected harboring mutation in *APC* gene, they are common types of lesions in MAP patients (48). Finally, *APC* genetic testing for this group of patients with serrated polyps wouldn't be informative. Tumors in MAP patients show a high

frequency of distinctive somatic G:C to T:A mutations in the *APC* and *Kras* genes (2,30). GAA sequences in *APC* gene are the target sites for truncating mutations and this site is frequently mutated during tumorigenesis (2, 30, 49). *APC* has 216 GAA sites in which G:C→T:A mutations could happen and result in a termination codons (2). In contrast *TP53*, *PTCH*, *RB1*, *NF1* and *VHL* have fewer target sites and this makes the APC the best target than the other genes for mutagenesis in MAP tumors (49). It is notable that in *Kras* gene the hot spot codon is c.34G>T at codon12 (50, 51).

Screening and Management

Early detection, genetic counseling and *MUTYH* mutation screening are important in affected individuals and their siblings. Based on National Comprehensive Cancer Network NCCN recommendation, Colonoscopy starts at age 25 and patients with more than 10 adenomas should be referred for genetic counseling and testing procedure (52). Patients with less than 10 adenomas should be referred for follow up screening and genetic testing for this group of patients is not necessary.

Other surveillance protocol for MAP patients recommends similar screening program similar to AFAP patients. Patients undergo colonoscopy every 2 years starting at 18-20 years and upper gastrointestinal endoscopy starts when the affected patient is between 25 and 30 years of age (53, 54).

MUTYH mutation screening is recommended for people who are diagnosed with MAP and patients who have a recessive mutation transmission and phenotype similar to AFAP. The affected gene may not be seen in every generation and usually have a normal parents (55,56).

First of all, the two putative codons Y165C or G382D are examined for their high incidence rate in majority of populations. Then PCR sequencing is performed for the entire coding region and intron–exon boundaries of *MUTYH*. The genetic testing for *MUTYH* in patients with multiple serrated polyps without adenomas is not recommended by NCCN (52).

There haven't been any reports to define molecular screening in patients with MAP in Iran yet and research is ongoing to determine APC and *MUTYH* variants in FAP patients. However, screening of mutations in other genes associated with CRC carried out and other repair genes like *MLH1* and *MSH6* have been studied (57-60).

Prevalence

Approximately 0.3%–1% of all colorectal cancers is associated with MAP (41, 42).

It is estimated that 1% to 2% of the general population has a mutation in *MUTYH*. There isn't a peculiar criterion to classify nonpolyposis *MUTYH*-associated CRC phenotype. Therefore, it has been recommended that all early-onset CRC cases should be evaluated for *MUTYH* mutations (61). Several Studies demonstrated that up to 30% of biallelic *MUTYH* mutation carriers develop CRC although they do not present a polyposis condition (62). There has also been reported some cases with MAP and no polyps, whereas in some cases more than 500 colorectal polyps observed (44). *APC* germline mutations are not present in 10–30% of FAP patients and in up to 90% of AFAP patients (63). In other words, *APC* mutations are detected in 10–22% of AFAP cases and biallelic germline mutation of *MUTYH* were identified in 15–30% of AFAP patients and approximately 7–22% of FAP patients. Biallelic *MUTYH* mutations can be detected in 30% of *APC* mutation-negative patients. (30, 55, 64, 65).

Biallelic germline mutations in *MUTYH* are common in patients with negative *APC* related FAP patients and in MAP cases. Howver, recently studies are focused on monoallelic *MUTYH* variants in CRC patients, which try to identify the

association between monoallelic mutation susceptibility to CRC (66-68).

Monoallelic *MUTYH* mutation carrier's account for 1% to 2% of the general population since biallelic mutations observed in less than 1% of all CRCs and the frequency of this mutation in patients with 10 to 100 polyps are 28% and in individuals with 100 to 1000 polyps are 14% (44, 69).

First degree relatives of MAP patients with biallelic mutation in *MUTYH* gene are considered as obligate carriers who carry at least one *MUTYH* mutation. Both parents are carriers of a biallelic mutation and each child has 25% chance of inheriting two mutations. Whether monoallelic *MUTYH* carriers (heterozygote) are at high risk for developing CRC is still not clear. Compared with the general population there is evidence that obligate monoallelic *MUTYH* mutation carriers have a modest risk for colorectal cancer (47, 66). Some authors believe that heterozygote mutation carriers should be considered as low penetrance alleles, although a consensus surveillance guideline for this subgroup needs to be developed.

MUTYH and other cancers

Extracolonic manifestations are common in patients with MAP which include duodenal cancer and related polyposis, cancers such as gastric, small intestinal, endometrial, liver, ovarian, bladder, thyroid, breast and skin cancers including melanoma, squamous epithelial, and basal cell carcinomas (70,71). Other manifestations such as osteomas, dental cysts and congenital hypertrophy of the retinal pigment epithelium (CHRPE) are also seen in this group of patients (32, 71, 72). These extra colonic manifestations are also reported in FAP patients and the occurrence is less in MAP than in FAP or AFAP patients (73). The association between breast cancer and *MUTYH* gene is not defined clearly so far (71). Since Frequency of biallelic *MUTYH* mutations in breast cancer seems to be low (74,75) in a valuable paper by Wasielewski et al. reported heterozygote mutations in *MUTYH* gene in families with both CRC and breast cancer. Moreover, they have also reported that there was an increased risk for breast cancer in female MAP patients (76). The association of many malignancies such as endometrial (77,78), gastric (79,80), bladder (81), lung (82) and diabetes (83) with MAP have reported respectively. Screening of somatic *MUTYH* gene mutations in sporadic CRC patients doesn't seem to be informative since the majority of papers revealed no association between *MUTYH* and sporadic colorectal cancer (84, 85) except one study detected two *MUTYH* mutations in Tunisian patients (86).

Immune system response

The immune system of patients with deficiency in DNA mismatch repair genes is more active than individuals without DNA repair defects. It is proposed that the accumulation of peptide elements and mutant proteins in surface of the tumor cells in patients with high MSI or aberrant expression of MMR genes, makes the immune system more active, which results in better diagnosis and survival (87, 88). This active immune system could affect tumorigenesis while antigens presenting in the tumor cell surface (89). Infact The accumulation of peptides and neoantigenes in such patients simulate anti-tumor immune response (90) and in comparison with other lesions they show increased survival rates (91). High Infiltration of lymphocytes in MAP tumors reported in several studies previously (43, 51). Human leukocyte antigen class I complexes mediates in targeting of tumor cells by CD8+ and loss of expression of this antigen is a common event in MSI-H and MAP tumors (92-94). When HLA is lost then tumors hide from immune system due to deficiency in recognition and elimination (95, 96).

Conclusion

Although the *MUTYH* mutations gaining attention in diagnosis and counseling of patients with CRC

and polyposis, many questions about diagnostic and screening protocols still remains unanswered. Detection of *MUTYH* gene variants and their association with polyposis and non polyposis CRC and study of immune system molecules and their involvements in tumorigenesis of MAP patients will be worthwhile for better diagnosis and further screening schedule for MAP patients.

Acknowledgements

The Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran financially supported this work with grant number 707.

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