Deficient DNA mismatch repair: a common etiologic factor for colon cancer

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Hereditary non-polyposis colon cancer (HNPCC), the most common form of hereditary colon cancer, is a syndrome of deficient DNA mismatch repair (MMR). Five, possibly six, human MMR genes have been identified that, when mutated in the germline, cause susceptibility to this syndrome. To date, more than 300 different predisposing mutations are known, mainly affecting the MMR genes MLH1 (~50%), MSH2 (~40%) and MSH6 (~10%). Genetically predisposed individuals carry a defective copy of an MMR gene in every cell. Somatic inactivation of the remaining wild-type copy in a target tissue, typically colon, gives rise to a profound repair defect, progressive accumulation of mutations and cancer. Instability at short tandem repeat sequences, microsatellites, is a typical manifestation of MMR deficiency and apart from HNPCC tumors, occurs in ~15% of sporadic colon and other tumors. The majority of the latter cases are attributable to one particular MMR gene, MLH1, and unlike HNPCC, an epigenetic rather than a genetic mechanism plays an important role in the inactivation of this gene. The present review provides an update of the genetics of HNPCC and more generally, of cancer development driven by deficient MMR. Recent discoveries suggest that apart from post-replication repair, MMR proteins have several other functions that are highly relevant to carcinogenesis. Knowledge of the complex interplay between the MMR system and other cellular pathways allows us to better understand the phenotypic manifestations of HNPCC and other cancers with deficient MMR.

DNA MISMATCH REPAIR

Several recent reviews are available on the basic process of mammalian DNA mismatch repair (MMR) (1–3). The primary function of this system is to eliminate base–base mismatches and insertion–deletion loops which arise as a consequence of DNA polymerase slippage during DNA replication (Fig. 1). The former lesions typically affect non-repetitive DNA and lead to single base substitutions (for example, G→T). Insertion–deletion loops affect repetitive DNA and involve gains or losses of short repeat units (CA) within microsatellites [(CA)12 in Fig. 1], which is referred to as microsatellite instability (MSI). In humans, at least six different MMR proteins are required. For mismatch recognition, the MSH2 protein forms a heterodimer with two additional MMR proteins, MSH6 or MSH3 (the resulting complexes are called hMutSα and hMutSβ, respectively) depending on whether base–base mispairs or insertion–deletion loops are to be repaired. In the former case, MSH6 is required whereas in the latter, MSH3 and MSH6 have partially redundant functions (4,5). A heterodimer of MLH1 and PMS2 (hMutLβ) coordinates the interplay between the mismatch recognition complex and other proteins necessary for MMR. These proteins may include at least proliferating cell nuclear antigen, DNA polymerases δ and ε, single-stranded DNA-binding protein and possibly helicase(s). Besides PMS2, MLH1 may heterodimerize with two additional proteins, MLH3 and PMS1. According to recent findings (6), the MLH1–MLH3 complex (like the MLH1–PMS2 complex) primarily functions in the repair of insertion–deletion loops whereas the role (if any) of the MLH1–PMS1 complex (hMutLβ) in MMR is yet to be characterized (7,8).

HEREDITARY NON-POLYPOISIS COLON CANCER—AN MMR DEFICIENCY SYNDROME

Hereditary non-polyposis colon cancer (HNPCC) is the most common form of hereditary colon cancer, accounting for 5–8% of all colon cancers (9). According to the international diagnostic criteria (Amsterdam criteria I) at least three close relatives should be affected with colon cancer in two successive generations and the age at diagnosis should be <50 years in at least one (10). In addition to colon cancer, HNPCC patients often have an excess of extra-colonic cancers, notably endometrial cancer, and to a lesser extent other cancers, including cancers of the small bowel, ureter and renal pelvis. The diagnostic criteria were recently revised to take these extra-colonic cancers into account (Amsterdam criteria II) (11). From the first clinical description of HNPCC (12) it took 80 years before the first HNPCC locus (MSH2) was genetically mapped (13) and 7 years later, researchers finally reported the identification of the actual predisposing mutation in the Warthin’s family (14).


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To date, the database contains information on more than 300 different predisposing mutations that occur in over 500 HNPCC families from all parts of the world. The majority of the mutations affect two genes, $MSH2$ and $MLH1$, whose protein products are indispensable for MMR (Fig. 1). These mutations commonly impair the necessary protein–protein or protein–DNA interactions.

$MSH2$ and $MLH1$ mutations often (albeit not invariably) give rise to ‘classical’ HNPCC families that fulfil the Amsterdam criteria I and have a high degree of MSI in tumors. According to international criteria, high-degree of MSI is defined as instability at $\geq 2/5$ loci, or $\geq 30–40\%$ of studied loci, whereas instability at fewer loci is referred to as MSI-low (16). In recent years, the number of reported $MSH6$ mutations has steadily increased (Table 1; 17–22).

$MSH6$ mutations often occur in clinically less typical HNPCC families with one or more of the following features: late onset, frequent occurrence of endometrial cancer and low degree of microsatellite instability in tumor tissue. Finally, preliminary observations suggest that the newly discovered MMR gene $MLH3$ may also be mutated in the germline in some suspected HNPCC families with a variable degree of MSI in tumor tissue (23).

**Table 1.** Number of different MMR gene germline mutations considered pathogenic and reported to the database of the International Collaborative Group on HNPCC

<table>
<thead>
<tr>
<th>MMR gene</th>
<th>Chromosomal location</th>
<th>Number of mutations (%) 1996</th>
<th>Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>$MSH2$</td>
<td>2p21</td>
<td>48 (38%)</td>
<td>125 (38%)</td>
</tr>
<tr>
<td>$MLH1$</td>
<td>3p21–23</td>
<td>75 (60%)</td>
<td>164 (49%)</td>
</tr>
<tr>
<td>$PMS1$</td>
<td>2q31–q33</td>
<td>1 (1%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>$PMS2$</td>
<td>7p22</td>
<td>2 (2%)</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>$MSH6$</td>
<td>2p21</td>
<td>0 (0%)</td>
<td>30 (9%)</td>
</tr>
<tr>
<td>$MLH3$</td>
<td>14q24.3</td>
<td>0 (0%)</td>
<td>7 (2%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>126 (101%)</td>
<td>332 (100.3%)</td>
</tr>
</tbody>
</table>

The present number is compared with the year 1996 when the database was established (15).

**SPORADIC COLON CANCERS WITH MSI**

MSI, a hallmark of HNPCC, occurs in ~15\% of sporadic tumors from the colorectum and other organs as well (16). Sporadic counterparts of tumors of the HNPCC spectrum (24), endometrial and gastric cancer, for example, typically show a high degree of instability (an MSI-low subset also exists) whereas MSI-low is a predominant pattern in tumors not belonging to the HNPCC spectrum. The majority of MSI-high tumors are due to inactivation of $MLH1$, which mostly results from promoter hypermethylation rather than somatic mutations or loss of heterozygosity (25). Studies on cell lines have shown that promoter hypermethylation is often biallelic (26). This DNA methylation disorder, the mechanism of which is unknown, is already present in colorectal adenomas and has several other gene targets besides $MLH1$ (27,28). The MMR genes $MLH1$ and $MSH2$ do not seem to be implicated in the MSI-low subset (29,30). Aberrant DNA methylation that may
involve a DNA repair pathway separate from MMR has been proposed to be a possible underlying defect in this group (31).

TUMORIGENESIS IN THE CONTEXT OF MMR DEFICIENCY

Mutation rates in tumor cells with MMR deficiency are 100–1000-fold as compared with normal cells (32,33). Apart from anonymous microsatellites, these mutations affect important growth-regulatory genes, especially those containing repeat sequences as mutation targets. In recent years, there has been a tremendous increase in the reported number of target genes whose mutations appear to be selected for in tumors with deficient MMR (Table 2). Some genes, such as the MMR genes MSH3 and MSH6 seem to be fairly commonly involved in microsatellite-unstable tumors of diverse origin (36), whereas others show considerable tissue specificity. For example, frameshift mutations in the TGFβRII (46) and TCF4 (39) genes are strongly selected for in gastrointestinal malignancies but not in endometrial cancer. Such tissue-specific selection may in part help explain the HNPCC tumor spectrum. Unusual manifestations (de novo neurofibromatosis type I, hematologic malignancies) in rare instances of homozygous MLH1 mutation carriers (47,48) provide further support to this model. Thus, a homozygous MMR gene mutation would generate constitutional genomic instability and downstream genes that by structural and other associated disorders.

As described above, MSI in sporadic colorectal cancer typically results from MLH1 inactivation that itself is part of a more primary defect, DNA methylation abnormality. Thus, MLH1 inactivation gives rise to MMR deficiency, which in turn induces secondary mutations in other growth-regulatory genes (Table 2). On the other hand, besides MLH1, other genes may be affected by aberrant DNA methylation, and these genes may be the same as or different from those susceptible to replication errors due to MMR deficiency. In individual tumors, several different mechanisms might therefore drive the tumorigenic process. For example, the APC gene, the ‘gatekeeper’ of cellular proliferation, may be inactivated in colon tumors by loss of heterozygosity (49), somatic mutations (50) or, as recently shown, by promoter hypermethylation (51,52). Loss of heterozygosity would indicate yet another mechanism of tumorigenesis. Interestingly, Esteller et al. (21) found that APC promoter hypermethylation was not more common in microsatellite-unstable than in microsatellite-stable tumors, which together with other observations by Toyota et al. (54) emphasizes that DNA methylation abnormality and MSI may indeed operate independently.

HNPPC—NOT A MERE MMR DEFICIENCY SYNDROME

More and more evidence is accumulating to indicate that the MMR system does not only function in post-replication repair but is directly or indirectly linked to several other essential biologic processes. Combining genetic information of HNPCC families, biochemical studies of MMR, and knowledge of other cellular functions of MMR may be helpful to establish
genotype–phenotype correlations in HNPCC. It has been known for some time that there is a connection between the MMR system and G1/S cell cycle checkpoint (55). It was recently shown that MMR gene mutation carriers who additionally have a variant form of cyclin D1 that is involved in G1/S control display a significantly lower age at onset of colon cancer than MMR gene mutation carriers without this variant (56). The variant form with a longer half-life may allow cells containing replication errors to pass through the G1/S checkpoint and proliferate instead of undergoing apoptosis; alternatively, a direct or indirect connection might exist between cyclin D1 and the MMR pathway (56).

Apart from biosynthetic errors, several other types of endogenous or exogenous DNA damage can serve as substrates for MMR proteins. For example, the MMR system can act on damage caused by heterocyclic amines (57), many of which are of dietary origin and therefore relevant to colon cancer. Enzymes that metabolize these compounds, including the N-acetyltansf erase 1 and 2, show polymorphic variation and, like cyclin D1, may modify the clinical phenotype of HNPCC (58,59). The MMR proteins also recognize and eliminate oxidative damage (60,61). Such damage may arise through the action of the cyclo-oxygenase 2 (COX2) enzyme whose expression levels are often elevated in inflammatory processes and cancer. COX2 inhibitors are presently being tested in international multicenter trials for their possible ability to reduce polyp formation in HNPCC (62). Even before the identification of human MMR genes, resistance to alkylating agents was described as a feature of MMR-deficient cells (reviewed in 63). Since then, the role of the MMR machinery in the correction of alkylation-induced DNA damage has aroused particular interest and has recently revealed interesting links to apoptosis. Among alkylating-derived base adducts, O6-methylguanine is one of the most cytotoxic and normally triggers a damage-signaling cascade that can lead to G2/M arrest or cell death; this requires a functional MMR system and demonstrates variable dependence on p53 (64,65). MMR-deficient cells fail to induce apoptosis and are therefore alkylation-tolerant. Mouse studies suggest that the persistence of mutagenic lesions generated by chemical DNA damage may be an important factor contributing to the HNPCC tumor spectrum (66).

### Table 3. Additional susceptibility genes to non-polypotic colon cancer identified by family studies

<table>
<thead>
<tr>
<th>Gene or locus</th>
<th>Chromosomal location</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβRHI</td>
<td>3p22</td>
<td>68</td>
</tr>
<tr>
<td>CHD1 (E-cadherin)</td>
<td>16q22</td>
<td>69</td>
</tr>
<tr>
<td>I1307K variant of APCa</td>
<td>5q21</td>
<td>70</td>
</tr>
<tr>
<td>E1317Q variant of APCa</td>
<td>5q21</td>
<td>72</td>
</tr>
<tr>
<td>Genes mapped but not yet identified</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMP5b</td>
<td>6q</td>
<td>73</td>
</tr>
<tr>
<td>CRAC1c</td>
<td>15q14–q22</td>
<td>74</td>
</tr>
</tbody>
</table>

aAssociated with a variable number of polyps. The number of polyps may not be higher than in the general population. Popula-
tion studies suggest that I1307K is unique to Ashkenazi Jews (71).

bHeredity mixed polyposis syndrome associated with atypical juvenile polyps.

cColorectal adenoma and carcinoma 1, identified in an Ashkenazi family.

### NON-POLYPTOTIC COLON CANCER FAMILIES WITHOUT DEMONSTRABLE MMR GENE MUTATIONS

Altogether, approximately two-thirds of all clinically typical HNPC families that meet the Amsterdam criteria I and show MSI in tumor tissue display germline mutations in the MMR genes shown in Table 1. This leaves one-third of such families molecularly unexplained. Some MMR gene alterations (especially in MLH1) consist of reduced mRNA or protein expression without demonstrable structural changes. The detection of even such alterations is possible with new techniques that allow the separation of the two parental homologues in monochromosomal hybrids (14). Although these new techniques are anticipated to reduce the molecularly unexplained fraction of HNPCC, linkage analyses (22,67) suggest that such non-polypotic colon cancer families exist that do not involve the presently known HNPCC loci and may therefore harbor novel predisposition genes. Table 3 shows examples of genes and mapped loci that may be associated with clinical phenotypes indistinguishable from HNPCC but without MSI in tumor tissue. Altogether, the nature of predisposition is yet to be discovered in a majority of familial aggregations of colon cancer (75) offering important challenges for future genetic research.

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### REFERENCES


738 Human Molecular Genetics, 2001, Vol. 10, No. 7
human molecular genetics, 2001, vol. 10, no. 7


